Satbir Singh Gosal
Shabir Hussain Wani *Editors*

Accelerated Plant Breeding, Volume 2

Vegetable Crops

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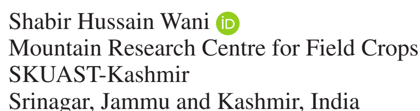
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Dr Lumpkin has specialized in research, education, international development and administration, originally within the areas of Asian agronomy and horticulture, ethnobotany, and marketing systems, and later within the international agriculture development community. His career evolved out of life experiences including growing up on a small farm in Eastern Washington,

Peace Corps in India, army in Japan and higher education at WSU and University of Hawaii, including field research in mainland China. He is widely known among the CGIAR, international agriculture donor agencies and national agriculture systems in the developed and developing world for his leadership in the rebuilding of two international agriculture research institutes (CIMMYT – International Maize and Wheat Improvement Center in Mexico and AVRDC – World Vegetable Center in Taiwan); establishment of a new international institute, i.e., The Borlaug Institute for South Asia; his books and publications on Azolla, Azuki, Edamame, Wasabi and global horticulture; and leadership to address malnutrition and poverty alleviation in the developing world. He became increasingly involved in administration and international development, beginning with a position as Chair of the Department of Crop and Soil Sciences at Washington State University (currently Emeritus Professor), Director General of AVRDC – The World Vegetable Center (www.avrdc.org), and Director

General of the International Maize and Wheat Improvement Center (CIMMYT) (www.cimmyt.org), along with appointment as the first Director General of the newly created Borlaug Institute for South Asia (bisa.cimmyt.org) approved for UN status by the Cabinet of the Government of India. Over 7 years of his leadership, CIMMYT's budget grew from $44 to $150 million and from 70 to 250 scientists. On leaving CIMMYT, the Board of Trustees appointed him as the first Director General Emeritus. During his career he has received scholarships/fellowships from East-West Center, National Science Foundation and National Academy of Science and was presented alumni awards by WSU and UH, Friendship award by China and Swaminathan award by India. He currently lectures and advises on international agriculture development and is developing 10 small vineyards in the Columbia River Gorge National Scenic Area.

Keeping in view his enormous contributions to Research & Development, we feel honoured to dedicate this book to Dr Thomas A. Lumpkin.

Foreword

Vegetables play a vital role in the human diet, especially for vegetarians. The demand for vegetables is expected to increase dramatically in the coming years. Fresh vegetables are gifted with almost all of the nutritional principles that our body requires. Vegetables are rich sources of minerals like potassium, phosphorus, iron, calcium, sodium magnesium, iodine, zinc, copper and manganese; vitamins like vitamin-A, vitamin B-complex, vitamin-C, vitamin-E, vitamin-K, niacin and folate; and supplementary amounts of protein, fibre and calories essential to the human diet. Besides, coloured vegetables also possess high levels of antioxidants. Using conventional breeding approaches, such as introduction, selection, hybridization, mutation and polyploidy, an appreciable progress has been made in the vegetable crops and a series of improved varieties/hybrids have been developed the world over. However, the breeding has been a difficult task in the presence of high level of heterozygosity, especially in the vegetatively propagated species, cross-incompatibility, seedlessness, complex polyploidy, and lack of flowering under certain situations. In the current scenario of climate change, population pressure, competition for natural resources and WTO, there is need for rapid development of new varieties possessing resistance to various biotic and abiotic stresses, durable and multiple resistance, wider adaptability, early maturity, varieties with better water-use efficiency, better shelf life and processing quality, with improved minerals, vitamins, amino acids, proteins, antioxidants and bioactive compounds and suitable for mechanized harvesting which could result in new growth and productivity constraints. Plant breeders always look for new breeding tools to circumvent the recurring problems and to speed up the breeding process. In this regard, innovative techniques/technologies such as doubled haploidy, embryo culture, marker-assisted selection, transgenic breeding, speed breeding and genome editing are increasingly being used to supplement/complement the conventional approaches. *Accelerated Plant Breeding: Vegetable Crops*, edited by Drs Satbir Singh Gosal and Shabir Hussain Wani, includes chapters on wide range of crops grown in subtropical and temperate zones, in which different edible plant parts such as roots, stems, leaves, flowers, pods/fruits and seeds are consumed. Separate chapters have been included on important vegetable crops such as potato, tomato, onion, cole crops, pea,

temperate root vegetables, brinjal, *Capsicum*, cucumber, pumpkin and squashes, okra, muskmelon and cowpea. This volume provides state-of-the-art information especially on recent methods which hold significant promise to speed up the process of plant breeding. I applaud the editors of this book, Dr S.S. Gosal and Dr Shabir Hussain Wani, for compiling very useful information from the contributions of selected experts working on different vegetable crops. I am confident that this book will be of much help to research workers, seed men, teachers, and students alike. I appreciate the authors for their hard work.

ML Chadha

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Preface

Vegetable crops represent a genetically diverse group of plants, grown under tropical, subtropical, and temperate climates of the world. Various crops differ with respect to their life span (annual, biennial, perennial), mode of propagation (vegetative, seeds), growth habit (herbaceous, shrub, vine, tree), growing season (winter, spring, summer) and consumption of their different parts (roots, stems, leaves, flowers, fruits, seeds). Vegetables play an important role in the nutritional security by providing not only the energy-rich food but also the vital protective nutrients like minerals and vitamins. Vegetable crops are grown under different growing conditions such as field, green house, polynet house, low tunnel and in the kitchen gardens. Conventional breeding methods such as pure line selection, mass selection, hybridization followed by pedigree/backcross methods, polyploidy and induction of mutations have been the methods of choice and were widely and successfully used for crop domestication, germplasm enhancement and development of superior varieties. Likewise, heterosis breeding has been exploited to a larger extent, to develop hybrids in various vegetable crops. Non-conventional methods based on cellular, transgenesis and genomics hold significant promise for precision and rapid breeding. The main aim of this volume is to highlight the role of new techniques/technologies, when used in conjunction with conventional methods, in accelerating the breeding process for early release of varieties. Innovative methods such as doubled haploidy, micropropagation, embryo culture, marker assisted selection, marker-assisted background selection, genomic selection, high-throughput genotyping, high-throughput phenotyping, reverse breeding, transgenic breeding, shuttle breeding, speed breeding, and genome editing are now increasingly becoming important. This volume provides an authoritative review account of various conventional and unconventional approaches for the improvement of important vegetable crops. The book includes chapters prepared by specialists and subject experts on different crops/aspects in relation to accelerated breeding. Separate chapters have been included on important crops such as potato, tomato, onion, cole crops, pea, temperate root vegetables, brinjal, *Capsicum*, cucumber, pumpkin and squashes, okra, muskmelon and cowpea.

We earnestly feel that this book will be highly useful for students, research scholars, and scientists working in the in the area of plant breeding, genomics, cellular/molecular biology, and biotechnology at universities, research institutes, R&Ds of agricultural MNCs for conducting research, and various funding agencies for planning future strategies.

We are highly grateful to all learned contributors, each of whom has attempted to update scientific information of their respective area and expertise and has kindly spared valuable time and knowledge.

We apologize whole heartedly for any mistakes, omissions, or failure to acknowledge fully.

We would like to thank our families (Dr Satwant Kaur Gosal (wife of SSG), Sana Ullah Wani, Taja Begum, Sheikh Shazia, Yasir Wani, and Muhammad Saad Wani (father, mother, wife, brother, and son of SHW)) for their continuous support and encouragement throughout the completion of this book.

We highly appreciate the all-round cooperation and support of Springer Nature Switzerland AG, Gewerbestrasse 11, 6330 Cham, Switzerland, for their careful and speedy publication of this book.

Ludhiana, Punjab, India — Satbir Singh Gosal
Srinagar, Jammu and Kashmir, India — Shabir Hussain Wani

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About the Editors

Satbir Singh Gosal has been a Bursary holder of The Royal Society, London, is a former Director at the School of Agricultural Biotechnology and Ex-Director of Research at Punjab Agricultural University, Ludhiana, India. He was an Honorary Member of the Board of Assessors (Australian Research Council, Canberra), Biotechnology Career Fellow, The Rockefeller Foundation, USA, and President of the Punjab Academy of Sciences. He has published more than 200 research papers in refereed journals and 35 book chapters. He has coauthored one textbook and has coedited five books including three with Springer.

Shabir Hussain Wani received his PhD in Genetics and Plant Breeding from Punjab Agricultural University. He has published over 100 peer-reviewed papers and has edited 13 books on plant stress physiology, including 7 with Springer. He also served as a Review Editor for Frontiers in Plant Science from 2015 to 2018. He is currently an Assistant Professor at the Mountain Research Centre for Field Crops of the Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir in India..

Chapter 1
Major Paradigm Shifts in Potato Breeding

Salej Sood, Vinay Bhardwaj, and S. Sundaresha

1.1 Introduction

Potato is an important global food crop. Potato produces more food per unit of land in comparison to major crops, and its demand is growing continuously. The wealth of genetic resources in potato is huge, and the genus *Solanum* includes about 1400 species (Jansky and Spooner 2018). The tuber-bearing wild relatives of genus *Solanum* have been classified as section Petota. There are 107 wild species, 4 landraces or traditional cultivated species, and rest are all improved cultivars (Spooner et al. 2007; Ovchinnikova et al. 2011; Spooner 2016). All cultivated species have been combined into single species, *S. tuberosum*, divided into two cultivar groups (Ugent 1970; Ovchinnikova et al. 2011). The first group named as Andigenum group contains all diploid, triploid and tetraploid cultivated potatoes from Andes through Venezuela and Argentina. Second group Chilotanum contains tetraploid of cultivated species of lowland south-central Chile. It is speculated that polyploid wild and cultivated species are the result of sexual polyploidization through 2n gamete formation (Spooner et al. 2010; Jansky and Spooner 2018). It is likely that first cultivated potatoes were diploid, and extensive gene flow within and among different ploidy species resulted in production of present-day polyploid highly heterozygous potatoes.

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1.2 Early Efforts in Potato Breeding

Potato originated in Andean region of South America and later spread to different parts of the world. Andean farmers were early breeders who used to collect open-pollinated berries from landraces to improve the tuber yields after degeneration of tubers due to virus infection. They maintained high diversity in their fields for various reasons including plant survival against various biotic and abiotic stresses, mixture of flavours, shapes, etc. Natural gene flow among cultivated and wild species and intentional crosses by Andean farmers resulted in new genetic combinations. There was no progress in potato breeding for many years until Thomas Andrew Knight did cross-pollination in potato. However, his efforts did not gain widespread acceptance. The devastation of potato crop in whole Europe due to late blight epidemics of the mid-1840s generated interest in potato breeding. Cross-pollination followed by selection resulted best potato plants for growing. The first few well-known cultivars in USA were 'Rough purple Chili', 'Garnet Chili' and 'Early Rose'. 'Russet Burbank' derived from 'Burbank' was the predominant cultivar of the USA and Canada and still occupies considerable area in the USA. The potato breeders learnt that each potato seedling derived from F_1 seed is different from each other. 'Daber' a Chilean potato cultivar is a prominent parent of many European cultivars (Jansky and Spooner 2018).

In India, the potato is believed to have been introduced towards beginning of seventeenth century most probably by the Portuguese traders or by British missionaries (Pushkarnath 1976). The earliest potato introductions in India resembled subsp. *andigena* and were adapted to short days, had long dormancy and were capable of withstanding high temperatures in country stores. Between 1924 and the end of World War II, a large number of European varieties were introduced with a view to selecting those suitable for local conditions. These, however, failed to make an impact as potato is grown in India in conditions very different from those in Europe.

The potato breeding programme initiated in India in 1935 at the Potato Breeding Station (PBS), Shimla under the Imperial (now Indian) Agricultural Research Institute, New Delhi. Variety improvement in potato was a challenge as the introduced European varieties were all long-day adapted, their multiplication in Indian conditions was characterized by progressive accumulation of viral diseases resulting in concomitant decrease in yield, and limitations of tuber storage and utilization in hot and humid Indian conditions. The available collections were evaluated for direct introduction and/or for use as parental lines in potato breeding programme. Since potato flowers only under long days, potato hybridization was initiated in hills at Kufri (Shimla), Himachal Pradesh. Crosses were made between indigenous cultivars and promising exotic introductions to breed new high-yielding hybrids suitable for subtropical conditions in plains and temperate conditions of the hills (Fig. 1.1) Initial attempts to breed high-yielding potato varieties for subtropical plains were, however, unsuccessful due to degeneration of progenies during evaluation period in the plains. To circumvent this problem, the seedlings were raised and maintained in hills at Kufri with only a few tubers of each clone being sent for

Fig. 1.1 Potato parental lines for hybridization at Shimla

evaluation in the plains. This plan too proved unsatisfactory due to dormancy of hill seed and limitation of land in the hills at Kufri.

The major breakthrough in potato improvement programme came in 1963 with the development of ‘Seed Plot Technique’, which made it possible to raise, evaluate, select and multiply breeding material under disease-free conditions in Indian plains itself. This led to development of a system, wherein crosses were made in hills and the segregating populations evaluated and maintained in disease-free condition in the plains. This approach has yielded rich dividends, and till date 62 indigenous improved potato varieties have been released by the ICAR-Central Potato Research Institute for different agro-climatic zones of the country.

1.3 Constraints in Potato Breeding

Cultivated potato varieties are autopolyploid and highly heterozygous. In potato breeding, heterozygous varieties are crossed with donor heterozygous genotypes for transfer of desired traits, and desired genotypes are selected in F_1 generation itself and released as new varieties. In potato, the hybrid cannot be reconstituted again in the form of TPS by crossing the same parental lines as each and every TPS in F1 generation is genetically different in potato. The F_1 hybrid varieties in potato are maintained in-vitro and are propagated clonally. This is the major difference in potato breeding in comparison to major food crops like rice, wheat and maize,

where homozygosity is first attained to release new varieties as open-pollinated homozygous lines or hybrid varieties. Attempts to develop homozygous lines in potato even at the diploid level failed earlier due to high inbreeding depression in tetraploid species and self-incompatibility in diploid species. Sterility and poor vigour prevented many lines from advancing beyond the S_2 generation in tetraploid potatoes. Sterility and incompatibility also complicated the introgression of useful biotic and abiotic stress-resistant genes from wild species into cultivated types. These barriers further restricted the base broadening of cultivated potatoes. Even today, only few species are found in the pedigree of most of the cultivated potato varieties across the globe (Bradshaw 2009). Although new beneficial alleles have been added in the modern-day potato cultivars, these alleles have not become fixed because selection was not effective against their deleterious counterparts (Vos et al. 2015). The autotetraploids like potato undergo twice as many mutations as diploids. The mutation load in tetraploids is therefore twice that of diploids (Otto 2007). Polysomic inheritance poses serious challenges in breeding:

(i) Genetic studies are difficult.
(ii) Mendelian ratios do not apply to genes distal to centromere.
(iii) Unexpected homozygotes appear due to double reduction.
(iv) Transgressive segregant identification requires large families.
(v) Deleterious recessive alleles are retained behind the functional allele.
(vi) Continuous variation observed even for single gene traits.
(vii) Epistatic interactions are magnified.
(viii) Allele dosage has great importance and interactions with environment are complex (Chase 1963a, b; Rowe 1967; Sanford 1983; Gopal 2015; Jansky and Spooner 2018).

These complexities associated with polyploid inheritance lead to slow genetic gains and poor response to selection. Since the breeding system in potato is still fundamentally unchanged, i.e. making crosses and phenotypic selection (MacKay 2005), to date there is no increase in the frequency of large set of desirable alleles.

1.4 Potato Breeding Programme

The conventional potato breeding programme is based on biparental crossing followed by selection. Rarely, the interspecific hybridization is followed to transfer the disease or insect resistance genes from other species which is followed by one to two backcrosses. The desired plant type is fixed in F_1 generation itself through clonal propagation from tubers. The chances of reassorting genes of heterozygous tetraploid parents into better combination are low, and a large number of progeny plants need to be evaluated. Although there is only single sexual cycle that is followed in potato, the time period required for development of a new variety in potato is 10 or more years (Fig. 1.2). This is due to time required for seed increase from

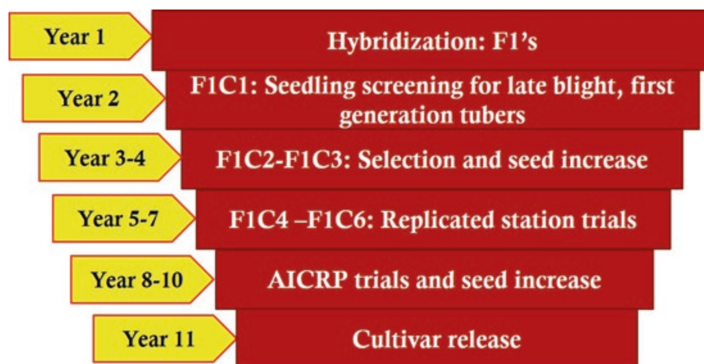

Fig. 1.2 Schematic representation of potato breeding programme

single selected plant for evaluation. The details of the process are given below in the flow chart.

1.5 Methods to Accelerate Potato Breeding

As evident from previous sections that potato breeding is complex, gains are slow in comparison to major field crops, and breeding cycle is long. In order to speed up the breeding process and enhance genetic gains, various breeding methods are being used.

1.5.1 Marker-Assisted Breeding

Developments in molecular biology during the last 30 years have allowed better understanding of genetic regions associated with resistance and susceptibility to diseases and pests. Most of the genes controlling monogenic (major *R* genes) and polygenic resistance (QTL's) to late blight have been mapped and located on the 12 potato chromosomes. Breeding for resistance in potato through pyramiding (incorporation of several different genes) started long time back, but exact quantification of the achieved resistance effect was not reported. Pentland Dell and Escorts were some early potato cultivars developed through such breeding strategies (Bradshaw 2009). Marker-assisted selection has been successfully employed in disease resistance in potato. The research on resistance to late blight in recent years has been focussed on stacking of several *R* genes in one cultivar/genotype which might increase both durability and level of resistance. Stacking of two *R* genes has been reported to improve durability of late blight resistance, besides delaying the onset of late blight disease. The additive effect of pyramiding *P. infestans* resistance genes

Rpi-mcd1 and *Rpi-ber* was studied by introgression in diploid *S. tuberosum* population. Sarpo Mira with at least five reported *R* genes (*3a, 3b, 4, Smira1, 2*) is one of the few potato cultivars, developed through conventional breeding, which expresses significant levels of durable late blight resistance (Kim et al. 2012).

At CPRI, parental lines selected through genotyping of indigenous and exotic potato germplasm collection of CPRI repository by molecular markers tightly linked to R1, R2 and R3 genes are being utilized for pyramiding these genes in single potato host background for providing enhanced durable late blight resistance (Fig. 1.3). Major QTLs for late blight resistance have been identified in diploid wild species *Solanum chacoense* using amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers (Chakrabarti et al. 2014). Similarly for all major viruses and potato cyst nematode, MAS is being used. Presently there are four different *R* genes, namely, Ry_{adg}, Ry_{sto}, Ry_{hou} and Ry_{chc}, known to confer extreme resistance (ER) to PVY. Additionally, four *N* genes, viz. Ny_{chc}, Ny_{dms}, Nc_{tbr} and Ny_{adg}, also confer high resistance (HR) to PVY. The gene Ny_{adg} conferring HR to PVY is epistatic to Ry_{adg}. As a result, the genotypes carrying both Ry_{adg} and Ny_{adg} exhibited ER to PVY. In India, a triplex (YYYy) potato parental line with Ry_{adg} gene having extreme resistance to PVY has been developed using MAS (Kaushik et al. 2013). The R genes Rx_{adg} (*Rx1*), Rx_{tbr}, Rx_{acl} (*Rx2*) and $Rx_{HB}^{scr}/Rx_{CP}^{scr}$ confer extreme resistance to PVX. A single dominant gene *Ns* was reported which confers HR for PVS

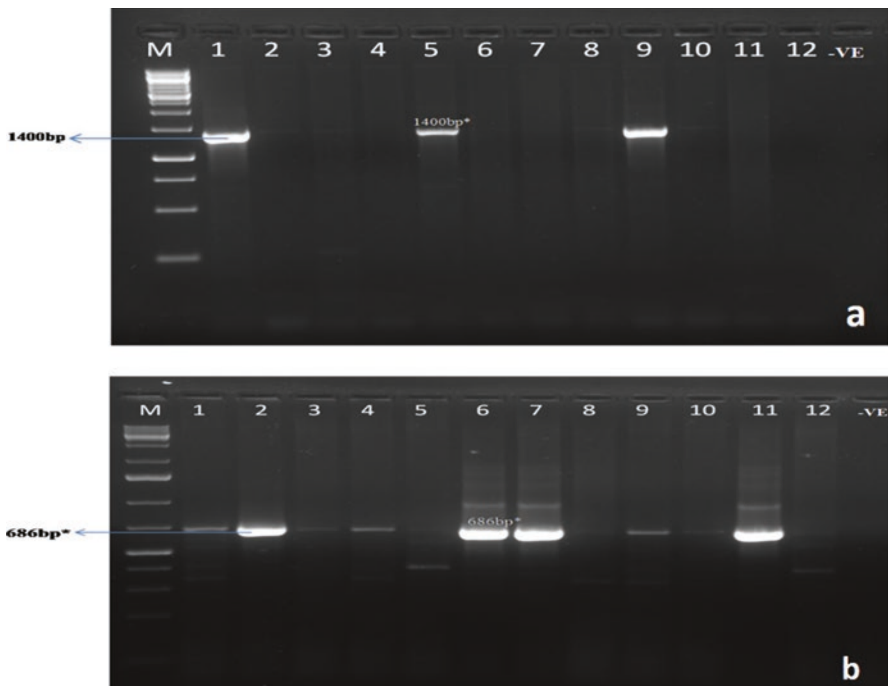

Fig. 1.3 Screening of parental lines and hybrids for late blight resistance genes (a-R1 and b-R2)

in *S. tuberosum* ssp. *andigenum*. Genotypes with *Ns* gene remain symptomless and no virus (PVS) titters develop in enzyme-linked immunosorbent assay (ELISA). The markers SCG17321, UBC811660 and SC811260 have proved to be highly accurate to select PVS-resistant potato genotypes through MAS (Witek et al. 2006). Genes for resistance to PVM were reported in wild and cultivated *Solanum* species. The PVM resistance gene *Rm* originates from *S. megistacrolobum* and is responsible for a hypersensitive response of potato plants to PVM infection. The dominant gene *Gm* was derived from *S. gourlayi* and confers a different type of PVM resistance than the *Rm* gene. The *R* genes which confer ER to PVA were Ry_{sto}, Ra_{sto}, Ra_{adg} and Ry_{ho}, whereas *N* genes were Na_{adg}, Na_{sto}, Ny_{chc}, Na_{dms}, Ny_{dms}, Na_{tbr} and $NaKE_{tbr}$ conferring HR to PVA (Cockerham 1970; Barker 1996; Hamalainen et al. 1998). Molecular markers associated with Ry_{adg} are also used to select genotype having PVA resistance. In 2009 a single gene from *S. etuberosum* (Rlr_{etb}) was mapped to chromosome IV which confers resistance to PLRV, besides some German potato cultivars were derived by introgression of genes from *S. demissum*. Recently, a sequence characterized amplified region (SCAR) marker designated as 'RGASC850' was derived from resistance gene analog (RGA), which is highly predictive of Rl_{adg}-based resistance. In addition, a cleaved amplified polymorphic sequence (CAPS) marker based on 'RGASC850' was developed for distinguishing the genotypes carrying Rl_{adg} gene.

For PCN, researchers have proved the complementary effect of the two resistance sources, *S. tuberosum* ssp. *andigena* and *S. vernei*, and therefore the joint effect of multiple *R* genes will result in broad spectrum and high level of resistance. Several mapped loci that harbour resistance to *G. pallida* and *G. rostochiensis* have been mapped from various wild and cultivated potato species. Molecular markers are available for selection genotypes resistant to PCN. So far, 19 genes including major and minor QTLs have been placed on potato chromosome map, conferring resistance against PCN (Dalamu et al. 2012).

1.5.2 Diploid Hybrid Breeding

Several efforts were made to broaden the narrow genetic base of cultivated tetraploid potato in the last century (Bradshaw and MacKay 1994; Bradshaw 2009; Jansky and Spooner 2018). Selection was also carried out in diploid cultivated potatoes, particularly *phureja* and *stenotomum* species. Clones producing 2n gametes were hybridized with cultivated tetraploid potatoes for transfer of genes from these species. Famous maize breeder Chase (1963a) proposed a diploid breeding scheme in potato to harness the genetic diversity from wild potato relatives. The scheme was proposed by first reducing the ploidy to diploid level of cultivated potatoes, hybridization at diploid level with diploid species and polyploidization of selected diploids back to tetraploid level.

Heterozygous diploids were found to be superior to homozygous diploids derived from doubling of monoploids generated through anther culture (Uijtewaal et al.

1987). The results indicated that increasing ploidy without adding new genetic variation could not lead to increased productivity in potato. In diploid and tetraploid potatoes, gene action and heterozygosity may have a greater effect on plant vigour and productivity than ploidy level (Rowe 1967). Diploids have shown high tuber yield potential than tetraploids in earlier studies (Carroll 1982; Hutten 1994; De Maine 1996; Watanabe et al. 1996; Simmonds 1997) suggesting ploidy reduction and breeding potatoes at diploid level for exploiting heterosis as explored in maize. For last two decades, superiority of tetraploid and its advantages have been questioned in comparison to diploids (Jansky and Spooner 2018). However, it is easy to increase ploidy; ploidy reduction is difficult.

Genetic variation is the foundation of breeding programme, and inbreeding has the capability of binding the entire genotype together (Allard 1999). The homozygosity in diploids by inbreeding is expected to be much faster than their tetraploid count parts. Many agronomically important traits in potato are expressed as recessive traits, but the homozygous recessive condition in tetraploid potatoes is practically impossible. The recessive traits can be easily expressed in diploids.

It is important to note that cultivated tetraploid species are self-compatible and amenable to selfing but inbreeding depression does not allow selfing for more than two generations in most cases. The diploid species on the other hand are self-incompatible. Two approaches have been explored to develop inbred potato lines in diploids and even in cultivated tetraploids. Androgenesis is one method to develop monoploid (1x) and subsequently homozygous diploidized plants (Veilleux 1990). This method is tedious and response to androgenesis is poor in most potato genotypes. Second method is based on natural self-incompatibility inhibitors, i.e. *sli* gene discovered in *S. chacoense*, which is sexually compatible with diploid cultivated potato (Phumichai et al. 2005). Inbred lines in potato have been developed using *sli* gene by crossing *sli* gene carrying *S. chacoense* and diploid cultivated *phureja* in Japan (Phumichai et al. 2005). They observed reduced vigour as selfing generations advanced, but individuals with good tuber yield were also observed in S_4 generation. A breakthrough paper appeared later, where Lindhout et al. (2011) reported development of diploid potato lines with acceptable tuber traits. They intercrossed F3 plants uniform for tuber traits to make F1 hybrids to exploit heterosis in potato. The proof of concept for diploid hybrid potato in this study sets the stage for future developments in this unexplored area in potato breeding. Afterwards, many studies appeared with development of vigorous fertile diploid inbred lines in potato (Jansky et al. 2014a, b, 2015; Endelman and Jansky 2016).

We at ICAR-CPRI, Shimla, have also started development of diploid inbred lines (Fig. 1.4) through transfer of self-incompatibility gene (*sli* gene) diploid semi-cultivated species and importing diploid population from USDA (Fig. 1.5). A series of diploid inbred lines with favourable genes for various tuber and quality traits in cultivated potato will result in desired F1 combinations. There are several advantages of generating inbred lines maintained as TPS rather than tubers. True potato seed can be easily shared among research programmes, each of which is likely to collect phenotypic data on a different set of traits. Phenotypic data are cumulative for each inbred line and can be aligned with genotype data. True potato seed does

Fig. 1.4 Selfed berries of diploid potato, *S. tuberosum*

Fig. 1.5 Maintenance of different wild potato species under glass house at Shimla

not carry any economically important fungal, oomycete, or bacterial pathogens or major potato viruses. TPS can be rapidly increased for agronomic and storage trials when a potential new hybrid cultivar is identified.

Targeted and predictable breeding has now become possible, enabling the development of new innovative varieties within a time period of <5 years compared to >10 years in traditional breeding, with beneficial traits. Furthermore, 30 grams of seed are sufficient to produce enough seedling tubers to plant 1 hectare of potatoes,

whereas 2500 kilos of conventional seed tubers are required for the same area. Thus, the propagation via True Potato Seeds will speed up the process of potato propagation by more than 400% and simultaneously decrease the costs with more than 300%.

1.5.3 Speed Breeding

Speed breeding is another emerging technology suitable for potato breeding where in four-six generations can be easily taken up under controlled conditions for accelerating the genetic gain in breeding and reducing the generation time of a breeding cycle for many years to 3–4 years. Rapid generation advancement towards reaching homozygosity would facilitate genetic gain for key traits and the rapid development of improved cultivars. Scientists in the University of Queensland, Australia, developed a speed-breeding platform by creating an artificial environment with enhanced light duration for longer daylight to speed up the breeding cycles of photo-insensitive crops. Results of speed breeding experiments conducted under controlled conditions in continuous light for extended daylight induced early reproduction and accelerated genetic gain. The yield and quality of the plants grown under artificial light were same as those to regular glasshouse conditions. Increasing human population needs more food and better crop varieties in future, capable of producing high yield with limited space and production window by completing more generations in lesser time. Speed breeding uses supplemental lighting to aid photosynthesis rate in intensive regimes of up to 22 h/day in a glasshouse environment that allows rapid generation cycling through single seed descent and potential for adaptation to larger-scale crop improvement programmes (Watson et al. 2018). The seed to seed cycle was completed in 8 weeks using this technique, allowing six generations of wheat each year. The technique has been extended in other grain crops like barley, pea and chickpea and canola. In potato, the speed breeding can be combined with aeroponics technology for disease-free tuber multiplication throughout the crop cycle (Fig. 1.6). Other existing technologies can also be amalgamated such as marker-assisted selection, genomic selection and CRISPR gene editing for rapidity and precision breeding.

1.5.4 Genomic Selection

Genomic selection (GS) has been proposed in 2001 as an alternative to 'marker-assisted selection' (MAS), when available molecular markers are enough to densely cover the genome of the animal or plant species. In genomic selection (GS), all markers are fitted together in a single step prediction model. Since the number of predictor variables (markers) usually exceeds the number of variables (traits phenotyped), the usual linear model framework is not applicable, and specific statistical models adapted to the 'big data' problem must be used. To 'train' the prediction

Fig. 1.6 Bulk seed tuber multiplication under aeroponic system in potato

model, we need a 'training' or reference population, with both genotypic and phenotypic data. In practise GS is always applied in a population that is different to the reference population where the marker effects are estimated. It might be that the selection candidates are from the same breed but are younger than the reference population, or they could be from a different selection line or breed.

A number of different factors influence the accuracy of genomic prediction models, such as trait heritability, size of training population and genetic relationship between individuals (Sverrisdóttir et al. 2017). The number of markers required for robust and unbiased predictions with GS in potato is around 8000 based on LD decay (Slater et al. 2016), while Sverrisdóttir et al. (2017) suggested 10,000 markers to obtain good prediction accuracies for dry matter and chipping quality. Recently, GS simulation studies have been made in tetraploid potato (Slater et al. 2016; Habyarimana et al. 2017; Sverrisdóttir et al. 2017, 2018; Endelman et al. 2018; Rodriguez et al. 2018; Stich and Inghelandt 2018), demonstrating that GS is a promising strategy for potato breeding. For a low heritability trait, i.e. breeders' visual preference, Slater et al. (2016) estimated nine times higher genetic gain over 20 years using a training population of 5000 individuals, and even when using merely 500 individuals, the expected genetic gain was more than double compared

to phenotypic selection. Higher genetic gains were estimated for other plant traits, disease resistance and quality traits in potato with moderate to high heritability (Sverrisdottir et al. 2017, 2018; Endelman et al. 2018; Rodriguez et al. 2018; Stich and Inghelandt 2018). Prediction accuracy can be increased by increasing the size of the training population, as more individuals will result in more observations per SNP allele, and thus the SNP effects will be estimated more accurately. For traits with low heritability, large numbers of individuals are required in order to achieve high prediction accuracies, while fewer individuals are necessary for high heritability traits (Sverrisdóttir et al. 2017). In addition, to maximize genomic estimated breeding value (GEBV) accuracy, the training population must be representative of selection candidates in the breeding programme to which GS will be applied (Heffner et al. 2009). Endelman et al. (2018) suggested that selection should be based on total genotypic value where inbred, F1 hybrid or vegetative clone is used as addition of nonadditive effects in the training population improved prediction accuracy of tuber yield and specific gravity. However, as nonadditive effects are not transmitted efficiently in progeny from parents, additive value should be considered for selection of parents.

1.5.5 Genome Editing

Random mutagenesis has many shortcomings and produces multiple undesirable rearrangements and mutations, which are expensive and very complex to screen. Genome editing uses engineered site-specific nucleases (SSNs) to delete, insert or replace a DNA sequence. Development of the engineered endonucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) paved the way for single nucleotide excision mechanism for crop improvement (Arora and Narula 2017). These genome editing technologies use programmable nucleases to increase the specificity of the target locus.

The clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated (Cas) system, which originated as a bacterial adaptive immune system, has become a revolutionary tool for basic research and crop genetic improvement. In CRISPR/Cas9- mediated genome editing, a guide RNA (gRNA) is used to direct Cas9 to target specific DNA sites. This simple RNA-guided DNA targeting system can be easily reprogrammed to target a desired gene and precisely modify the genome sequence. Due to its simplicity, low cost and adaptability, CRISPR/Cas9 has been rapidly repurposed to many innovative applications including precise base editing. Currently, numerous applications have been reported for CRISPR/Cas9-mediated genome engineering in various organisms (Butler et al. 2018). Mutation frequencies differ depending on the Cas9 expression cassette used, and using an expression vector harbouring the best combined Cas9/gRNA expression cassette results in an improved frequency of targeted mutagenesis (Mikami et al. 2015). In addition to the high-level expression of the *CAS9* gene, efficient translation of the

mRNA may also allow the generation of a large amounts of Cas9 protein, resulting in increased efficiency towards the targeted mutagenesis. Moreover, the precision of genome editing allows sequence changes to be made without altering the genetic background, in contrast to traditional genome-wide mutagenesis platforms (Butler et al. 2018).

Potato has a tetraploid genome and commonly shows a vegetative reproduction manner. It implies that there are large difficulties in obtaining homozygous mutants in which all of the target genes have been altered. Many challenges have to be overcome to establish potato mutants through one-step engineering processes using programmable nucleases. In recent years, genome editing through either TALEN or CRISPR-Cas9 has been used to study and develop commercially important traits in potato, traits which would otherwise be difficult to include through traditional breeding technologies (Sawai et al. 2014; Andersson et al. 2017, 2018). The first successful case of targeted mutagenesis created a mutant for which SSR2 gene was knocked out by TALENs (Sawai et al. 2014). To date, genome engineering techniques have been applied to create various gene mutants (Butler et al. 2015; Nicola et al. 2015; Wang et al. 2015; Andersson et al. 2017). The granule bound starch synthase I (GBSSI) gene is typically a model target for genetic engineering in potato. A GBSSI-deficient mutant shows amylose-free starch in potato tubers. Amylose-free starch is commercially valuable and used for food industries and manufacturing paper. To establish a potato mutant, a powerful and innovative tool is required because site-specific mutations must be present on all four target gene alleles. Use of dMac3 translational enhancer was highly effective in increasing the mutagenesis efficiency (Andersson et al. 2018). The acetolactate synthase (ALS) gene in potato was recently modified using genome editing and was shown to confer reduced susceptibility to certain herbicides (Butler et al. 2015). Similarly, self-compatible diploid potatoes have been developed by knocking out the self-incompatibility gene *S-RNase* using genome editing, opening new avenues for diploid potato breeding (Ye et al. 2018).

References

Allard RW (1999) History of plant population genetics. Annu Rev Genet 33:1–27

Andersson M et al (2017) Efficient targeted multiallelic mutagenesis in tetraploid potato (*Solanum tuberosum*) by transient CRISP-Cas9 expression in protoplasts. Plant Cell Rep 36:117–128

Andersson M, Turesson H, Olsson N, Fält AS, Ohlsson P, Gonzalez MN, Samuelsson M, Hofvander P (2018) Genome editing in potato via CRISPR-Cas9 ribonucleoprotein delivery. Physiol Plant 164:378–384

Arora L, Narula A (2017) Gene editing and crop improvement using CRISPR-Cas9 system. Front Plant Sci 8:1932. https://doi.org/10.3389/fpls.2017.01932

Barker H (1996) Inheritance of resistance to potato viruses Y and a in progeny obtained from potato cultivars containing gene Ry: evidence for a new gene for extreme resistance to PVA. Theor Appl Genet 93:710–716

Bradshaw JE (2009) Potato breeding at the Scottish plant Breeding Station and the Scottish crop research institute: 1920–2008. Potato Res 52:141–172

Bradshaw JE, MacKay GR (1994) Breeding strategies for clonally propagated crops. In: Bradshaw JE, MacKay GR (eds) Potato Genetics. CAB International, Wallingford, pp 467–497
Butler NM, Atkins PA, Voytas DF, Douches DS (2015) Generation and inheritance of targeted mutations in potato (*Solanum tuberosum* L.) using the CRISPR/Cas system. PLOS One 10:e0144591
Butler NM, Jiang J, Stupar RM (2018) Crop improvement using genome editing. Plant Breeding Review 41:55–101
Carroll CP (1982) A mass-selection method for the acclimatization and improvement of edible diploid potatoes in the United Kingdom. J Agric Sci 99:631–640
Chakrabarti SK, Singh BP, Thakur G, Tiwari JK, Kaushik SK, Sharma S, Bhardwaj V (2014) QTL mapping underlying resistance to late blight in a diploid potato family of *Solanum spegazzinii* × *S. chacoense*. Potato Res 57:1–11
Chase SS (1963a) Analytic breeding in *Solanum tuberosum* L. -a scheme utilizing parthenotes and other diploid stocks. Can J Genet Cytol 5:389–395
Chase SS (1963b) Analytic breeding of amphipolyploid plant varieties. Crop Sci 4:334–337
Cockerham G (1970) Genetical studies on resistance to potato viruses X and Y. Heredity 25:309–348
Dalamu BV, Umamaheshwari R, Sharma R, Kaushik SK, Joseph TA, Singh BP, Gebhardt C (2012) Potato cyst nematode (PCN) resistance: genes, genotypes and markers-an update. SABRAO J Breed Genet 44(2):202–228
De Maine MJ (1996) An assessment of true potato seed families of *Solanum phureja*. Potato Res 39:323–332
Endelman JB, Jansky SH (2016) Genetic mapping with an inbred line-derived F_2 population in potato. Theor Appl Genet 129:935–943
Endelman JB, Carley CAS, Bethke PC, Coombs JJ, Clough ME et al (2018) Genetic variance partitioning and genome-wide prediction with allele dosage information in autotetraploid potato. Genetics 209:77–87
Gopal J (2015) Challenges and way-forward in selection of superior parents, crosses and clones in potato breeding. Potato Res 58:165–188
Habyarimana E, Parisi B, Mandolino G (2017) Genomic prediction for yields, processing and nutritional quality traits in cultivated potato (*Solanum tuberosum* L.). Plant Breed 136:245–252. https://doi.org/10.1111/pbr.12461
Hamalainen JH, Sorii VA, Watanabe KN, Gebhardt C, Valkonen JPT (1998) Molecular examination of a chromosome region that controls resistance to potato Y and a potyviruses in potato. Theor Appl Genet 96:1036–1043
Heffner EL, Sorrells ME, Jannink JL (2009) Genomic selection for crop improvement. Crop Sci 49:1–12. https://doi.org/10.2135/cropsci2008.08.0512
Hutten R (1994) Basic aspects of potato breeding via the diploid level. PhD Thesis, Wageningen, p 93
Jansky SH, Spooner DM (2018) The evolution of potato breeding. Plant Breed Rev 41:169–214
Jansky S, Bethke P, Spooner DM (2014a) Yield gains in potato: contributing factors and future prospects. In: Smith S, Diers B, Carver B, Specht J (eds) Yield gains in major U.S. field crops. Crop Science Society of America, Madison, pp 195–217
Jansky S, Chung Y, Kittipadukal P (2014b) M6: a diploid potato inbred line for use in breeding and genetics research. J Plant Reg 8:195–199
Jansky SH, Dawson J, Spooner DM (2015) How do we address the disconnect between genetic and morphological diversity in germplasm collections? Am J Bot 102:1213–1215
Kaushik SK, Sharma R, Garg ID, Singh BP, Chakrabarti SK, Bhardwaj V, Pandey SK (2013) Development of a triplex (YYYy) parental potato line with extreme resistance to potato virus Y using marker assisted selection. J Hortic Sci Biotechnol 88:580–584
Kim HJ, Lee HR, Jo KR, Mortazavian SMM, Huigen DJ, Evenhuis B, Kessel G, Visser RGF, Jacobsen E, Vossen JH (2012) Broad spectrum late blight resistance in potato differential set plants MaR8 and MaR9 is conferred by multiple stacked R genes. Theor Appl Genet 124:923–935

Lindhout P, Meijer D, Schotte T, Hutten RCB, Visser RGF, vanEck HJ (2011) Towards F1 hybrid seed potato breeding. Potato Res 54:301–312

MacKay G (2005) Propagation by traditional breeding methods. In: Razdan M, Mattoo A (eds) Genetic improvement of Solanaceae crops, volume 1, potato. Science Publishers, Inc., Enfield, pp 65–81

Mikami M, Toki S, Endo M (2015) comparison of CRISPR/Cas9 expression constructs for efficient targeted mutagenesis in rice. Plant Mol Biol 88:561–572

Nicola A et al (2015) Targeted gene mutation in tetraploid potato through transient TALEN expression in protoplasts. J Biotechnol 204:17–24

Otto SP (2007) The evolutionary consequences of polyploidy. Cell 131:452–462

Ovchinnikova A, Krylova E, Gavrilenko T, Smekalova T, Zhuk M, Knapp S, Spooner DM (2011) Taxonomy of cultivated potatoes (Solanum section Petota: Solanaceae). Bot J Linn Soc 165:107–155

Phumichai C, Mori M, Kobayashi A, Kamijima O, Hosaka K (2005) Toward the development of highly homozygous diploid potato lines using the self-compatibility controlling *Sli* gene. Genome 48:977–984

Pushkarnath (1976) Potato in sub-tropics. Orient Longman Ltd., New Delhi, p 289

Rodriguez FE, Douches D, Lopez-Cruz M, Coombs J, de los Campos G (2018) Genomic Selection for Late Blight and Common Scab Resistance in Tetraploid Potato (*Solanum tuberosum*). G3 Genes Genomes Genet 8:2471–2481

Rowe PR (1967) Performance and variability of diploid and tetraploid potato families. Am Potato J 44:263–271

Sanford JC (1983) Ploidy manipulations. In: Moore JN, Janick J (eds) Methods in fruit breeding. Purdue University Press, Purdue, pp 100–123

Sawai S et al (2014) Sterol side chain reductase 2 is key enzyme in the biosynthesis of cholesterol, the common precursor of toxic steroidal glycoalkaloids in potato. Plant Cell 26:3763–3774

Simmonds N (1997) A review of potato propagation by means of seed, as distinct from clonal propagation by tubers. Euphytica 40:191–214

Slater AT, Cogan NOI, Forster JW, Hayes BJ, Daetwyler HD (2016) Improving genetic gain with genomic selection in autotetraploid potato. Plant Genome 9:3

Spooner DM (2016) Species delimitations in plants: lessons learned from potato taxonomy by a practicing taxonomist. J Syst Evol 54:191–203

Spooner DM, Nunez J, Trujillo G, Herrera MDR, Guzman F, Ghislain M, Nunez J, del Rosario HM, Guzma F (2007) Extensive simple sequence repeat genotyping of potato landraces supports a major re-evaluation of their gene pool structure and classification. Proc Natl Acad Sci U S A 104:19398–19403

Spooner DM, Gavrilenko T, Jansky SH, Ovchinnikova A, Krylova E, Knapp S, Simon R (2010) Ecogeography of ploidy variation in cultivated potato (*Solanum* sect. Petota). Am J Bot 97:2049–2060

Stich B, Van Inghelandt D (2018) Prospects and potential uses of genomic prediction of key performance traits in tetraploid potato. Front Plant Sci 9:159. https://doi.org/10.3389/fpls.2018.00159

Sverrisdóttir E, Byrne S, Sundmark EHR, Johnsen HO, Kirk HG et al (2017) Genomic prediction of starch content and chipping quality in tetraploid potato using genotyping-by-sequencing. Theor Appl Genet 130:2091–2108

Sverrisdottir E, Sundmark EHR, Johnsen HO, Kirk HG, Asp T, Janss L, Bryan G, Nielsen KL (2018) The value of expanding the training population to improve genomic selection models in tetraploid potato. Front Plant Sci 9:1118. https://doi.org/10.3389/fpls.2018.01118

Ugent D (1970) The potato: what is the origin of this important crop plant, and how did it first become domesticated? Science 170:1161–1166

Uijtewaal BA, Jacobsen E, Hermsen JGT (1987) Morphology and vigour of monohaploid potato clones, their corresponding homozygous diploids and tetraploids and their heterozygous diploid parent. Euphytica 36:745–753

Veilleux R (1990) *Solanum phureja*: anther culture and the induction of haploids in a cultivated diploid potato species. In: Bajaj Y (ed) Biotechnology in agriculture and forestry 12: haploids in crop improvement I. Springer, New York, pp 530–543

Vos PG, Uitdewilligen J, Voorrips RE, Visser RGF, van Eck HJ (2015) Development and analysis of a 20K SNP array for potato (*Solanum tuberosum*): an insight into the breeding history. Theor Appl Genet 128:2387–2401

Wang S et al (2015) Efficient targeted mutagenesis in potato by the CRISPR/Cas9 system. Plant Cell Rep 34:1473–1476

Watanabe K, Orrillo M, Perez S, Crusado J (1996) Testing yield of diploid potato breeding lines for cultivar development. Breed Sci 46:245–249

Watson A, Ghosh S et al (2018) Speed breeding is a powerful tool to accelerate crop research and breeding. Nat Plants 4:23–29. https://doi.org/10.1038/s41477-017-0083-8

Witek K, Strzelczyk-Żyta D, Hennig J, Marczewski W (2006) A multiplex PCR approach to simultaneously genotype potato towards the resistance alleles Ry-f sto and ns. Mol Breed 18(3):273–275

Ye M, Peng Z, Tang D, Yang Z, Li D, Xu Y, Zhang C, Huang S (2018) Generation of self-compatible diploid potato by knockout of S-RNase. Nature Plants 4(9):651–654

Chapter 2
A Rapid Disease Resistance Breeding in Tomato (*Solanum lycopersicum* L.)

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2.1 Introduction

Tomato (*Solanum lycopersicum* L.) is an autogamous diploid ($2n = 2\times = 24$) vegetable of the nightshade family (Solanaceae) and the genus *Solanum*, which comprises several species divided into two subgenera. The red-fruited species is *Eulycopersicon* and green fruited is *Eriopersicon.* Tomato is believed to be originated in mountainous regions of the Andes in Peru, Ecuador and Bolivia. Tomato is one of the important vegetable crops cultivated in tropical and sub-tropical regions of the world. Globally, tomatoes are cultivated in an area of 4.85 million hectares with annual production of 182.30 million tons (FAOSTAT 2017). The major tomato-cultivating countries are China, India, the USA, Italy, Turkey and Egypt. In India, tomato ranks third in terms of area and production after potato and onion. India produces 22.33 million tons of tomatoes from an area of around 0.8 million hectares (NHB data base 2017–2018). Tomatoes are well adapted to tropical and sub-tropical regions of the world. Its versatility in fresh and processed forms has played a major role in its rapid and widespread adoption as an important nutritious food crop. Tomatoes are consumed as fresh fruit and salad or in processed forms such as ketchup, juice, sauce, paste, soup and pickle and canned as whole fruit. Tomato is rich in vitamins A and C and provides sufficient quantity of antioxidants, lycopene that protects the human body against chronic and cardiac diseases. As a result, its cultivation has become more intensive and also highly remunerative during scarcity. Both acidic and regular segment varieties and F_1 hybrids are commercially adopted by the tomato growers in the country. Intensive and continuous cultivation of tomato in recent years has led to the prevalence of diseases and insect pests, which have become major production constraints in the country. Cultivated tomato has narrow

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genetic diversity because of extensive selection during breeding and domestication. Therefore, tomato crop is more vulnerable to disease epidemics.

The management of diseases by application of plant protection chemicals is not cost-effective and also ineffective in some pathogens. Therefore, it is important to exploit genetic resistance against plant pathogens by developing resistant cultivars/hybrids. Additionally, resistant cultivar/hybrids should also possess other economic characters like yield, firmness and quality to make them viable for commercial cultivation. Success in biotic stress resistance breeding strategy depends on the accessible to resistant source(s), which can be directly utilized for resistance breeding program. Efforts have been made for identification of sources of resistance and efficient utilization by many researchers using conventional and molecular breeding approaches. So far, conventional plant breeding approaches that utilize selection breeding strategies and breeding cycles have led to improvement of tomato crop. However, these strategies are less precise and time-consuming, especially for improving complex quantitative traits. With the advent of DNA marker tools, it is now comparatively easy to identify the genomic regions and assess their phenotypic effects on various polygenic traits of interest. Despite decades of breeding and selection efforts, there are still some biotic pathogens, viz. fungal, viral, bacterial pathogenic diseases, that challenge the tomato cultivation in various regions of the world. Recent developments in the area of plant genomics, including functional and structural genomics, have paved ways for adoption of important tools for tomato breeding. These tools are being utilized for crop improvement and genetic analysis of the crop in a number of plants including vegetable crops. With the advent of molecular markers, it facilitate indirect selection of a phenotype based on the selection of marker linked to important traits, thus helping in the selection and speeding up of selection cycles in crop improvement programs (Varshney and Tuberosa 2007). These can be precisely and efficiently utilized for selection of such traits which have low heritability and also for gene introgression from wild germplasm (Gupta et al. 1996) and for identification of quantitative trait loci (QTLs) that control important agronomic traits (Dudley 1993). Marker-assisted selection provides plant breeders a quick and precise alternative strategy to conventional selection schemes which are based on extensive phenotyping for improving cultivars with respect to qualitative and quantitative traits like resistance to biotic and abiotic stresses, yield and adaptability. In this chapter, we will discuss the use of resistant source(s), availability of resistant gene(s) and conventional and marker-assisted breeding approaches including new tools for understanding and improving disease resistance in tomato.

2.2 Breeding for Resistance to Fungal Pathogens

Tomato is affected by more than 200 diseases. Among them major important fungal diseases which cause serious threats in tomato production are foliar diseases like late blight (*Phytophthora infestans*), early blight (*Alternaria solani*), powdery

mildew (*Oidium lycopersicum*), septoria leaf spot (*Septoria lycopersici*) and leaf mold (*Cladosporium fulvum*). Other fungal diseases that infect tomato include fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*), verticillium (*Verticillium dahlia*), anthracnose (*C. coccodes* and *C. dematium*) and corky root (*Pyrenochaeta lycopersici*). These foliar diseases are serious threats which cause major crop loss worldwide because of prevailing favourable weather conditions which determine the incidence and severity of the disease.

2.2.1 Late Blight (LB)

Phytophthora infestans is a plant destroyer best known for Irish famine in Ireland in 1845 which resulted in death and emigration of over one million people. Due to its destructive economic and social impact, this disease has been a major pathological and genetic research since the occurrence of the Irish potato famine in the 1840s. Late blight (*Phytophthora infestans* (Mont.) de Bary) is one of the most important production constraints of tomato in India and worldwide which causes substantial yield reduction up to 40–100% (Nowakowska et al. 2014; Ohlson and Foolad 2015). The disease appears when weather conditions are cool and humid. *Phytophthora infestans* can devastate an entire unprotected crop within 7–10 days in the field (Nowicki et al. 2012).

The first complete dominant gene *Ph-1*, providing resistance to *P. infestans* (race T-0), was reported in wild *S. pimpinellifolium* accessions West Virginia 19 and 731 (Bonde and Murphy 1952; Gallegly and Marvel 1955; Peirce 1971). In 1962, a late blight-resistant variety Rockingham, carrying *Ph-1* gene, was notified and released (Rich et al. 1962). Subsequently, *Ph-1* gene was mapped in the same cultivar on chromosome 7 through morphological markers (Peirce 1971). Currently *Ph-1* gene has been overcome by occurrence of new highly aggressive races of *P. infestans*, and race T-0 is no longer found.

A second tomato late blight resistance gene, *Ph-2*, was identified and mapped in chromosome 10 in *S. pimpinellifolium* accession West Virginia 700 (Gallegly and Marvel 1955; Moreau et al. 1998). This incomplete dominant gene is also reported to be race specific and provides narrow resistance to some races by slowing the rate of disease progress rather than preventing the pathogen development. Further, *Ph-2* is often unbaled to provide resistance reaction against more aggressive *P. infestans* isolates (Goodwin et al. 1995; Black et al. 1996). However, *Ph-2* has been introgressed into several cultivars such as Fline, Flora Dade, Heinz 1706, Campbell 28 and Europeel (Gallegly 1960; Laterrot 1994) through marker-assisted breeding. Markers linked to LB resistance genes and resistance sources are listed in Table 2.1.

A broad-spectrum resistance gene, namely, *Ph-3*, was identified and mapped onto chromosome number 9 in wild species *S. pimpinellifolium* accession L3708; *Ph3* was identified and is the most effective LB resistance gene, which exhibits broad-spectrum resistance against a wide range of *P. infestans* isolates (Black et al. 1996; Kim and Mutschler 2005; Zhang et al. 2013).

Table 2.1 List of fungal disease-resistant genes and linked molecular markers for marker-assisted selection

Resistant gene (Chromosome)	Marker	Marker sequence	Marker type	References
(a) Late blight (*Phytophthora infestans*)				
Ph-2 (10)	dTG63	F: CTACTCTTTCTATGCAATTTGAATTG R: AATAATTTTCAACCATAGAATGATT	CAPS (*Hinf1*)	Panthee and Foolad (2012)
		F: TCGATCGTATGTAGACGATG R: AGGCAAATCTTGAAGAAGCA	CAPS (*MspI*)	Jung et al. (2015)
	Dtg422	F-TGACATGAGAAGGAAAAGACTTAAG R-GTCAATAATTTTCAACCATAGAATGATT	CAPS (*Hinf1*)	Panthee and Foolad (2012)
Ph-3 (9)	Ph-3-GLR/S	F: GCTACCTTAATTATTTACAATTTTCCT R: TTGTGAAACAGAAAAGGTAAATATCA		Ren et al. (2019)
	NC-LB-9-6676	F: ACAGAAAAGTGCACGAAGTT R: ATTTGAATGTTCTGGATTGC	SCAR	Panthee and Foolad (2012)
(b) Leaf mold (*Cladosporium fulvum*)				
Cf-*9* (10)		F: TGGGAATTTACCCGAAAGAA R: CCCAATCATTCAGTGCGTTA	SCAR	Truong et al. (2011)
(c) Verticillium wilt (*Verticillium* spp.)				
Ve (9)		F: CGAACTTGAC TACATTGACC CTG R: CAGTCTTGAAAGGTTGCTCAGCC	CAPS (*Xba*I)	Jung et al. (2015)
Ve (9)		F: GAC TAC ATT GAC CCT GGG CTC TTG R: TGA GAG CAC CTT AAG CTT TTC AAT	CAPS (*Xba*I)	Kuklev et al. (2009)
(d) Fusarium wilt (*Fusarium* spp.)				
I1 (11)	At2	F: CGAATCTGTATATTACATCCGTCGT R: GGTGAATACCGATCATAGTCGAG	SCAR	Arens et al. (2010)
I2 (11)	Z1063	F: ATTTGAAAGCGTGGTATTGC R: CTTAAACTCACCATTAAATC	SCAR	Arens et al. (2010)
I3 (7)	P7-43D	F: GGTAAAGAGATGCGATGATTATGTGGAG R: GTCTTTACCACAGGAACTTTATCACC	SCAR	Barillas et al. (2008)

I7 (8)	Solyc08g077740	F: AAGAAGTTCCCTTCTTCCCTTA R: GGAATAACCAAGGGGGTGTT	CAPS (*Age*I)	Gonzalez-Cendales et al. (2015)
(e) Grey leaf spot				
Sm (10)	CT55	F- CATCTGGTGAGGCGGTGAAGTA R- TCCCCCAAACAAAACAGTAATA	CAPS (*Ddel*)	Ji and Scott (2009)
	Sm-InDel	F: CTACACTTTCTCGTTCCCAATG R: ATCGCCAAACCAATC AAATC	InDel	Su et al. (2019)
(f) Bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*)				
Rx-3 *(5)*	Rx3-L1	F: CTCCGAGCGAAGAGTCTAGAGTC R:GAAGGCAAAAGGAAAAGGAGAAGGATGG	CAPS (*Bsr*BI)	Yang et al. (2005)
Rx-4 *(11)*	pcc12	F: TCCACA TCAAA TGCGTTTCT R: TTCCAATCCTTTCCATTTCG	InDel	Pei et al. (2012)
Bs4 (5)	Bs4-A03/B03	F: GGGTTGGAGTCCGAAGAGCAGG R: GACTAACCAACGCAAGTTATTGGACAGG	CAPS (*Msp*I)	Schornack et al. (2004)
(g) Bacterial speck (*Pseudomonas syringae* pv. *tomato*)				
Pto		F: ATCTACCCACAATGAGCATGAGCTC R: GTGCATACTCCAGTTTCCAC	CAPS (*Rsa*I)	Yang and Francis (2005)

Thus so far, no major LB resistance genes have been identified and characterized in other tomato species. However, several minor/major QTLs conferring broad-spectrum resistance have been identified in tomato wild species *S. habrochaites* (Robert et al. 2001; Brouwer et al. 2004), *S. pennellii* and *S. pimpinellifolium* (Frary et al. 1998). Several advanced breeding lines carrying *Ph-2*, *Ph-3* and *Ph-2* + *Ph-3* have been developed conferring resistance to late blight in tomato (Hanson et al. 2016). Recently, ICAR-Indian Horticultural Research Institute, Bengaluru, India, has released a F_1 hybrid Arka Abhed resistant to late blight conferred by *Ph-2* + *Ph-3* gene. At ICAR-IIHR, several advanced breeding lines have been developed by pyramiding *Ty-2*, *Ty-3*, *Ph-2* and *Ph-3* genes for combined resistance to late blight and tomato leaf curl virus.

2.2.2 Early Blight

Early blight is caused by *Alternaria solani* and *A. tomatophila* which can infect leaves, stems and fruits which eventually leads to fruit rot. Cultivated tomato is mostly susceptible to *Alternaria solani* which can cause up to 80% reduction in yield (Yadav and Dabbas 2012), and very few resistance accessions are available. Resistance to early blight has been found in cultivated and wild species *viz.*, *Solanum habrochaites- PI 127827* (Locke 1949); Targinni Red, Devon Surprise (Moore and Reynard 1945); Red cherry (Lodha 1977); *Solanum habrochaites* line -PI 134417 (Datar and Mayee 1980); *Solanum habrochaites* -PI 126445 (Gardner 1984) and NCEBR-1 and NCEBR-2 (Gardner 1988). A number of commercial cultivars with moderate but sufficient level of resistance have been reported and released (Table 2.2).

S. habrochaites line PI 134417 was found to be strongly resistant to both leaf blight and stem blight of *Alternaria solani* under severe epidemic conditions. *S. pimpinellifolium* has showed moderate resistance to leaf blight and was also found to be resistant to stem blight. Resistance to early blight has been identified in wild sources, viz. *S. peruvianum*, *S. habrochaites* and *S. pimpinellifolium*, and has been utilized to develop resistant pre-breeding lines in tomato crop improvement program (Foolad et al. 2008). A wild species, *S. habrochaites*, is found to be the richest source of resistant genes among other wild species of tomato, but this species often brings negative traits like late maturing, indeterminate growth habit and low in yield. Some highly resistant *S. habrochaites* accessions are PI390516, PI390658, PI390660, PI390662, PI390663 and LA2100. Amarnath et al. (2019) identified six genotypes (NCEBR-1, NCEBR-4, Arka Alok, Arka Rakshak, Arka Saurabh and *S. habrochaites* accession LA1777) as highly resistant against early blight through detached leaf assay.

Several advanced breeding lines resistant to early blight have been developed using resistant wild species (Foolad et al. 2000; Sadashiva et al. 2017) (Table 2.2). Breeding for early blight resistance through conventional approach is difficult and challenging because of the polygenic (QTLs) nature of resistance and the linkage of

Table 2.2 List of important tomato diseases and their sources of resistance

Disease	Resistant sources	References
Late blight (*Phytophthora infestans*)	*S. pimpinellifolium, S. cheesmaniae, S. pennellii,* L3708, Arka Abhed	Lukyanenko (1991), Smart et al. (2007)
Early blight (*Alternaria solani*)	*S. habrochaites* "LA1777"*, S. pimpinellifolium, S. peruvianum,* NCEBR-1, NCEBR-2, hybrids: Arka Rakshak, Arka Samrat, Plum Dandy, Mountain Magic, Mountain Merit and Mountain Supreme	Sadashiva et al. (2017), Gardner (1988, 2000)
Bacterial spot	*S. lycopersicum* "H7981" and *S. pimpinellifolium* (accessions "PI126932" and "PI128216"), *S. pennellii* "LA716"	Wang et al. (2011), Astua-Monge et al. (2000)
Bacterial specks	*S. habrochaites* "LA1777"	Thapa et al. (2015)
Leaf mold	*S. pimpinellifolium, S. habrochaites*	Parniske et al. (1997)
Fusarium wilt	*S. pimpinellifolium* accession "PI79532", *S. pennellii* "LA716", *S. pennellii* "PI414773" Pan American	Bohn and Tucker (1940), Scott and Jones (1989), Lim et al. (2006), Gonzalez-Cendales et al. (2015)
Verticillium wilt	*S. pimpinellifolium, S. esculentum "*Peru*"*	Kuklev et al. (2009)
Buckeye rot	*S. pimpinellifolium* "EC54725"	Rattan and Saini (1979)
Sclerotium wilt	AVTO1173	The World Vegetable Centre, Taiwan
Anthracnose	PI 272636	Barksdale (1972)
Septoria leaf spot	*S. habrochaites, S. peruvianum, S. pimpinellifolium* and *S. glandulosum* "PI 126448"	Kurozawa and Balmer (1977), Kalloo (1986)
Grey leaf spot *(Stemphylium solani* Weber)	*S. pimpinellifolium,* Florida basket, Florida Lanai, Florida Petite and Flora-Dade	Andrus et al. (1942), Stevens and Rick (1986), Augustine et al. (1981), Hernandez (1985)
Corky root or brown root rot (*Pyrenochaeta lycopersici*)	*S. hirsutum (PI*129157), *S. peruvianum, S. chilense,* PI 262906, PI 198601, Mobohir 72–12 and Moboglan 2	Laterrot (1978), Veliev and Sviridova (1987), Jones et al. (1989)

resistance with several undesirable traits such as late flowering and fruit maturity. Chaerani and Voorrips (2006) attempted a cross between *S. habrochaites* and *S. lycopersicum* to obtain resistance, but it was observed that resistant plants were late in maturity and low in yield. In some cases where breeding lines were derived from *S. pimpinellifolium* accession LA2093 and tomato advanced breeding line NCEBR-1, resistance to early blight was high with desirable traits.

It has been reported that younger leaves show more resistance to early blight compared to older leaves because of presence of high total sugar content and glycoalkaloids like solanine, chaconine and solanidine (Sinden et al. 1972; Rotem 1994). It is possible that a late maturing tomato cultivar may exhibit early blight resistance without even having any resistant genes or QTLs because of the high

sugar content and glycoalkaloids in it (Johanson and Thurston 1990; Chaerani and Voorrips 2006). A negative correlation between early blight resistance and early maturity made it difficult to breed early blight resistance using conventional methods.

Progress in early blight resistance breeding has been slow due to lack of well-characterized, effective resistance genes (Vakalounakis 1991; Poysa and Tu 1996; Banerjee et al. 1998; Vloutoglou 1999) and polygenic inheritance of the resistance and lack of molecular markers linked to EB (Barksdale and Stoner 1977; Maiero et al. 1989; Thirthamallappa and Lohithaswa 2000).

Resistance to early blight is mainly governed by polygenes, and several QTLs have been mapped to different locations on chromosomes with the help of DNA markers. Many efforts are being conducted to identify markers linked to QTL governing early blight disease resistance.There is limited success in developing molecular markers linked to QTL for the selection of early blight resistance because of minor QTLs that were utilized in breeding programs.

2.2.3 Leaf Mold

Leaf mold caused by fungus *Cladosporium fulvum* (*Fulvia fulvum* eke) is a serious threat of tomato cultivation especially of greenhouse tomatoes, which may result in 40–90% yield loss. Several resistant cultivars/breeding lines have been developed in Canada (Vagabong, Vetomold, Vulcan, Vinequeen), Great Britain (Eurocross A, Eurocross B, Paghamcross, Antimold B, Pipo), the Netherlands (Sonato, Sobeto, Revermun, Extase, Panase), USSR (Pionerskii, Leningradskii osennii, Dunai, Solnyshko, Turist 714) and other countries (Table 2.2). But due to mutation, more aggressive pathogenic races of the fungus break down the resistance, and cultivars exhibited susceptibility to highly aggressive newly emerged races soon after release. Several dominant resistance genes against *C. fulvum* have been identified which are designated from *Cf*1 to *Cf24* (Kerr et al. 1971; Laterrot 1981). Gene *Cf2* had shown immunity to two races of pathogen. The gene *Cf*1 exhibited broad-spectrum resistance against most of the races of *Cladosporium fulvum.*

Genes *Cf*4, Cf6 and *Cf7* have shown resistance to many races of *C. fulvum* which is located on chromosome 1 (Kerr and Bailey 1964) and *Cf2* located on chromosome 6 (Kerr et al. 1980). *Cf*1 and *Cf4* have been mapped onto chromosome 1, and *Cf*3 gene has been mapped onto chromosome 11. Cf 5 and Cf 21 are located on chromosome 4, Cf 6 and Cf *11* on chromosome 12, *Cf7* and Cf 8 on chromosome 9, *Cf9* on chromosome 10, *Cf10* on chromosome 7, *Cf*14 on chromosome 3, *Cf*19 and *Cf20* on chromosome 2, *Cf*22 on chromosome 1 and *Cf*24 on chromosome 5 (Kanwar et al. 1980a,b; Kalloo 1986). The resistance to many races of pathogen governed by the gene Cf 1, which is only partial, can be considered as race-nonspecific or horizontal resistance to C. *fulvum* (Boukema 1974).

Most of the sources of resistance have been found in wild species: genes *Cf2*, *Cf3*, *Cf*5 to *Cf10* were derived from *L. pimpinellifolium* (Kerr 1977; Boukema 1978, 1981; Laterrot 1981); gene *Cf*4 was derived from *L. hirsutum* f. *glabratum* and

L. peruvianum. Solanum pennellii according to data presented by Stamova et al. (1984) is an additional source of the gene Cf 4 as well as *Cf2*. Resistance governed by a major single gene is often not stable and durable because of occurrence of highly aggressive races. The best strategy for achieving durable and broad-spectrum resistance is pyramiding of multiple genes into a single cultivar or genotype for leaf mold resistance.

2.2.4 Anthracnose

Anthracnose in tomato is caused by several species of *Colletotrichum,* but majorly by *C. coccodes*, *C. dematium* (Barksdale 1972) and *C. truncatum* (Saini et al. 2017; Villafana et al. 2018). It is a serious disease in processing tomatoes where ripe fruits are held on the plant for synchronous harvesting resulting in post-harvest fruit rot, which severely affects marketability of the fruits and processing quality. This is the most significant ripe fruit rot in the USA and as well as in India. Initially, symptoms appear on ripe fruit as minute, sunken, water-soaked circular lesions. The lesions increase in area and become more depressed, and the central portion darkens. The darkened rotten area comprises many small, dark, fungal structures from which spores are released under moist weather. Anthracnose lesions on a fruit often coalesce and result in rotting of fruits. The disease is developed rapidly when temperature is 25° to 30 °C, high relative humidity (>90%) with intensive light. In tomatoes, high level of resistance to six anthracnose fungi was found in accession PI 272636 (Barksdale 1972), which has been introgressed to advanced breeding lines of tomatoes (Stevenson et al. 1978). Resistance to anthracnose is mainly governed by polygenes with incomplete dominance and epistatic interaction (Miller et al. 1983).

2.2.5 Powdery Mildew

Powdery mildew disease is caused by *Oidium lycopersici* and *Leveillula taurica* (Lév.) Arnaud (asexual state *Oidiopsis taurica* (Lév.) Salmon). It has become a globally important fungal disease since 1986, when it was first reported in the Netherlands (Paternotte 1988). Later this foliar disease was quickly spread over all tomato-cultivated areas in the world. Most of the modern tomato cultivars are susceptible to powdery mildew; however, resistance to *Oidium lycopersici* has been reported in many wild species (Ciccarese et al. 1998; Lindhout et al. 1994). Resistance to *Leveillula taurica* has been found in accessions of *S. lycopersicum* var. *cerasiforme* and many wild species, viz. *S. habrochaites*, *S. peruvianum* and *S. chilense* (Palti 1988; Hernandes and Stamova 1990). The dominant resistance gene *Lv* has been mapped onto chromosome 12 in *S. chilense* accession LA1969 which confers high level of resistance against *L. taurica* by inducing hypersensitive response (Yordanov et al. 1975; Stamova and Yordanov 1987; Chungwongse et al.

1997). A recessive ol-2 gene has been identified in *S. lycopersicum* var. *cerasiforme* and mapped onto chromosome 4 (Ciccarese et al. 1998; Bai et al. 2008) which confers incomplete resistance to *L. taurica* (Zheng et al. 2013). In addition to major genes, several QTLs have been reported which confer high resistance to *Oidium lycopersici*.

2.2.6 *Fusarium* Wilt

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* Sacc. (*Fol*) is a destructive soil-borne disease causing 10–50% yield loss in many tomato-growing areas of the world and is serious in a hot humid weather and in sandy soils with acidic pH (5.5). Optimum temperature for fusarium infection is around 28 °C. The disease symptoms are yellowing of the leaves, starting with the lower leaves to upward. Infected leaves later show downward curling, followed by browning and discolouration of vascular tissue and drying. Three races of *Fusarium*, races 1, 2 and 3, have been identified (Bohn and Tucker 1940; Grattidge and O'Brien 1982). Resistance against *Fol* has been identified in various wild species of tomato. First resistance gene *I* was identified on chromosome 11 in *S. pimpinellifolium* accession "PI79532" which provides resistance to *Fol* race 1 (Bohn and Tucker 1940). Second, *I-2* resistance gene was identified on chromosome 11 which confers resistance to *Fol* race 2, in *S. lycopersicum* × *S. pimpinellifolium* hybrid "PI126915", and this gene was reported to encode a coiled-coil, nucleotide-binding (CC-NB)-LRR resistance protein (Stall and Walter 1965). A third resistance gene *I-3* was identified and mapped in chromosome 7 in *S. pennellii* "LA716" which provides resistance to *Fol* race 3 (Scott and Jones 1989), and this encodes a protein called S-receptor-like kinase (SRLK) (Lim et al. 2008; Catanzariti et al. 2015). Furthermore, a partial dominant resistance gene *I-7* was identified on chromosome 8 in *S. pennellii* "PI414773" which provides broad-spectrum resistance to *Fol* races 1, 2 and 3 and encodes a typical extracellular LRR receptor-like protein (LRR-RLP) (Lim et al. 2006; Gonzalez-Cendales et al. 2015). Molecular markers for these resistance genes and source of resistance are listed in Tables 2.1and 2.2, respectively, which can be used for marker-assisted breeding for fusarium wilt resistance. Five tomato breeding lines (CLN3241S, CLN3241R, CLN3241P, CLN3241Q and CLN3241H-27) resistant to *Fol* race1 and race 2 were developed using conventional and marker-assisted selection (Hanson et al. 2016).

2.2.7 *Verticillium* Wilt

Verticillium wilt in tomato is caused by soil-borne fungi *Verticillium dahliae* and *V. alboatrum*, which also cause vascular wilt in over 200 plant species (Fradin and Thomma 2006). This disease can reduce the yield of susceptible plant in the field up

to 40–47% by race 1 isolates and by 19–31.2% by race 2 isolates (Bender and Shoemaker 1984). A single dominant gene *Ve* was identified and mapped onto chromosome 9 and reported to confer high resistance to *V. alboatrum* race 1 (Schaible 1962; Diwan et al. 1999). The *Ve* locus was confirmed to contain two closely associated, inversely oriented genes, *Ve1* and *Ve2* (Kawchuk et al. 2001; Fradin et al. 2009). *Ve1* resistance gene is reported to encode for extracellular LRR receptor-like protein class of disease resistance proteins (Kawchuk et al. 2001), which is critical for switching on effector-triggered immunity in the host plant. Fradin et al. (2009) reported that *Ve1*-mediated resistance signalling partially overlaps with signalling mediated by Cf (*C. fulvum*) proteins, type members of the receptor-like protein class of resistance proteins. Jung et al. (2015) developed gene-based molecular markers (Table 2.1) for verticillium wilt resistance which can be used for marker-assisted selection for disease resistance in tomato breeding programs.

2.2.8 Septoria Leaf Spot

Septoria leaf spot (*Septoria lycopersici* Speg.) is one of the major fungal diseases of tomato worldwide. The fungus affects the foliage, stems and the green fruits. Cultivars "Targinnie Red" (Andrus and Reynard 1945) and "Bounty", "Campbell 1327", "Heinz 1350" and "Rutgers" (Benedict 1972) were found to be resistant. Resistance was found in wild species (Table 2.2): *S. habrochaites*, *S. peruvianum*, *S. pimpinellifolium* and *S. glandulosum* PI 126448 (Kurozawa and Balmer 1977). The resistance is governed by a single dominant gene "Se" (Barksdale and Stoner 1978). An advanced breeding line AVTO1173 was found to be highly resistant to septoria leaf spot (Gul et al. 2016). Satelis et al. (2010) reported five breeding lines with high level of resistance in *S. peruvianum* accessions (PI-306811, CNPH-1036, LA-1910, LA-1984 and LA-2744). The resistance sources to septoria leaf spot are listed in Table 2.2.

2.2.9 Grey Leaf Spot

Grey leaf spot is an important fungal disease caused by *Stemphylium solani* Weber which is epidemic in some areas where continuous high temperature and humidity occur. Tomato wild species *S. pimpinellifolium* showed high level of resistance to *S. solani* (Andrus et al. 1942; Stevens and Rick 1986). Resistance to grey leaf spot is governed by a dominant gene *Sm* which is closely associated with the *I* gene and mapped onto short arm of chromosome 11 (Yang et al. 2017). Marker linked to incomplete dominant gene Sm is listed in Table 2.1. Various cultivars with resistance to grey leaf spot disease have been developed: Florida Basket, Florida Lanai and Florida Petite (Augustine et al. 1981). Cultivars Campbell 28, Rotella, Caraibe, Ont. 7620 and Flora-Dade were found to be highly resistant to all three races and were recommended as sources for resistance in resistance breeding (Hernandez 1985).

2.3 Breeding for Resistance to Root-Knot Nematodes

Another major production constraint is root-knot nematodes (RKN) (*Meloidogyne* spp.), and extent of economic losses caused in tomato by RKN infestation range between 20.6 and 85% (Sasser 1989). Use of nematicides in commercial fields is impractical due to high costs and to health, soil and environment hazards. Breeding resistant cultivars is the only practical approach to manage these nematodes in fields. Exploitation of hybrid vigour for yield improvement in tomato began a century ago (Hedrick and Booth 1907), and till recently many varieties/hybrids resistant to bacterial wilt and root-knot nematodes have been released from private as well as public sector, but varieties/hybrids having combined resistance to both these diseases are not available. Losses to plants by nematodes account for an estimated 14% worldwide, which cause loss of almost $100 billion dollars annually. Among plant pathogenic nematodes, root-knot nematodes are the major devastating nematode. They can substantially reduce crop yields by producing some of dramatic symptoms on plant. Root-knot nematode occurs worldwide, affecting thousand of plant species.They have the capacity to survive in temperate climates and can devastate crops grown in the tropical region. Symptoms of root-knot nematodes are more dramatic; nematode infection on root tissue resulted in large galls or “knots” which can be seen throughout the roots of infected plants. Under severe infection condition, plants show reduction in plant growth and yield with unacceptable quality of produces. The severity of gall formation generally depends on nematode population, virulence of nematodes and susceptibility to host plant. While the most root-knot nematode damages occur below ground plant parts, various nematode infection symptoms can also be observed above ground plant parts. Because galled roots are unable to absorb and transport water and nutrients from the soil, subsequently, plant may also exhibit nutrient deficiency symptoms (chlorosis). On severe infection plants may wilt slowly even in the presence of sufficient soil moisture, especially during the afternoon. Stunting in growth is often observed on tomato grown in root-knot nematode-infested soil.

Resistance to root-knot nematodes (RKN) in tomato was first reported by Bailey (1941) a wild species *S. peruvianum* (PI-128657). Smith (1944) introgressed root-knot nematode resistance into *S. lycopersicum* by crossing “Michigan State Forcing” with “PI 128657” through embryo rescue technique. Three major single dominant resistance genes have been identified in the wild species *S. peruvianum*, which include *Mi-1*, *Mi-3* and *Mi-5* and have been mapped in chromosomes 6, 12 and 12, respectively (Yaghoobi et al. 1995; Veremis and Roberts 1996; Milligan et al. 1998). One heat stable gene *Mi-9* has been identified in *S. arcanum* and mapped onto chromosome no. 6 (Ammiraju et al. 2003) (Table 2.3. *Mi-1*, introgressed from *S. peruvianum* L., provides broad-spectrum resistance to three species of root-knot nematodes, *Meloidogyne arenaria*, *M. incognita* and *M. javanica*, and also to the potato aphid (*Macrosiphum euphorbiae*) (Dropkin 1969; Rossi et al. 1998). Inheritance of heat-stable resistance to the root-knot nematode has been reported to be monogenic (single dominant gene) in *Lycopersicon peruvianum* (PI 270435

crossed with PI 126440) (Cap et al. 1993). Shrestha et al. (2012) also reported single dominant resistance in Pusa 120. Four CAPS markers (Aps, C8B, REX-1 and CT119) linked to root-knot nematodes have been identified (Ammiraju et al. 2003), and four SCAR markers (Mi23, PMi, TG-180 and TG-263) linked to root-knot nematodes have also been identified (Yaghoobi et al. 2005; Seah et al. 2007; Arens et al. 2010). One SSR marker (SSR-W415) linked to *Mi* gene also has been identified (Wang et al. 2013a, b). Resistant cultivars against root-knot nematodes developed at various centres are listed in Table 2.4.

2.4 Breeding for Resistance to Bacterial Diseases

2.4.1 Bacterial Wilt

Bacterial wilt caused by *Ralstonia solanacearum* is the major bacterial disease which is severe production constraint to tomato cultivation in India. This pathogen has a wide range of host >200 species covering over 50 plant families (Aliye et al. 2008) which cause up to 75–100% yield loss in the tropics and subtropics (Denny 2006). This disease is the most severe in tropical and sub-tropical climates where high temperatures coupled with high rainfall occur. The disease develops rapidly when the soil temperature is higher than 20 °C or soil moisture is high. The pathogen *Ralstonia solanacearum* prefers acidic soils (soil pH < 7.0) for its growth and pathogenesis. Bacterial wilt can occur on all types of soil, including sandy and clay types. Infestation of root-knot nematode (*Meloidogyne* spp.) accelerates the wilt disease development. Bacterial wilt is difficult to manage due to high pathogen variability, high survival rate of pathogen under diverse environmental conditions and its wide host range. Managing bacterial wilt by chemical treatments is not effective. Therefore, managing bacterial wilt by utilizing genetic resistance to *Ralstonia solanacearum* would be the most effective and economically and environment friendly approach (Denny 2006).

Wilting first starts appearing on younger leaves of plants during the day with high temperatures, and infected plants may show recovery in wilting symptoms temporarily, in the evening when temperatures are cooler. A few days later, a sudden and permanent wilt occurs. Roots and collar region of the stem show browning and discoloration of vascular system. The infected roots may rot due to secondary infection by bacteria. For confirmation, the stems of the infected plants are cut and placed in a small container of water which shows yellowish or greyish bacterial ooze oozing out from the cut portion. Symptoms of bacterial wilt are different from those of bacterial canker, which causes leaf chlorosis, stem cankers and bird's-eye spots on fruits. The symptoms of bacterial wilt can be distinguished from those of fusarium wilt because of the rapidity of the occurrence of wilt, under congenial conditions, for the former, and the drier, firmer stem rot of the latter.

Table 2.3 Molecular markers associated with resistance to root-knot nematodes in tomato

Gene	Marker	Marker type	Marker ID	Forward/reverse	Restriction enzyme	Product size (bp)		References
						R	S	
Mi	CAPS	Codominant	Aps	F- GGAACGTGGGTAGCATATGA R- GCCAATGCTCATCAATGTGA	Taq I	1 and 0.6 kb	Uncleaved (N-1.6 KB)	Ammiraju et al. (2003)
Mi	CAPS	Codominant	C8B	F- TACCCACGCCCCATCAATG R- TTGCAAGAGGGTGAATATTGAGTGC	Taq	115, 292	407	Ammiraju et al. (2003)
Mi	CAPS	Codominant	REX-1	F- TCGGAGCCTTGGTCTGAATT R- GCCAGAGATGATTCGTGAGA	Taq	N-750 570, 162	Uncleaved	Ammiraju et al. (2003)
Mi	CAPS	Codominant	CT119	F- TCAGGTATCGAACCAAAC R- TAAAAGGTTCATCCTAATA	Mse I	N-450 280, 170	250, 170	Ammiraju et al. (2003)
Mi-1	SCAR	Codominant	Mi23	F-TGG AAA AAT GTT GAATTT CTT TTG R-GCA TAC TAT ATG GCTTGT TTA CCC	380		430	Seah et al. (2007)
Mi-1	SCAR	Codominant	PMi	F-GGTATGAGCATGCTTAATCAGAGCTCTC R-CCTACAAGAAATTATTGTGCGTGTGAATG	550		350	Arens et al. (2010)
Mi-3	SCAR	Codominant	TG-180	F-ATACTTCTTTGCAGGAACAGCTCAC R-CACATTAGTGATCATAAAGTACCAG	1.2 kb		0.9 kb	Yaghoobi et al. (2005)
Mi-3	SCAR	Codominant	TG-263	F-GCTGAGAAATAAAGCTCTTGAGG R-TACCCTTAATGCTTCGGCAGTGG	0.9 kb		0.75 kb	Yaghoobi et al. (2005)
Mi-HT	SSR	Codominant	SSR-W415	F: AAGTCTTATCTAATTGCCTAT R: ATTTCCGTAATGATATGATCT	670		550	Wang et al. (2013)
Mi-HT	CAPS	Codominant	Mi	F: GACCAACTTCCCCCTCCAAGATCA R: CTGTTCTAACCGGTTGCGGAGTAAA	Kpn I	80/570	650	SGN
Mi-HT	CAPS	Codominant	W737	F: GCCCTGATGAACTATTAGATGTC R: ACTCGCAACTTTACAAGATCC	Hind II	281/638/919	919	Wang et al. (2013)

Table 2.4 *RKN*-resistant sources in tomato

Sl. No.	Varieties/F_1 hybrids	Sources
1	Arka Vardhan	IIHR, Bengaluru
2	Pusa-120, Pusa Hybrid-2 and Pusa Hybrid-4	IARI, New Delhi
3	Punjab NR-7, Sel-1-6-1-4	PAU, Ludhiana
4	Hisar Lalit	HAU, Hisar
5	Narendra Tomato-2	NDUA&T, Faizabad, UP
6	CO-3	TNAU, Coimbatore, TN

The tomato line, Hawaii 7996 (*Solanum lycopersicum*), exhibited strong and stable resistance against *R. solanacearum* bred by J.C. Gilbert in the 1970s (Wang et al. 1998). Researches at the World Vegetable Centre, Taiwan, have developed bacterial wilt resistance breeding lines (CLN1462, CLN1463) with heat-tolerance (Opena et al. 1989). Several resistance sources against bacterial wilt have been reported in wild species *S. pimpinellifolium* and *S. lycopersicum* var. *cerasiforme* and in cultivated tomato. However, the first identified source of resistant was accession PI127805A (*S. pimpinellifolium*) in 1964 (Acosta et al. 1964; Yin et al. 2005; Yang and Francis 2007; Alsam et al. 2017). Resistance in current cultivars is mostly derived from three major sources, PI127805A (*S. pimpinellifolium*), CRA66 Sel A (*S. lycopersicum* var. *cerasiforme*) and PI129080 (*S. pimpinellifolium*), and some other additional resistant sources (Hanson et al. 1998).

The inheritance of resistance to bacterial wilt could be dominant, partially dominant or recessive in nature depending on the resistant sources and bacterial strains used (Yue et al. 1995; Li et al. 2001; Wang et al. 2004; Yang and Francis 2007). The genetics of resistance to bacterial wilt are complex and controlled mostly by qualitative traits. Inheritance of bacterial wilt resistance has been reported to be controlled by polygenic (Mangin et al. 1999 and Hanson et al. 1998), whereas a single dominant resistance gene was reported by Scott et al. (1988) and Grimault et al. (1995). Seven QTLs have been identified from the resistant line Hawaii 7996 on six chromosomes 3, 4, 6, 8, 10 and 12 and three QTLs on three chromosomes (6, 7 and 10) from a *S. lycopersicum* var. *cerasiforme* accession L285 (Yang and Francis 2007). The QTLs on chromosome 6 identified from both resistant sources are located in the same region, which could be a resistant gene cluster with different loci conferring resistance to different strains, or could be one locus conferring resistance to all three tested strains. This QTL exhibited stable field resistance (Wang et al. 2004) and may be utilized for marker-assisted selection (MAS) to develop cultivar resistance to bacterial wilt disease. Single nucleotide polymorphism (SNP) markers tightly linked to two QTLs Bwr-6 (chromosome 6) and Bwr-12 (chromosome 12) have been identified recently (Kim et al. 2018). These markers will facilitate and speedup the breeding cycle for developing resistant cultivars/hybrids using MAS. Wang et al. (2013a, b) identified two SSR markers (SLM12–2 and SLM12–10) linked to bacterial wilt (Table 2.5).

Table 2.5 BW-resistant varieties/hybrids and breeding in tomato

Sl. No.	Varieties/F_1 hybrids	Sources
1	Arka Alok, Arka Abha, Arka Abhijit, Arka Ananya, Arka Shreshta, Arka Rakshak ($F_{1)}$, Arka Samrat (F_1)	ICAR-IIHR, Bengaluru
2	Sakthi, Mukthi, Anagha, Vellayani Vijay	KAU, Kerala
3	Swarna Lalima, Swarna Naveen, Swarna Samridhi (F_1), Swarna Sampada ($F_{1)}$, Swarna Deepti (F_1), Swarna Vijaya (F_1), Swarna Anmol (F_1)	HARP, Ranchi (research station of ICAR-RCER, Patna)
4	Utkal Pallavi, Utkal Kumari, Utkal Urbashi	OUA&T, Bhubaneswar
5	Megha, DMT-2	UAS, Dharwad
6	Sonali	BSKVV, Dapoli, MH
7	VC-48-1	AAU, Jorhat

2.4.2 Bacterial Speck

Bacterial speck caused by *Pseudomonas syringae* pv. *tomato* (Pst) is an important disease that primarily occurs in cool and moist weather condition. Although this disease has been reported in the early 1930s, it emerged as a devastating disease in the late 1970s, when serious yield loss was recorded (Goode and Sasser 1980). Symptoms of bacterial speck includes minute blackish to brownish necrotic lesions (specks) that have been surrounded by chlorotic circular ring caused by the bacterial toxin which is known as coronatine (Boch et al. 2002). Specks can also be seen in the on both unmature and ripe fruits, which leads to decrease in marketability of the fruits (Jones 1991). Bacterial speck incidence in the field can vary from 15% to 100% (Zhao et al. 2001; Deng et al. 2008; Wang et al. 2015). Resistant sources against bacterial speck have been identified in various tomato wild species and also in cultivated tomato. Most investigations on inheritance of resistance suggested that the resistance to bacterial speck is simply inherited. Four incomplete dominant resistant genes have been identified, genes *Pto* (*Pto-1*) from *S. pimpinellifolium* accession PI370093, *Pto-2* from *S. pimpinellifolium* accession PI126430 and *Pto-3* from *S. habrochaites* accession PI134417 which confers resistance to *Pst*race 0, whereas gene *Pto-4* from PI134417 exhibits resistance to race 1 (Yang and Francis 2007, Table 2.1). Polygenic resistance has also been reported in *Solanum habrochaites* accession LA1777, and QTLs conferring resistance to *Pseudomonas syringae* pv. *tomato* race 1 have been reported (Thapa et al. 2015, Table 2.2). The resistance gene *Pto* has been cloned and well characterized (Martin et al. 1993) which provides effective resistance against race 0 (Pedley and Martin 2003). Therefore, this gene has been widely used in resistance breeding programmes, and cultivars carrying *Pto* gene are commercially available worldwide (Yang and Francis 2007). Molecular markers tightly linked to Pto gene (Table 2.1) have also been developed (Sun et al., 2011c) and have been used for marker-assisted selection.

2.4.3 Bacterial Canker

Bacterial canker is a vascular disease caused by the gram-positive bacteria *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) (Davis et al. 1984). This disease was first identified in the USA during 1909 (Smith 1910) and has emerged as a devastating disease of tomato worldwide, which caused huge yield loss (Yang and Francis 2007; Sen et al. 2015; Chalupowicz et al. 2017). Resistance sources against bacterial canker have been identified in *Solanum* wild species and have been incorporated into cultivated cultivars in several countries including the USA, the Netherlands and Bulgaria (Yang and Francis 2007; Sen et al. 2015). *Solanum nigrum* was identified as a resistant source to bacterial canker and incorporated into cultivated background through backcrossing (Li et al.2012). Genetic analysis studies suggested that the resistance to bacterial canker is controlled by polygenic genes; therefore development of resistant cultivars can be facilitated by marker-assisted pyramiding of QTLs, which can help minimize the undesirable linkage drag from wild species and can accelerate the resistance breeding programme. Molecular markers linked to these QTLs conferring resistance to bacterial canker have been identified in *S. peruvianum* accession LA2157 and *S. habrochaites* accession LA407 (Yang and Francis 2007; Sen et al. 2015). These markers might provide a tool for facilitating transfer of resistance from wild species into cultivated tomatoes. However, the linkage between the markers and resistance is not very tight. Therefore, fine mapping or cloning of genes/QTLs conferring resistance to the disease is necessary to identify molecular markers tightly linked to the resistance or develop functional markers of genes/QTLs.

2.4.4 Bacterial Spot

Bacterial spot is a complex disease and has been reported to cause by at least four species of *Xanthomonas* (*X. euvesicatoria*, *X. vesicatoria*, *X. perforans* and *X. gardneri*) (Jones et al. 2004) though recent classification suggested that *X. euvesicatoria* and *X. perforans* can be considered same species (Barak et al. 2016; Cui 2016). Bacterial spot is a serious disease of tomato especially in humid tropic regions of the world (Stall et al. 2009; Jones et al. 2014). This disease was first noticed in China during the mid-1940s (Li 1991). Bacterial spot incidence under field can be varied from 29% to 100% (Sun et al. 1991; Ding et al. 1996; Li et al. 1997; Guo et al. 2008; Wang and Ying 2008). Sources of resistance to the four species of *Xanthomonas* have been reported in both wild and cultivated tomato species (Yang and Francis 2007; Scott et al. 2013; Liabeuf et al. 2015, Table 2.2). Resistance in Hawaii-7998, Hawaii-7981, PI114490 (*S. lycopersicum* var. *cerasiforme*) and PI128216 (*S. pimpinellifolium*) has been incorporated into breeding programs, and cultivars with resistance to at least one of the four species have been developed (Francis and Miller 2005; Zhang et al. 2009; Hutton et al. 2010a, b; Scott et al. 2013; Liabeuf 2016).

2.5 Breeding for Resistance to Viral Diseases

The tomato hosts more than 200 species of a wide variety of pests and pathogens that can cause significant economic losses. Among these about 146 viruses belonging to 33 genera are reported to infect tomato around the world (Dhaliwal and Sharma 2016). Some of the major ones are tomato leaf curl disease/*Tomato yellow leaf curl virus* (ToLCD/TYLCD), *Groundnut bud necrosis tospovirus* (GBNV), *Tomato mottle virus* (ToMoV), *Tomato mosaic virus* (ToMV), *Cucumber mosaic virus* (CMV), *Pepino mosaic virus* (PepMV), *Tomato chlorosis virus* (ToCV)/*Tomato infectious chlorosis virus* (TICV), *Potato virus X*, *Potato virus Y*, *Capsicum chlorosis virus* (CaCV), *Watermelon bud necrosis tospovirus* (WBNV), *Tomato ring spot virus* (TRSV) and *Tomato torrado virus*. In India two virus diseases, viz. tomato leaf curl disease/tomato yellow leaf curl disease and groundnut bud necrosis disease, are the major constraints in tomato production. These two viruses alone cause significant economic loss in tomato cultivation, which globally made them the top two viral diseases of tomato. In the following paragraphs, these two diseases are described briefly.

2.5.1 Tomato Leaf Curl Disease/Tomato Yellow Leaf Curl Disease

Tomato leaf curl is a devastating viral disease of tomato caused by whitefly (*Bemesia tabaci*)-transmitted begomoviruses (family *Geminiviridae*). Virus has twinned icosahedral (geminate) particles and are either monopartite with a genome of a single circle of single-stranded DNA of about 2.7 kb or bipartite with two circles of ssDNA both of about 2.7 kb. Begomoviruses are also sometimes associated with satellite molecules of circular ssDNA: alphasatellites and/or betasatellites. The monopartite begomoviruses were originally considered to be originated in the Old World, while the bipartite begomoviruses were predominantly originated in the New World. Tomato leaf curl disease was first reported in the 1930s in Israel, and it was found to be caused by a whitefly-transmitted virus in 1960s. Since then, tomato leaf curl disease has been reported from across the world. It has been a global constraint to tomato (*Solanum lycopersicum*) production since the 1980s (Moriones and Navas-Castillo 2000). TYLCD/ToLCD can be characterized by the presence of yellowing, curling and cupping of leaves and severe stunting and abortion of flowers and fruits, all of which can lead to yield reduction of up to 100% (Abhary et al. 2007) on the infected susceptible tomato plants. To date, several hundred isolates of tomato yellow leaf curl-/tomato leaf curl-causing viruses have been sequence characterized from different parts of the world indicating high genetic diversity and wide geographical distribution of the viruses. In India tomato leaf curl disease was first reported in 1948 by Vasudeva and Samraj. Since then this disease has widely spread in almost all tomato-growing regions of the country which became a serious production constraint due to its regular outbreaks. Ten monopartite tomato-infecting

Begomovirus species are known to be associated with tomato leaf curl disease in India. Two bipartite viruses that have been reported which infect tomato are *Tomato leaf curl New Delhi virus* (ToLCNDV) and *Tomato leaf curl Palampur virus* (ToLCPalV).

2.5.1.1 Breeding for Resistance to Tomato Leaf Curl Disease

Management of whitefly-transmitted *Tomato leaf curl virus* by means of chemical control is practically not effective due to its wide host range and fast mobility of the insect vector. Genetic resistance against *Tomato leaf curl virus* is considered as an economically viable, environmentally friendly approach to manage the disease, and its deployment through breeding tomato cultivars resistant to the virus has gained importance in recent years. Cultivated tomatoes are susceptible to TYLCV infection, but resistance to TYLCV has also been reported in wild species of tomato (Ji et al. 2007b). Resistant sources against TYLCV have been identified in wild tomato species which include *S. pimpinellifolium*, *S. peruvianum*, *S. chilense*, *S. habrochaites*, *S. cheesmaniae* and *S. pennellii.* Since the early 1970s, traditional breeding programmes have resulted in introgression of *Tomato leaf curl virus* resistance from wild species into different cultivars. Programs to develop *Tomato leaf curl virus*-resistant/*Tomato leaf curl virus*-tolerant cultivars were started in Israel in 1974 and later in Egypt and France using several wild species. The wild species *S. pennellii* (LA 716) has exhibited multiple pest and disease resistance due to the presence of type IV glandular trichomes which secretes the acyl sugars. Banerjee and Kalloo (1987) evaluated a large number of germplasm/wild species and have identified *S. hirsutum f. glabratum* 'B6073', *S. hirsutum f. typicum* 'A1904', and an accession of *S. peruvianum* as highly resistant to the virus.

Currently six independently inherited *Ty* resistance genes have been reported and mapped onto different chromosomes. So far, three TYLCV resistance genes have been cloned and characterized. Resistance genes *Ty-1* and *Ty-3* originated from *S. chilense* accessions LA1969 and LA2779, respectively (Zamir et al. 1994; Ji et al. 2007a). They are allelic in nature of the same gene that is located on the long arm of tomato chromosome 6 and encode an RNA-dependent RNA polymerase (RDR) (Verlaan et al. 2011, 2013). *Ty-2* gene has originated from wild species *S. habrochaites* accession B6013 and mapped onto long arm of chromosome 11 and encodes an NB-LRR gene (Yang et al. 2014; Yamaguchi et al. 2018). A recessive gene *ty-5* is a mutant allele of the *pelota* gene located on chromosome 4. The mutation in *ty-5* is caused by a T-to-G transversion in the coding region, which occurred in cultivated tomato (Lapidot et al. 2015). Recessive resistance gene *ty-5* is originated from a complex of *S. peruvianum* accessions (Anbinder et al. 2009). In addition to these cloned genes, two resistance loci *Ty-4* and *Ty-6* have been mapped. *Ty-4* is identified from *S. chilense* LA1932. This locus is located on the long arm of chromosome 3 and has a minor effect towards TYLCV resistance (Ji et al. 2009). Recently, a resistance locus *Ty-6* has been identified on the long arm of chromosome 10, presumably originating from *S. chilense* accessions LA1938 and LA2779 (Hutton and Scott 2014). Molecular markers linked to each of the Ty resistance gene have been

Table 2.6 Molecular markers for selecting ToMV resistance genes, Tm1 and Tm22

Resistant locus	Marker information		References
Tm1	CCACTGTATGATTTCTGCTAGTGAA AGCTTTAACAAATATAAGAATAAAGAC GCAAGCTAAGGTTTACATATATGCC	SCAR (gene-based)	Ishibashi et al. (2007)
$Tm2^2$	F: GAGTTCTTCCGTTCAAATCCTAAGCTTGAGAAG R: CTACTACACTCACGTTGCTGTGATGCAC	CAPS (gene-based)	Lanfermeijer et al. (2003,2005)
	SCN131000 (AccI) F: AGCGTCACTCCATACTTGGAATAA R: AGCGTCACTCAAAATGTACCCAAA	CAPS (linked marker)	Sobir et al. (2000)

developed (Table 2.6). *Ty-1*, *Ty-2* and *Ty-3* have been used widely for pyramiding into cultivated tomato in resistance breeding programs. Utilizing marker-assisted selection (MAS), the resistance genes have been successfully introgressed into the commercial cultivars. Punjab Agricultural University, Ludhiana, has developed cultivars 'Punjab Varkha Bahar 1', 'Punjab Varkha Bahar 2' and 'Punjab Varkha Bahar 4' resistant to local isolates of *Tomato leaf curl virus*, and MAS is being employed to develop the resistant cultivars with multiple Ty genes. Recent study showed that *Ty-2*-based resistance can be overcome by TYLCV-related *Tomato yellow leaf curl Sardinia virus* (TYLCSV) (Barbieri et al. 2010) and breakdown of *Ty-2*-based resistance has been demonstrated recently by an isolate of the Mild strain of TYLCV (TYLCV-Mld) (Ohnishi et al. 2016). *Ty-1*-mediated resistance is not stable and under high temperature and high disease pressure leads to resistance breakage in some cases (García-Cano et al. 2008). Breakage of resistance facilitates the TYLCD epidemics, which necessitates plant breeders to continuously look for effective novel sources of resistance in the tomato gene pool. Continuous effort from the breeders and pathologists led to the identification of numbers of wild resistance species, including *S. arcanum*, *S. cheesmaniae*, *S. chilense*, *S. chmielewskii*, *S. corneliomulleri*, *S. galapagense*, *S. habrochaites*, *S. neorickii*, *S. pennellii*, *S. peruvianum* and *S. pimpinellifolium* (Ji et al. 2007b; Vidavski et al. 2008; De la Peña et al. 2010; Pereira Carvalho et al. 2010; Tomás et al. 2011; Yan et al. 2018).

2.5.2 *Bud Necrosis Disease of Tomato*

Bud necrosis virus disease caused by *Groundnut bud necrosis tospoviruses* (GBNV) belongs to the family *Bunyaviridae*, and the genus *Tospovirus* has become emerging limiting factor for the sustainable production of tomato in India. The genome of tospoviruses is of negative/ambisense polarity and consists of three enveloped isometric RNA segments that are denoted as large (L, ~8.9 kb), medium (M, ~4.8 kb) and small (S, ~2.9 kb) according to their sizes. GBNV is transmitted by thrips (Thysanoptera) in a propagative manner. Tospoviruses have become a serious threat

to tomato cultivation as well as other horticultural crops. *Tomato spotted wilt virus* emerged as one of the ten most important plant viruses because of its impact on economic losses and wide range of hosts and distribution around the world (Dhaliwal and Sharma 2016). Due to their economic impact on a wide range of important crops in India, most of the research was carried out in the Indian subcontinent. Historical perspective of disease symptoms similar to those induced by tospoviruses has been described in India since the 1960s on several vegetable crops such as brinjal, chilli, cowpea, pea, potato and tomato (Mandal et al. 2012). Since *Tospovirus* was known as a monotypic genus until 1990, with *Tomato spotted wilt virus* as the sole species, tospoviral diseases described in India were initially considered to be caused by *Tomato spotted wilt virus*. It was in 1992 that, based on serology, the bud necrosis disease of groundnut caused by a *Tospovirus* different from *Tomato spotted wilt virus* was named *Groundnut bud necrosis virus* (Reddy et al. 1992).Until the mid-2000s, the occurrence of *Groundnut bud necrosis virus* was limited to a few regions. Beginning in 2001, epidemics of *Groundnut bud necrosis virus* were reported in several tomato-growing regions of India, including Hoskote and Nasik regions of Karnataka and Maharashtra States, respectively, where up to 100% of disease incidence was recorded. Subsequently, GBNV is recognized as the most economically important *Tospovirus* of tomato, as losses due to GBNV alone have been estimated at more than US$89 million per annum in Asia. Other tospoviruses reported to infect tomato in India include *Watermelon bud necrosis virus* and *Capsicum chlorosis virus* (Kunkalikar et al. 2011).

2.5.2.1 Resistance Breeding to Bud Necrosis Disease

GBNV symptoms appear like necrotic rings on leaves, stems and patchy colour ring on fruits (Manjunatha 2008; Mandal et al. 2012). Necrosis on the foliage often results in collapse of stem, or the whole plant shows symptoms of blight. Generally, plants collapse and die when infected at an early stage (Akram 2012). Genetically, resistance cultivars of tomato against GBNV would be the most sustainable solution to manage the virus infection in tomato. TSWV is the most widely spread *Tospovirus* across the world and has been targeted for genetic resistance approaches. In the last three decades, genetic resistance sources and resistance genes have been identified and introgressed into commercial cultivars. Among these resistance genes, dominant (Sw1a and Sw1b) and recessive (sw2, sw3 and sw4) genes have been identified; however, their resistance was broken down due to occurrence of severe and more aggressive isolates of with TSWV. The tomato cultivars when evaluated in field condition of India and the cultivars with Sw-5 (BL 1022) and Sw-7 (CK-12) genes were found highly susceptible to GBNV (Sain and Chadha 2016). Search for resistant cultivars/accessions/varieties of GBNV has been carried out by several workers; EC 5888 was highly resistant, while EC 8630 and EC 26512 were found to be resistant by Venkataramana et al. (2010), and resistance in them was found to be governed by the single dominant gene. Sain and Chadha (2016) reported high level of field resistance (>80%) in seven lines, viz. *S. peruvianum* (L00735, L00671,

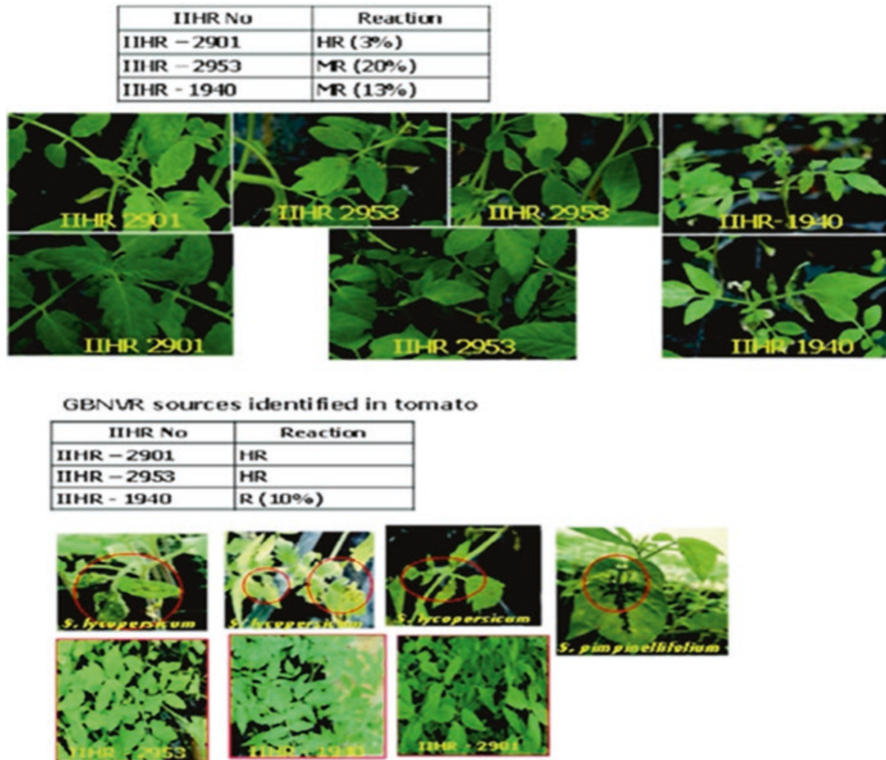

Fig. 2.1 Tomato lines and wild species identified as resistant to GBNV disease at ICAR-IIHR, Bengaluru

L00887, L06138), *S. chilense* (TL02226) and *S. pimpinellifolium* (L03708, TL02213). Recently, at ICAR-IIHR, Bengaluru, IIHR-2988 (*S. chilense*), IIHR-1940 (*S. peruvianum*) and IIHR-2901 were identified as resistant while IIHR-2953, IIHR-2809 (*S. peruvianum*) and IIHR-2101 (*S. habrochaites*) as moderately resistant to GBNV after mechanical sap inoculation (Fig. 2.1). To incorporate GBNV resistance into different elite backgrounds of tomato effectively, more studies need to be directed towards understanding the genetic basis of its inheritance. This will further help in determining the breeding strategies for the successful introgression of these genes into different backgrounds. These lines may also be utilized for development of molecular markers linked with GBNV resistance.

2.5.3 Tomato Mosaic Virus *(Tomv) Resistance*

Tomato mosaic virus (ToMV) belongs to the family *Virgaviridae* and the genus *Tobamovirus* and constitutes a positive-sense RNA genome. ToMV infects the whole plant like stems, petioles, leaves and fruit. The symptoms on foliage upon

ToMV infection generally cause mottling or mosaic appearance. In case of severe infection, leaves may look stunted, akin to ferns surrounded with dark green regions. Infected plants showed reduction in fruiting, and dotted with yellow blotches, necrotic spots can be found with brownish interior fruit tissue. Two resistance genes against ToMV, namely, Tm-1 and Tm-2, have been identified which confer resistance to ToMV and have been introgressed to cultivated tomatoes from wild species. A partial dominant gene Tm-1 was originally identified in *S. habrochaites* "PI126445" (Pelham 1966; Watanabe et al. 1987). Resistance protein coded by Tm-1 gene does not show similarity in any functional domain with previously reported resistance proteins, but it physically binds and functionally inhibits the replication proteins of ToMV (Ishibashi et al. 2007). A strong resistance gene Tm-2 was identified and characterized in wild species *S. peruvianum* which exhibit a high degree of resistance compared to Tm-1. The Tm-2 and Tm-2^2 resistance genes are reported to be allelic in nature (Pelham 1966; Young and Tanksley 1989), and the Tm-22 resistance gene has been shown to be more stable and durable than the Tm-2 (Fraser 1990). Currently, Tm-2^2 is identified as strong, broad-spectrum resistance gene; thus, it is widely utilized as a ToMV resistance source in tomato breeding programs. ToMV linked molecular markers are listed in Tables 2.7 and 2.8.

2.6 Development of Multiple Disease-Resistant Advanced Breeding Lines

Wild species of *Solanum lycopersicum* are the important resistance sources of several biotic and biotic stresses. But direct utilization of wild sources in crop improvement is very difficult than breeding with cultivated species due to incompatibility in crossing. Pre-breeding aims to identify desired traits (e.g. disease resistance) from wild species and introgressing them into breeding materials that are more readily crossable with modern, elite cultivars. Development of pre-breeding lines resistant to disease enables breeders to utilize them directly in breeding program for transferring the disease resistance in the background of cultivated variety of tomato. Marker-assisted gene pyramiding strategy has been widely adopted for development of pre-breeding or advanced breeding lines with resistant to diseases. For plant-virus interaction, e.g. *Solanum lycopersicum-Tomato leaf curl virus* (ToLCV; Prasanna et al. 2014). Pyramiding for broad-spectrum combined resistance to fungal and virus-host interaction has been also achieved, e.g. tomato late blight and *Tomato spotted wilt virus* (Robbins et al. 2010) *Phaseolus vulgaris*- anthracnose and potyvirus resistance (Ferreira et al. 2012). Combined resistance to bacterial spot and bacterial speck in tomato has been achieved through marker-assisted selection for bacteria-host interaction (Yang and Fransis 2005). At ICAR-IIHR, Bengaluru, several breeding lines (Fig. 2.2) with triple disease resistance to early blight, bacterial wilt and tomato leaf curl virus disease coupled with good horticultural traits have been developed. These breeding lines have been successfully utilized in hybrid development program.The pedigree selection breeding scheme adopted to develop triple disease-resistant breeding lines is illustrated in Fig. 2.2.

Table 2.7 Molecular markers for selecting resistant tomatoes against TYLCV/ToLCV

Resistant locus (chromosome)	Marker information		References
Ty1/3 (ch 6)	F: ATGAAGACAAAAACTGCTTC R: TCAGGGTTTCACTTCTATGAAT (SspI, BglII or TaqI)	CAPS (gene-based)	Jung et al. (2015)
Ty3 (ch 6)	SCAR 1 F: GCTCAGCATCACCTGAGACA R: TGCAGGAACAGAATGATAGAAAA	SCAR (gene-based)	Dong et al. (2016)
Ty2 (ch 11)	T0302 F: TGG CTC ATC CTG AAG CTG ATA GCG C R: AGT GTA CAT CCT TGC CAT TGA CT	SCAR	Yang et al. (2014)
Ty4 (ch 3)	C2_At4g17300 (AflI) F: ATTTAACCGTGTCTGGGCAACTCAATGG R: GCTCACTTTGCAAATCACATCCCCATTTCACC C2_At5g60160 (NlaIII) F: TTCTCGCGGCCTTTTCTCCTC R: TCGTGATCGCAAACATATACTCGC	CAPS	Ji et al. (2009)
ty5 (ch 4)	F: TTCAAGTCCTTCTTCAAC R: ATAGATTTAAACAACAATTATAGAGATAAAAAAGTTACCTGT (RsaI)	dCAPS (gene-based)	Lapidot et al. (2015)
	F-GGTGCTCATGGATAGCTTAC R-CTATATAGGCGATAGCACCAC	(SSR) TM273	Kardivel et al. (2013)
Ty6 (ch 10)	UF_10.61192 F: CATAAAGTTCCGGCGAGTGT R: TCCATTCCAAACCAAGTGAAG (BssHII)	CAPS	Gill et al. (2019)

Table 2.8 Resistant sources identified for some other viruses infecting tomatoes

S.No.	Viruses	Reported resistant source	References
1.	*Pepino mosaic virus* (PepMV)	*S. habrochaites* (LA 1731)	Ling and Scott (2007)
2.	*Potato virus Y*	*S. habrochaites* (L.03683 and L.03684), PI 247087	Oliveira et al. (2018), Thomas and McGrath (1988)
3.	*Tomato mottle virus* (ToMoV)	*S. chilense* (LA 1932, LA 1938, LA 1969 and LA 2779)	Griffiths and Scott (1998)
4.	*Tomato chlorosis virus* (ToCV)	*S. habrochaites* PI 127827, *S. lycopersicum* LT05, *S. peruvianum* (sensu lato) CGO 6711&*S. chilense* LA1967	Arcos et al. (2018)

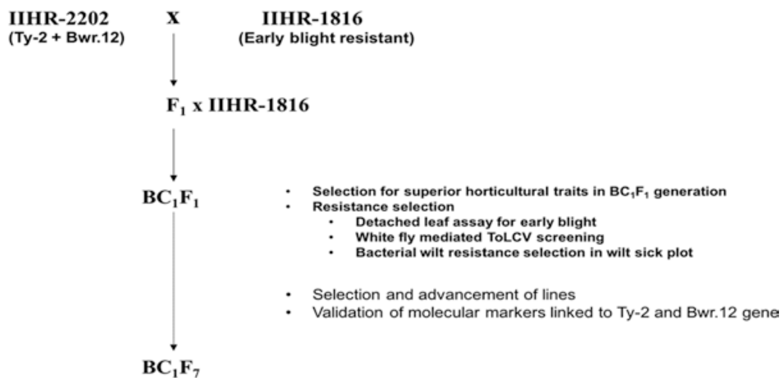

Fig. 2.2 Breeding strategy for triple disease resistance in tomato

2.7 Development of Tomato F_1 Hybrids with Multiple Disease Resistance

Hybrids are popular especially in Solanaceae vegetable crops to achieve ealiness, high yield potential and quality of produce. Heterosis in tomato has been observed for several traits, viz. fruit yield, number of fruits per plant, fruit firmness and shelf life. At ICAR-IIHR, Bengaluru, all the 7 lines were crossed with elite tomato lines received from The World Vegetable Centre, Taiwan, to develop more than 60 hybrids. Of these, two F_1hybrids, viz. Arka Samrat (H-240) and Arka Rakshak (H-241), were found to be highly promising for yield, fruit quality attributes with triple disease resistance to ToLCV+BW + EB (Figs. 2.3 and 2.4). Due to their high yield potential, excellent fruit quality and triple disease resistance, both these hybrids have been widely adopted by Indian farmers.

Fig. 2.3 Arka Samrat: A high-yielding triple disease-resistant tomato F_1 hybrid

Fig. 2.4 Arka Rakshak: A high-yielding triple disease-resistant tomato F_1 hybrid

Further, molecular-assisted breeding (MAB) program on tomato at ICAR-IIHR, Bengaluru, has also resulted in the development of first multiple disease-resistant tomato F_1 hybrid "Arka Abhed" (H-397) in the country (Fig. 2.5). Arka Abhed is suitable for both fresh market and processing with multiple disease resistance to tomato leaf curl disease (*Ty-2* + *Ty-3*), bacterial wilt (*Bwr*.12), early blight and late blight (*Ph-2* + *Ph-3*) (Fig. 2.6). Arka Abhed has also exhibited field tolerance to bipartite *Tomato leaf curl New Delhi virus* (ToLCNDV) under field conditions when tested at IARI, New Delhi.

2.8 Breeding Tomatoes for Processing Quality with Resistance to Diseases

ICAR-IIHR has also developed two triple disease (ToLCD+BW + EB)-resistant tomato F_1 hybrids, viz Arka Apeksha (H-385) and Arka Vishesh (H-391), suitable for processing with high TSS (4.5-5^0Brix) and high lycopene (10–12 mg/100 g).

Fig. 2.5 Arka Abhed: First multiple disease-resistant dual-purpose tomato F_1 hybrid

Fig. 2.6 Reaction of Arka Abhed against late blight under field condition

Both the hybrids are high yielding (80–90 t/ha) with firm (7 kg/cm^2), deep red, medium large (75–80 g), oblong, jointless (j2) fruits suitable for mechanical harvesting (Fig. 2.7).

Fig. 2.7 Arka Apeksha and Arka Vishesh: High-yielding processing F_1 hybrids

References

Abhary M, Patil BL, Fauquet CM (2007) Molecular biodiversity, taxonomy, and nomenclature of tomato yellow leaf curl-like viruses. In: Czosnek H (ed) Tomato yellow leaf curl virus disease: management, molecular biology, breeding for resistance. Springer, Dordrecht, pp 85–118. https://doi.org/10.1007/978-1-4020-4769-5_6

Acosta JC, Gilbert JC, Quinon VL (1964) Heritability of bacterial wilt resistance in tomato. Proc Am Soc Hortic Sci 84:455–462

Akram M (2012) Biological characterization and variability of the nucleocapsid protein gene of groundnut bud necrosis virus isolates infecting pea from India. Phytopathol Mediterr 51:266–275

Aliye N, Fininsa C, Hiskias Y (2008) Evaluation of rhizosphere bacterial antagonists for their potential to bioprotect potato (*Solanum tuberosum*) against bacterial wilt (*Ralstonia solanacearum*). Biol Control 47:282–288

Amarnath M, Ravishankar KV, Satish D, Sriram S, Sadashiva AT (2019) Screening of tomato genotypes against early blight pathogen *Alternaria solani* using detached leaf assay method. Pest Manage Horticult Ecosyst 25(1):93–101

Ammiraju JS, Veremis JC, Huang X, Roberts PA, Kaloshian I (2003) The heat-stable root-knot nematode resistance gene *Mi-9* from *Lycopersicon peruvianum* is localized on the short arm of chromosome 6. Theor Appl Genet 106:478–484

Anbinder I, Reuveni M, Azari R, Paran I, Nahon S, Shlomo H et al (2009) Molecular dissection of tomato leaf curl virus resistance in tomato line TY172 derived from Solanum peruvianum. Theor Appl Genet 119:519–530. https://doi.org/10.1007/s00122-009-1060-z

Andrus CF, Reynard CB (1945) Resistance to Septoria leaf spot and its inheritance in tomatoes. Phytopathology 35:16–24

Andrus CF, Reynard GB, Wade BL (1942) Relative resistance of tomato varieties, selections and crosses to defoliation by *Alternaria* and *Stemphylium*. USDA Circ 652:23

Arcos MG ´l, de Noronha Fonseca ME, Arruabarrena A, Lima MF, Michereff-Filho M, Moriones E, Fernandez-Munoz R, Boiteux LS (2018) Identification of genetic sources with attenuated Tomato chlorosis virus-induced symptoms in Solanum (section Lycopersicon) germplasm. Euphytica 214:178. https://doi.org/10.1007/s10681-018-2253-9

Arens P, Mansilla C, Deinum D, Cavellini L, Moretti A, Rolland S, van der Schoot H, Calvache D, Ponz F, Collonnier C, Mathis R, Smilde D, Caranta C, Vosman B (2010) Development and evaluation of robust molecular markers linked to disease resistance in tomato for distinctness, uniformity and stability testing. Theor Appl Genet 120:655–664

Aslam MN, Mukhtar T, Hussain MA, Raheel M (2017) Assessment of resistance to bacterial wilt incited by *Ralstonia solanacearum* in tomato germplasm. J Plant Dis Prot 124:585–590

Astua-Monge G, Minsavage GV, Stall RE, Vallejos CE, Davis MJ, Jones JB (2000) Xv4-vrxv4: A new gene-for-gene interaction identified between *Xanthomonas campestris pv. vesicatoria* race T3 and the wild tomato relative *Lycopersicon pennellii*. Mol Plant-Microbe Interact 13(12):1346–1355

Augustine JJ, Harbaugh BK, Crill JP (1981) Florida basket. A dwarf tomato for hanging baskets, vol 283. Agricultural Experiment Stations, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, p 2

Bai Y, Pavan S, Zheng Z, Zappel NF, Reinstädler A, Lotti C, De Giovanni C, Ricciardi L, Lindhout P, Visser R, Theres K (2008) Naturally occurring broad-spectrum powdery mildew resistance in a central American tomato accession is caused by loss of Mlo function. Mol Plant-Microbe Interact 21(1):30–39

Bailey DM (1941) The seedling method for root-knot nematodes resistance. Proc Am Hortic Sci 38:573–575

Banerjee MK, Kalloo G (1987) Sources and inheritance of resistance to leaf curl virus in Lycopersicon. Theor Appl Genet 73:707–710

Banerjee MK, Chhabra ML, Saini PS (1998) Responses of tomato cultivars to *Alternaria* blight. Tests Agrochem Cultiv 19:50–51

Barak JD, Vancheva T, Lefeuvre P, Jones JB, Timilsina S, Minsavage GV, Vallad GE, Koebnik R (2016) Whole-genome sequences of *Xanthomonas euvesicatoria* strains clarify taxonomy and reveal a stepwise erosion of type 3 effectors. Front Plant Sci 7:1805

Barbieri M, Acciarri N, Sabatini E, Sardo L, Accotto GP, Pecchioni N (2010) Introgression of resistance to two Mediterranean virus species causing tomato yellow leaf curl into a valuable traditional tomato variety. J Plant Pathol 92:485–493

Barillas AC, Mejia L, Sanchez-Perez A, Maxwwell DP (2008) CAPS and SCAR markers for detection of I-3 gene introgression for resistance to *Fusarium oxysporum* f. sp. *lycopersici* race 3. Rep Tomato Genet Coop 58:11–17

Barksdale TH (1972) Resistance in tomato to six anthracnose fungi. Phytopathology 62:660–663

Barksdale TH, Stoner AK (1977) A study of the inheritance of tomato early blight resistance. Plant Dis Rep 61:63–65

Barksdale TH, Stoner AK (1978) Resistance in tomato to *Septoria lycopersici*. Plant Dis Rep 62(10):844–847

Bender CG, Shoemaker PB (1984) Prevalence of *Verticillium* wilt of tomato and virulence of *Verticillium* dahliae race 1 and race 2 isolates in Western North Carolina. Plant Dis 68:305–309

Benedict WG (1972) Influence of light on peroxidase activity associated with resistance of tomato cultivars to *Septoria lycopersici*. Can J Bot 50:1931–1936

Black LL, Wang TC, Hanson PM, Chen JT (1996) Late blight resistance in four wild tomato accessions: effectiveness in diverse locations and inheritance of resistance. Phytopathology 86:S24

Boch J, Joardar V, Gao L, Robertson TL, Lim M, Kunkel BN (2002) Identification of *Pseudomonas syringae* pv. *tomato* genes induced during infection of Arabidopsis thaliana. Mol Microbiol 44:73–88

Bohn GW, Thcker CM (1940) Studies on Fusarium wilt of tomato. I. Immunity in *Lycopersicon pimpinellifolium* mill. and its inheritance in hybrids. Mo Agric Exp Station Res Bull 311:1–82
Bonde R, Murphy EF (1952) Resistance of certain tomato varieties and crosses to late blight. Maine Agric Exp Station Bull 497:5–15
Boukema IW (1978) Races of *Cladosporium fulvum (Fulvia fulva)* in the Netherlands and resistant genes in the genus *Lycopersicon*. In: Genotype and environment in glasshouse tomato breeding. Proc Eucarpia Tomato Working Group, Leningrad, 150–155
Brouwer DJ, Jones ES, Clair DAS (2004) QTL analysis of quantitative resistance to *Phytophthora infestans* (late blight) in tomato and comparisons with potato. Genome 47:475–492
Boukema IW (1981) Races of *Cladosporium fulvum Cke.(Fulvia fulva)* and genes for resistance in the tomato (*Lycopersicon Mill.*). In Genetics and breeding of tomato: proceedings of the meeting of the Eucarpia Tomato Working Group, Avignon-France, May 18–21, 1981. Versailles, France: Institut national de la recherche agronomique, 1981
Cap GB, Roberts PA, Thomason IJ (1993) Inheritance of heat-stable resistance to *Meloidogyne incognita* in *Lycopersicon peruvianum* and its relationship to the mi gene. Theor Appl Genet 85:777–783
Catanzariti AM, Lim GT, Jones DA (2015) The tomato I-3 gene: a novel gene for resistance to Fusarium wilt disease. New Phytol 207(1):106–118
Chaerani R, Voorrips R (2006) Tomato early blight (*Alternaria solani*): the pathogen, genetics, and breeding for resistance. J Gen Plant Pathol 72:335–347
Chalupowicz L, Barash I, Reuven M, Dror O, Sharabani G, Garte-mann KH, Eichenlaub R, Sessa G, Manulis-Sasson S (2017) Differential contribution of *Clavibacter michiganensis ssp. michiganensis* virulence factors to systemic and local infection in tomato. Mol Plant Pathol 18:336–346
Chunwongse J, Doganlar S, Crossman C, Jiang J, Tanksley SD (1997) High-resolution genetic map of the Lv resistance locus in tomato. Theor Appl Genet 95(1–2):220–223
Ciccarese F, Amenduni M, Schiavone D, Cirulli M (1998) Occurrence and inheritance of resistance to powdery mildew (*Oidium lycopersici*) in *Lycopersicon* species. Plant Pathol 47:417–419
Cui Z (2016) Detection and analysis of *Xanthomonas* associated with bacterial spot in tomato and pepper seeds. M.D. dissertation. China Agricultural University, Beijing
Datar VV, Mayee CD (1980) Breeding for early blight resistance in tomato. Ind Phytopatho l33:151
Davis MJ, Gillaspie AG, Vidaner AK Jr, Harris RW (1984) *Clavibacter* : a new genus containing some phytopathogenic coryneform bacteria, including *Clavibacter xyli* subsp. *xyli* sp. *nov*., subsp. *nov*. and *Clavibacter xyli* subsp. *cynodontis* subsp. *nov*., pathogens that cause ratoon stunting disease of sugarcane and bermudagrass stunting disease. Int J Syst Bacteriol 34:107–117
De la Peña R, Kadirvel P, Venkatesan S, Kenyon L, Hughes J (2010) Integrated approaches to manage Tomato Yellow Leaf Curl Viruses. In: Hou CT, Shaw J-F (eds) Biocatalysis and Biomolecular Engineering. Wiley, Hoboken, pp 105–132. https://doi.org/10.1002/9780470608524-ch8
Deng G, Qu X, Chen XR, Yang CD, Xue L (2008) Pathogen identification of bacterial leaf speck of tomato based on 16S rDNA, physiology and biochemistry in Gansu Province. Plant Prot 34:47–51
Denny TP (2006) Plant pathogenic *Ralstonia* species. In: Gnanamanickam SS (ed) Plant-associated bacteria. Springer, Dordrecht, pp 573–644
Dhaliwal MS, Sharma A (2016) Breeding for resistance to virus diseases in vegetable crops. In: Peter KV (ed) Innovations in horticultural sciences. New India Publishing Agency, New Delhi, pp 303–327
Ding AY, Shi CK, Zheng JF, Zhu HC, Jin XD (1996) Preliminary study on bacterial spot to tomato in Shandong. J Shandong Agric Univ 27:207–208
Diwan N, Fluhr R, Eshed Y, Zamir D, Tanksley SD (1999) Mapping of Ve in tomato: a gene conferring resistance to the broadspectrum pathogen, *Verticillium dahliae* race 1. Theor Appl Genet 98:315–319

Dong P, Han K, Siddique MI, Kwon J-K, Zhao M, Wang F, Kang B-C (2016) Gene-based markers for the tomato yellow leaf curl virus resistance gene Ty-3. Plant Breed Biotechnol 4:79–86
Dropkin VH (1969) Cellular responses of plants to nematode infections. Annu Rev Phytopathol 7:101–122
Dudley JW (1993) Molecular markers in plant improvement: manipulation of genes affecting quantitative traits. Crop Sci 33:660–668
David M. Francis, Sally Miller, Ohio 9834 and Ohio 9816: Processing Tomato Breeding Lines with Partial Resistance to Race T1 of Bacterial Spot. HortScience 40 (5):1566-1568
FAOSTAT (2017) Food and Agriculture Organization of the United Nations (FAO) Statistical Data-bases. http://faostat.fao.org/
Ferreira JJ, Campa A, Pérez-Vega E, Rodríguez-Suárez C, Giraldez R (2012) Introgression and pyramiding into common bean market class fabada of genes conferring resistance to anthracnose and potyvirus. Theor Appl Genet 124(4):777–788
Foolad MR, Ntahimpera N, Christ BJ, Lin GY (2000) Comparison of field, greenhouse, and detached leaflet evaluations of tomato germplasm for early blight resistance. Plant Dis 84:967–972
Foolad MR, Merk HL, Ashrafi H (2008) Genetics, genomics and breeding of late blight and early blight resistance in tomato. Crit Rev Plant Sci 27(2):75–107
Fradin EF, Thomma BPHJ (2006) Physiology and molecular aspects of Verticillium wilt diseases caused by *V. dahliae* and *V. alboatrum*. Mol. Plant Pathol 7:71–86
Fradin EF, Zhang Z, Juarez Ayala JC, Castroverde CD, Nazar RN, Robb J, Liu CM, Thomma BP (2009) Genetic dissection of Verticillium wilt resistance mediated by tomato *Ve1*. Plant Physiol 150:320–332
Frary A, Graham E, Jacobs J, Chetelat RT, Tanksley SD (1998) Identification of QTL for late blight resistance from *L. pimpinellifolium* L3708. Tomato Genet Coop Rep 48:19–21
Fraser RSS (1990) The genetics of resistance to plant viruses. Annu Rev Phytopathol 28:179–200
Gallegly ME (1960) Resistance to the late blight fungus in tomato. In: Proceedings of plant sci seminar. Campbell Soup Co, pp 113–135
Gallegly ME, Marvel ME (1955) Inheritance of resistance to tomato race-O of Phytophthora-Infestans. Phytopathology 45:103–109
García-Cano E, Resende RO, Boiteux LS, Giordano LB, FernándezMuñoz R, Moriones E (2008) Phenotypic expression, stability, and inheritance of a recessive resistance to monopartite begomoviruses associated with tomato yellow leaf curl disease in tomato. Phytopathology 98:618–627. https://doi.org/10.1094/PHYTO-98-5-0618
Gardner RG (1984) Use of *Lycopersicon hirsutum* PI 126445 in breeding early blight resistant tomatoes (Abstr). HortScience 19(2):208
Gardner RG (1988) NC EBR-1 and NC EBR-2 early blight resistant tomato breeding lines. HortScience 23:779–781
Gill U, Scott JW, Shekasteband R, Ogundiwin E, Schuit C, Francis DM, Sim S-C, Smith H, Hutton SF (2019) Ty-6, a major begomovirus resistance gene on chromosome 10, is effective against tomato yellow leaf curl virus and tomato mottle virus. Theor Appl Genet 132(5):1543–1554
Gonzalez-Cendales Y, Catanzariti AM, Baker B, Mcgrath DJ, Jones DA (2015) Identification of *I-7* expands the repertoire of genes for resistance to Fusarium wilt in tomato to three resistance gene classes. Mol Plant Pathol. https://doi.org/10.1111/mpp.12294
Goode MJ, Sasser M (1980) Prevention-the key to controlling bacterial spot and bacterial speck of tomato. Plant Dis 64:831–834
Goodwin SB, Sujkowski LS, Fry WE (1995) Rapid evolution of pathogenicity within clonal lineage of the potato late blight disease fungus. Phytopathology 85:669–676
Grattidge R, O'Brien RG (1982) Occurrence of a third race of Fusarium wilt of tomatoes in Queensland. Plant Dis 66:165–166
Griffiths PD, Scott JW (1998) Inheritance and Linkage of Tomato Mottle Virus Resistance Genes Derived from Lycopersicon chilense Accession LA 1932. Journal of the American Society for Horticultural Science 126(4):462–467

Grimault V, Prior P, Anais G (1995) A monogenic dominant resistance of tomato to bacterial wilt in Hawaii-7996 is associated with plant colonization by *Pseudomonas-solanacearum*. J Phytopathol 143:349–352

Gul Z, Ahmed M, Khan ZU, Khan B, Iqbal M (2016) Evaluation of tomato lines against Septoria leaf spot under field conditions and its effect on fruit yield. Agric Sci 7(04):181

Guo SC, Zhuang JR, Li Q, Dong QC (2008) Investigation and integrative management of bacterial spot in Fei County. China Veg 5:60–61

Gupta PK, Balyan HS, Sharma PC, Ramesh B (1996) Microsatellites in plants: a new class of molecular markers. Curr Sci 70:45–54

Hanson PM, Licardo O, Hanudin, Wang JF, Chen JT (1998) Diallel analysis of bacterial wilt resistance in tomato derived from different sources. Plant Dis 82:74–78

Hanson P, Lu SF, Wang JF, Chen W, Kenyon L, Tan CW, Tee KL, Wang YY, Hsu YC, Schafleitner R, Ledesma D (2016) Conventional and molecular marker-assisted selection and pyramiding of genes for multiple disease resistance in tomato. Sci Hortic 201:346–354

Hedrick WP, Booth NO (1907) Mendalian characters in tomatoes. Proc Am Soc Hortic Sci 5:19–24

Hernandes R, Stamova L (1990) Resistance to *Leveillula taurica* in tomato lines from Costa Rica. Tomato Genet Coop Rep 40:15

Hernandez MM (1985) Comparison of the effects of three isolates of *Stemphylium solani* weber, obtained from different regions of the province of Havana on lines of tomato *(Lycopersicon esculentum* mill). Cult Trop 7(3):3–11

Hutton SF, Scott JW (2014) Ty-6, a major begomovirus resistance gene located on chromosome10. Rep Tomato Genet Coop 64:14–18

Hutton SF, Scott JW, Jones JB (2010a) Inheritance of resistance to bacterial spot race T4 from three tomato breeding lines with differing resistance backgrounds. J Am Soc Hortic Sci 135:150–158

Hutton SF, Scott JW, Yang W, Sim SC, Francis DM, Jones JB (2010b) Identification of QTL associated with resistance to bacterial spot race T4 in tomato. Theor Appl Genet 121:1275–1287

Ishibashi K, Masuda K, Naito S, Meshi T, Ishikawa M (2007) An inhibitor of viral RNA replication is encoded by a plant resistance gene. Proc Natl Acad Sci U S A 104:13833–13838

Ji Y, Scott JW (2009) A CAPS marker linked to the tomato gray leafspot (*Stemphylium sp*.) resistance gene Sm. Rep Tomato Genet Coop 59:29–31

Ji Y, Schuster DJ, Scott JW (2007a) Ty-3, a begomovirus resistance locus near the tomato yellow leaf curl virus resistance locus ty-1 on chromosome 6 of tomato. Mol Breed 20:271–284. https://doi.org/10.1007/s11032-007-9089-7

Ji Y, Scott JW, Hanson P, Graham E, Maxwell DP (2007b) Sources of resistance, inheritance, and location of genetic loci conferring resistance to members of the tomato-infecting begomoviruses. In: Czosnek H (ed) Tomato yellow leaf curl virus disease: management, molecular biology, breeding for resistance. Springer, Dordrecht, pp 343–362. https://doi.org/10.1007/978-1-4020-4769-5_20

Ji Y, Scott JW, Schuster DJ, Maxwell DP (2009) Molecular mapping of ty-4, a new tomato yellow leaf curl virus resistance locus on chromosome 3 of tomato. J Am Soc Hortic Sci 134:281–288

Johanson A, Thurston HD (1990) The effect of cultivar maturity on the resistance of potato to early blight caused by *Alternaria solani*. Am Potato J 67:615–623

Jones JB (1991) Bacterial speck. In: Jones JB, Jones JP, Stall RE, Zitter TA (eds) Compendium of tomato diseases. APS Press, St. Paul, pp 26–27

Jones RA, Millet A, Gainnini C (1989) A greenhouse screening method for *Pyrenochaeta lycopersici* resistance in tomato. Euphytica 40:187–191

Jones JB, Lacy GH, Bouzar H, Stall RE, Schaad NW (2004) Reclassification of the xanthomonads associated with bacterial spot disease of tomato and pepper. Syst Appl Microbiol 27:755–762

Jones JB, Lacy GH, Bouzar H, Minsavage GV, Stall RE, Schaad NW (2005) Bacterial spot –worldwide distribution, importance and review. Acta Hortic 695:27–33

Jones JB, Zitter TA, Momol TM, Miller SA (2014) Compendium of tomato diseases and pests, 2nd edn. APS Press, Minnesota

Jung J, Kim HJ, Lee JM, Oh CS, Lee HJ, Yeam I (2015) Gene-based molecular marker system for multiple disease resistances in tomato against *Tomato yellow leaf curl virus*, late blight, and verticillium wilt. Euphytica 205:599–613

Kadirvel P, de la Peña R, Schafleitner R, Huang S, Geethanjali S, Kenyon L, Tsai W, Hanson P, (2013) Mapping of QTLs in tomato line FLA456 associated with resistance to a virus causing tomato yellow leaf curl disease. Euphytica 190(2):297–308

Kalloo G (1986) Tomato *(Lycopersicon esculentum* Miller). Allied Publishers Pvt, New Delhi, p 470

Kanwar JS, Kerr EA, Harney PM (1980a) Linkage of *Cft* to *Cftl* genes for resistance to tomato leaf mold (c. *fulvum* CKe). Rep Tomato Genet Coop 30:20–21

Kanwar JS, Kerr EA, Harney PM (1980b) Linkage of *Cft2* to *Cf24* genes for resistance to tomato leaf mold (c. *fulvum* CKe). Rep Tomato Genet Coop 30:22–23

Kawchuk LM, Hachey J, Lynch DR, Kulcsar F, van Rooijen G, Waterer DR, Robertson A, Kokko E, Byers R, Howard RJ, Fischer R, Prufer D (2001) Tomato *Ve* disease resistance genes encode cell surface-like receptors. Proc Natl Acad Sci U S A 98:6511–6515

Kerr EA (1977) Problems and complications of developing new greenhouse varieties. In: Proceedings of Can Mid- west greenhouse vegetable grower, Ontario, pp 45–47

Kerr EA, Bailey DL (1964) Resistance to *Cladosporium fulvum* Cke. Obtained from wild species of tomato. Can J Bot 42:1541–1542

Kerr EA, Patrick ZA, Bailey DL (1971) Resistance in tomato species to new races of leaf mold *(Cladosporium fulvum* Cke). Hortic Res 11:84–92

Kerr EA, Kerr E, Patrick ZA, Potter JW (1980) Linkage relation of resistance to *Cladosporium* leaf mould *(Cf2)* and root-knot nematodes *(Mi)* in tomato and a new gene for leaf mould resistance *(Cf11)*. Can J Genet Cyto I 22:183–186

Kim MJ, Mutschler MA (2005) Transfer to processing tomato and characterization of late blight resistance derived from *Solanum pimpinellifolium* L. L3708. J Am Soc Hortic Sci 130:877–884

Kim BY, Hwang IS, Lee HJ, Lee JM, Seo EY, Choi D, Oh CS (2018) Identification of a molecular marker tightly linked to bacterial wilt resistance in tomato by genome-wide SNP analysis. Theor Appl Genet. https://doi.org/10.1007/s00122-018-3054-1

Kuklev MY, Fesenko IA, Karlov GI (2009) Development of a CAPS marker for the Verticillium wilt resistance in tomatoes. Russ J Genet 45(5):575–579

Kunkalikar SR, Sudarsana P, Arun BM, Rajagopalan P, Chen TC, Yeh SD, Naidu RA, Zehr UB, Ravi KS (2011) Importance and genetic diversity of vegetableinfecting tospoviruses in India. Phytopathology 101:367–376

Kurozawa C, Balmer E (1977) Response of national and imported cultivars and wild species of tomato to *Septoria lycopersici* Speg. Summa Phytopathol 3:135–141

Lanfermeijer FC, Dijkhuis J, Sturre MJG, de Haan P, Hille J (2003) Cloning and characterization of the durable tomato mosaic virus resistance gene tm-22 from Lycopersicon esculentum. Plant Mol Biol 52:1037–1049

Lanfermeijer FC, Warmink J, Hille J (2005) The products of the broken tm-2 and the durable tm-2(2) resistance genes from tomato differ in four amino acids. J Exp Bot 56:2925–2933

Lapidot M, Karniel U, Gelbart D, Fogel D, Evenor D, Kutsher Y et al (2015) A novel route controlling begomovirus resistance by the messenger RNA surveillance factor pelota. PLoS Genet 11:e1005538. https://doi.org/10.1371/journal.pgen.1005538

Laterrot H (1978) Resistance aux maladies. B. *Pyrenochaeta lycopersici*. Rapp Activ Stn Amelion Plantes Moraic 1977–1978:102–103

Laterrot H (1981) Premier bilan de l'étude de l'efficacité envers *Cladosporium fulvum* de 24 nouvelles origines résistantes de tomate. In Genetics and breeding of tomato: proceedings of the meeting of the Eucarpia Tomato Working Group, Avignon-France, May 18–21, 1981. Versailles, France: Institut national de la recherche agronomique, 1981

Laterrot H (1994) NILs of tomato except for *Ph-2* gene. Tomato Coop Genet Rep 44:20–21

Li MY (1991) Identification and control of tomato diseases. Vegetables 3:26–27

Li C, Jin Q, Peng G (1997) Pathogen identification of tomato bacterial scab in Xinjiang. China Veg 4:4–6

Li HT, Zou QD, Lv SW, Mu X, Xu WK (2001) Study on inheritance to bacterial wilt in tomato. Liaoning Agric Sci 5:1–4

Liabeuf D (2016) Development of processing tomato lines resistant to *Xanthomonas gardneri*: from screening to breeding, Ph.D. dissertation. The Ohio State University, Columbus

Liabeuf D, Francis DM, Sim SC (2015) Screening cultivated and wild tomato germplasm for resistance to *Xanthomonas gardneri*. Acta Hortic 1069:65–70

Lim GTT, Wang GP, Hemming MN, Basuki S, McGrath DJ, Carroll BJ, Jones DA (2006) Mapping the *I-3* gene for resistance to Fusarium wilt in tomato: application of an *I-3* marker in tomato improvement and progress towards the cloning of *I-3*. Australas Plant Pathol 35:671–680

Lim GTT, Wang GP, Hemming MN, McGrath DJ, Jones DA (2008) High resolution genetic and physical mapping of the *I-3* region of tomato chromosome 7 reveals almost continuous microsynteny with grape chromosome 12 but interspersed microsynteny with duplications on *Arabidopsis* chromosomes 1, 2 and 3. Theor Appl Genet 118:57–75

Lindhout P, Pet G, Van der Beek H (1994) Screening wild *Lycopersicon* species for resistance to powdery mildew (*Oidium lycopersicum*). Euphytica 72:43–49

Ling KS, Scott JW (2007) Sources of resistance to Pepino mosaic virus in tomato accessions. Plant Dis 91(6):749–753

Locke SB (1949) Resistance to early blight and septoria leaf spot in the genus *Lycopersicon*. Phytopathology 39:824–836

Lodha PC (1977) Reaction of some tomato cultivars to culture filtrate of *Alternaria solani* (Ed et Mat) Jones et grout. Phytopathol Mediterr 16:36–37

Lukyanenko AN (1991) Disease resistance in tomato. In: Kalloo G (ed) Genetic improvement of tomato. Springer, Berlin/Heidelberg, pp 99–119

Maiero M, Ng TJ, Barksdale TH (1989) Combining ability estimates for early blight resistance in tomato. J Am Soc Hortic Sci 114:118–121

Mandal B, Jain RK, Krishnareddy M, Kumar NKK, Ravi KS, Pappu HR (2012) Emerging problems of Tospoviruses (Bunyaviridae) and their management in the Indian subcontinent. Plant Dis 96:468–479. https://doi.org/10.1094/PDIS-06110520

Mangin B, Thoquet P, Olivier J, Grimsley NH (1999) Temporal and multiple quantitative trait loci analyses of resistance to bacterial wilt in tomato permit the resolution of linked loci. Genetics 151:1165–1172

Manjunatha L (2008) Studies on bud blight disease of tomato caused by groundnut bud necrosis virus. Doctoral dissertation, UAS, Dharwad

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu TY, Earle ED, Tanksley SD (1993) Map-based cloning of a protein-kinase gene conferring disease resistance in tomato. Science 262:1432–1436

Miller AN, Ng T, Barksdale TH (1983) A diallel analysis of the inheritance of resistance to tomato anthracnose caused by *Colletotrichum dematium*. Phytopathology 73:968

Milligan SB, Bodeau J, Yaghoobi J, Kaloshian I, Zabel P, Williamson VM (1998) The root knot nematode resistance gene mi from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. Plant Cell 10:1307–1319

Moore WD, Reynard GB (1945) Varietal resistance of tomato seedlings to the stem -lesion phase of *Alternaria solani*. Phytopathology 35:933–935

Moreau P, Thoquet P, Olivier J, Laterrot H, Grimsley N (1998) Genetic mapping of Ph-2, a single locus controlling partial resistance to *Phytophthora infestans* in tomato. Mol Plant-Microbe Interact 11:259–269

Moriones E, Navas-Castillo J (2000) Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. Virus Res 71:123–134. https://doi.org/10.1016/S0168-1702(00)00193-3

Nowakowska M, Nowicki M, Kłosińska U, Maciorowski R, Kozik EU (2014) Appraisal of artificial screening techniques of tomato to accurately reflect field performance of the late blight resistance. PloS one 9(10):e109328

Nowicki M, Foolad MR, Nowakowska M, Kozik EU (2012) Potato and tomato late blight caused by *Phytophthora infestans*: an overview of pathology and resistance breeding. Plant Dis 96:4–17

Ohlson EW, Foolad MR (2015) Heritability of late blight resistance in tomato conferred by Solanum pimpinellifolium accession PI 224710. Plant Breed 134(4):461–467

Ohnishi J, Yamaguchi H, Saito A (2016) Analysis of the mild strain of tomato yellow leaf curl virus, which overcomes ty-2. Arch Virol 161:2207–2217. https://doi.org/10.1007/s00705-016-2898-4

Oliveira RM, Dianese ÉC, Lima MF, Resende RO, Inoue-Nagata AK, Boiteux LS (2018) Sources of resistance to potato virus Y and pepper yellow mosaic virus in Solanum (section Lycopersicon) germplasm. Eur J Plant Pathol 150(3):691–699

Opena RT, Green SK, Talekar NS, Chen JT (1989) Genetic improvement of tomato adaptability to the tropics: progress and future prospects. In: Green SK (Sc. ed) Griggs TD, Mc lean BT (Publ. eds) Tomato and pepper production in the tropics. Proceedings of the international symposium on integrated management practices. AVRDC, Shanhua, pp 71–85

Palti J (1988) The *Leveillula* mildews. Bot Rev 54(4):423–535

Panthee DR, Foolad MR (2012) A reexamination of molecular markers for use in marker-assisted breeding in tomato. Euphytica 184:165–179

Parniske M, Hammond-Kosack KE, Golstein C, Thomas CM, Jones DA, Harrison K, Wulff BB, Jones JD (1997) Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the Cf-4/9 locus of tomato. Cell 91:821–832

Paternotte SJ (1988) Occurrence and chemical control of powdery mildew (*Oidium* sp.) in tomatoes. Mededelingen van de Faculteit landbouwwetenschappen. Rijksuniversiteit Gent, 53(2b), pp.657–661

Pedley KF, Martin GB (2003) Molecular basis of Pto -mediated resistance to bacterial speck disease in tomato. Annu Rev Phytopathol 41:215–243

Pei C, Wang H, Zhang J, Wang Y, Francis DM, Yang W (2012) Fine mapping and analysis of a candidate gene in tomato accession PI128216 conferring hypersensitive resistance to bacterial spot race T3. Theor Appl Genet 124:533–542

Peirce LC (1971) Linkage tests with *Ph* conditioning resistance to race 0, *Phytophthora infestans*. Tomato Genet Coop Rep 21:30

Pelham J (1966) Resistance in tomato to tobacco mosaic virus. Euphytica 15:258–267

Pereira-Carvalho RC, Boiteux LS, Fonseca MEN, Díaz-Pendón JA, Moriones E, Fernández-Muñoz R et al (2010) Multiple resistance to Meloidogyne spp. and bipartite and monopartite Begomovirus spp. in wild Solanum (*Lycopersicon*) accessions. Plant Dis 94:179–185. https://doi.org/10.1094/PDIS94-2-0179

Poysa V, Tu JC (1996) Response of cultivars and breeding lines of *Lycopersicon* spp., to Alternaria solani. Can Plant Dis Surv 76:5–8

Prasanna HC, Sinha DP, Rai GK, Krishna R, Kashyap SP, Singh NK, Singh M, Malathi VG (2014) Pyramiding ty-2 and ty-3 genes for resistance to monopartite and bipartite tomato leaf curl viruses in India. Plant Pathol 64:256–264

Rattan RS, Saini SS (1979) Inheritance of resistance to fruit rot *(Phytophthora parasitica* Dast.) in tomato *(Lycopersicon escillennun* Mill.). Euphytica 28:315–317

Reddy DVR, Ratna AS, Sudarshana MR, Kiran KI (1992) Serological relationships and purification of bud necrosis virus, a tospovirus occurring in peanut (*Arachis hypogaea* L.) in India. Ann Appl Biol 120:279–286

Ren Z, You Z, Munir S, Zhang Y, Li H, Zhang J, Wang T, Zheng W, Ye Z (2019) Development of a highly specific co-dominant marker for genotyping the Ph-3 (tomato late blight resistance) locus by comparing cultivated and wild ancestor species. Mol Breed 39(3):45

Rich AE, Richards MC, Meader EM (1962) Rockingham, a new tomato variety resistant to late blight. Plant Dis Rep 46:130

Robbins MD, Masud MAT, Panthee DR, Gardner RG, Francis DM, Stevens MR (2010) Marker-assisted selection for coupling phase resistance to tomato spotted wilt virus and Phytophthora infestans (late blight) in tomato. Hortic Sci 45:1424–1428

Robert VJM, West MAL, Inai S, Caines A, Arntzen L, Smith JK, St Clair DA (2001) Marker-assisted introgression of blackmold resistance QTL alleles from wild *Lycopersicon cheesmanii* to cultivated tomato (*L. esculentum*) and evaluation of QTL phenotypic effects. Mol Breeding 8:217–233

Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM (1998) The nematode resistance gene Mi of tomato confers resistance against the potato aphid. Proc Natl Acad Sci USA 95: 9750–9754. Plant Dis 66:46–47

Rotem J (1994) The genus *Alternaria* biology, epidemiology, and pathogenicity, 1st edn. APS, St. Paul, pp 48–203

R.G. Gardner, `Plum Dandy', a Hybrid Tomato, and Its Parents, NC EBR-5 and NC EBR-6. HortScience 35 (5):962–963

Sadashiva AT, Hanson P, Reddy MK, Ravishankar KV, Prasad M, Prasanna HC, Reddy KM, Singh TH, Saritha RK, Hussain Z Mythili JB, Kaushal A (2017) Breeding tomato (*Solanum lycopersicum* L.) for resistance to biotic and abiotic stresses

Sain SK, Chadha ML (2016) Response of some wild species of tomato against Peanut bud necrosis virus under open-field conditions. Indian J Hortic 73(1):60–65

Sasser JN (1989) Plant parasitic nematodes. The Farmer's hidden enemy. Published by North Carolina State University Raleigh, North Carolina, p 115

Satelis JF, Boiteux LS, Reis A (2010) Resistance to *Septoria lycopersici* in Solanum (section Lycopersicon) species and in progenies of *S. lycopersicum* × *S. peruvianum*. Scientia Agricola 67(3):334–341

Schaible LW (1962) Fruit setting responses of tomato to high night temperature. In: Proceedings of plant science symposium, Canden, p 229

Schornack S, Ballvora A, Gürlebeck D, Peart J, Baulcombe D, Ganal M, Baker B, Bonas U, Lahaye T (2004) The tomato resistance protein Bs4 is a predicted non-nuclear TIR-NB-LRR protein that mediates defense responses to severely truncated derivatives of AvrBs4 and overexpressed AvrBs3. Plant J 37:46–60

Scott JW, and Jones JP (1989) Monogenic resistance in tomato to Fusarium oxysporum f. sp. lycopersici race 3. Euphytica 40(1–2):49–53

Scott JW, Somodi GC, Jones JB (1988) Bacterial spot resistance is not associated with bacterial wilt resistance in tomato. Proc Fla State Hortic Soc 101:390–392

Scott JW, Hutton SF, Shekasteband R, Sim SC, Francis DM (2013) Identification of tomato bacterial spot race T1, T2, T3, T4, and *Xanthomonas gardneri* resistance QTLs derived from PI 114490 populations selected for race T4. Acta Hortic 1069:53–58

Seah S, Williamson VM, Garcia BE, Mejia L, Salus MS, Martin CT, Maxwell DP (2007) Evaluation of a co-dominant SCAR marker for detection of the *Mi-1* locus for resistance to root-knot nematode in tomato germplasm. Rep Tomato Genet Coop 57:37–40

Sen Y, Derwolf JV, Visser RGF, Heusden SV (2015) Bacterial canker of tomato: current knowledge of detection, management, resistance, and interactions. Plant Dis 99:4–13

Shrestha S, Kumar R, Behera TK, Sharma HK (2012) Inheritance of resistance to root-knot nematode (*Meloidogyne incognita* race 1) in tomato (*Solanum lycopersicum* L.) 'Pusa 120'. J Hortic Sci Biotechnol 87(3):211–216

Sinden SL, Goth RW, O'Brien MJ (1972) Effect of potato alkaloids on the growth of *Alternaria solani* and their possible role as resistance factors in potatoes. Phytopathology 63:303–307

Smart CD, Tanksley SD, Mayton H, Fry WE (2007) Resistance to *Phytophthora infestans* in *Lycopersicon pennellii*. Plant Dis 91:1045–1049

Smith EF (1910) A new tomato disease of economic importance. Science (New Series) 31:794–796

Smith PG (1944) Embryo culture of a tomato species hybrid. Proc Am SOC Hortic Sci 44:413–416

Sobir OT, Murata M, Motoyoshi F (2000) Molecular characterization of the SCAR markers tightly linked to the tm-2 locus of the genus *Lycopersicon*. Theor Appl Genet 101:64–69

Stall RE, Walter M (1965) Selection and inheritance of resistance on tomato to isolates of races 1 and 2 of the Fusarium wilt organism. Phytopathology 55:1213–1215

Stall RE, Jones JB, Minsavage GV (2009) Durability of resistance in tomato and pepper to Xanthomonads causing bacterial spot. Annu Rev Phytopathol 47:265–284

Stamova L, Yordanov M (1987) Resistance to Leveillula taurica (Lev) Arn. Tomato Genet Coop Rep 37:73

Stamova L, Yordanov M, Stamova S (1984) Genes for resistance to *Cladosporium fulvum* Cke *(Fulvia vulva)* in *Solanum pennellii* Corell. In: A new era in tomato breeding. 9th Meet Eucarpia Tomato Working Group, 22–24 May 1984, Wageningen, pp 75–80

Stevens MA, Rick CM (1986) Geneties and Breeding. In: Atherton JG, Rudich I (eds) The tomato crop: a scientific basis for improvement. Chapman & Hall, London, pp 35–109

Stevenson WR, Evans GE, Barksdale TH (1978) Evaluation of tomato breeding lines for resistance to fruit anthracnose. Plant Dis Rep 62:937–940

Su X, Zhu G, Huang Z, Wang X, Guo Y, Li B, Du Y, Yang W, Gao J (2019) Fine mapping and molecular marker development of the Sm gene conferring resistance to gray leaf spot (*Stemphylium* spp.) in tomato. Theor Appl Genet 132(4):871–882

Sun FZ, Zhu H, Zheng JQ, Yi Q (1991) Occurrence of bacterial spot in Beijing and Datong areas. Plant Prot 17:50–51

Sun HJ, Liu XX, Li WH, Yang WC (2011a) Preliminary mapping of a gene in tomato accession LA1589 conferring resistance to race T3 of bacterial spot. J Agric Univ Hebei 34:65–69

Sun HJ, Zhang JY, Wang YY, Scott JW, Francis DM, Yang WC (2011b) QTL analysis of resistance to bacterial spot race T3 in tomato. Acta Hortic Sin 38:2297–2308

Sun WY, Zhao WY, Wang YY, Pei CC, Yang WC (2011c) Natural variation of Pto and fen genes and marker-assisted selection for resistance to bacterial speck in tomato. Agric Sci China 10:827–837

Thapa SP, Miyao EM, Davis RM, Coaker G (2015) Identification of QTLs controlling resistance to Pseudomonas syringae pv. Tomato race 1 strains from the wild tomato, *Solanum habrochaites* LA1777. Theor Appl Genet 128(4):681–692

Thirthamallappa, Lohithaswa HC (2000) Genetics of resistance to early blight (*Alternaria solani* Sorauer) in tomato (*Lycopersicum esculentum* L.). Euphytica 113:187–193

Thomas JE, McGrath DJ (1988) Inheritance of resistance to potato virus Y in tomato. Aust J Agric Res 39(3):475–479

Tomás DM, Cañizares MC, Abad J, Fernández-Muñoz R, Moriones E (2011) Resistance to tomato yellow leaf curl virus accumulation in the tomato wild relative *Solanum habrochaites* associated with the C4 viral protein. Mol Plant Microbe Interact 24:849–861. https://doi.org/10.1094/MPMI-12-10-0291

Truong HTH, Choi H, Cho MC, Lee HE, Kim JH (2011) Use of Cf-9 gene-based markers in marker-assisted selection to screen tomato cultivars with resistance to Cladosporium fulvum. Hortic Environ Biotechnol 52(2):204

Vakalounakis DJ (1991) Control of early blight of greenhouse tomato, caused by *Alternaria solani*, by inhibiting sporulation with ultraviolet-absorbing vinyl film. Plant Dis 75:795–797

Varshney RK, Tuberosa R (eds) (2007) Genomics-assisted crop improvement: genomics approaches and platforms, vol 1. Springer, Dordrecht, pp 1–12

Veliev RV, Sviridova IA (1987) Breeding tomato for resistance to corky root. Sel Semenovod USSR 2:26–27

Venkata Ramana C, Venkata Rao P, Prasada Rao RDVJ, Kumar SS, Reddy IP, Reddy YN (2010) Genetic analysis for Peanut bud necrosis virus (PBNV) resistance in tomato (*Lycopersicon esculentum* Mill.). In: III international symposium on tomato diseases, vol 914, pp 459–463

Veremis JC, Roberts PA (1996) Differentiation of *Meloidogyne incognita* and *M. arenaria* novel resistance phenotypes in Lycopersicon peruvianum and derived bridge-lines. Theor Appl Genet 93:960–967

Verlaan MG, Szinay D, Hutton SF, de Jong H, Kormelink R, Visser RGF et al (2011) Chromosomal rearrangements between tomato and *Solanum chilense* hamper mapping and breeding of the TYLCV resistance gene Ty-1. Plant J 68:1093–1103. https://doi.org/10.1111/j.1365-313X.2011.04762.x

Verlaan MG, Hutton SF, Ibrahem RM, Kormelink R, Visser RGF, Scott JW et al (2013) The tomato yellow leaf curl virus resistance genesTy-1and Ty-3are allelic and code for DFDGD-class RNA-dependent RNA polymerases. PLoS Genet 9:e1003399. https://doi.org/10.1371/journal.pgen.1003399

Vidavski F, Czosnek H, Gazit S, Levy D, Lapidot M (2008) Pyramiding of genes conferring resistance to tomato yellow leaf curl virus from different wild tomato species. Plant Breed 127:625–631. https://doi.org/10.1111/j.1439-0523.2008.01556.x

Villafana RT, Ramdass AC, Rampersad SN (2018) First report of Colletotrichum truncatum causing anthracnose in tomato fruit in Trinidad. Plant Dis 102(9):1857–1857

Vloutoglou I (1999) Evaluation of tomato cultivars and hybrids for resistance to *Alternaria solani* infection. Tests Agrochem Cultiv 20:48–49

Wang CX, Ying CQ (2008) Occurrence pattern and prevention methods for bacterial spot of tomato. Jilin Veg 4:50–51

Wang JF, Hanson P, Barnes JA (1998) Worldwide evaluation of an international set of resistance sources to bacterial wilt in tomato. In: Prior P, Allen C, Elphinstone J (eds) Bacterial wilt disease: molecular and ecological aspects. Springer, Berlin, pp 269–275

Wang GP, Lin MB, Wu DH (2004) Classical and molecular genetics of bacterial wilt resistance in tomato. Acta Hortic Sin 31:401–407

Wang H, Hutton SF, Robbins MD, Sim SC, Scott JW, Yang WC, Jones JB, Francis DM (2011) Molecular mapping of hypersensitive resistance from tomato cv. Hawaii 7981 to Xanthomonas perforans race T3. Phytopathology 101:1217–1223

Wang JF, Ho FI, Truong HTH, Hugan SM, Balatero CH, Dittapongpitch V, Hidayati N (2013a) Identification of major QTLs associated with stable resistance of tomato cultivar ‘Hawaii 7996’ to *Ralstonia solanacearum*. Euphytica 190:241–252

Wang Y, Yang W, Zhang W (2013b) Mapping of a heat-stable gene for resistance to southern root-knot nematode in *Solanum lycopersicum*. Plant Mol Biol Report 31(2):352–362

Wang YY, Chai AL, Li BJ (2015) Diversity of symptoms and integrative management of tomato bacterial speck. China Veg 11:82–84

Watanabe Y, Kishibayashi N, Motoyoshi F, Okada Y (1987) Characterization of tm-1 gene action on replication of common isolates and a resistance-breaking isolate of TMV. Virology 161:527–532

Wencai Yang, David M. Francis, Marker-assisted Selection for Combining Resistance to Bacterial Spot and Bacterial Speck in Tomato. Journal of the American Society for Horticultural Science 130 (5):716-721

Yadav OP, Dabbas MR (2012) Efficacy of fungicides in the management of early blight of tomato (Alternaria solani). Int J Plant Prot 5(2):413–416

Yaghoobi J, Kaloshian I, Wen Y, Williamson VM (1995) Mapping a new nematode resistance locus in *Lycopersicon peruvianum*. Theor Appl Genet 91:457–464

Yaghoobi J, Yates JL, Williamson VM (2005) Fine mapping of the nematode resistance gene Mi-3 in *Solanum peruvianum* and construction of a *S. lycopersicum* DNA contig spanning the locus. Mol Gen Genomics 274:60–69

Yamaguchi H, Ohnishi J, Saito A, Ohyama A, Nunome T, Miyatake K et al (2018) An NB-LRR gene, TYNBS1, is responsible for resistance mediated by the ty-2 Begomovirus resistance locus of tomato. Theor Appl Genet 131:1345–1362. https://doi.org/10.1007/s00122-018-3082-x

Yan Z, Pérez-de-Castro A, Díez MJ, Hutton SF, Visser RGF, Wolters A-MA, Bai Y, Li J (2018) Resistance to tomato yellow leaf curl virus in tomato germplasm. Front Plant Sci 9:1198. https://doi.org/10.3389/fpls.2018.01198

Yang W, Francis DM (2005) Marker-assisted selection for combining resistance to bacterial spot and bacterial speck in tomato. J Am Soc Hortic Sci 130:716–721

Yang WC, Francis DM (2007) Genetics and breeding for resistance to bacterial diseases in tomato: prospects for marker assisted se- lection. In: Razdan MK, Mattoo AK (eds) Genetic improvement of Solanaceous crops, 1. Tomato. Science Publishers, New Hamp-shire, pp 379–419

Yang X, Caro M, Hutton SF, Scott JW, Guo Y, Wang X et al (2014) Fine mapping of the tomato yellow leaf curl virus resistance gene ty-2 on chromosome11oftomato. Mol Breed 34:749–760. https://doi.org/10.1007/s11032-0140072-9

Yang H, Zhao T, Jiang J, Wang S, Wang A, Li J, Xu X (2017) Mapping and screening of the tomato *Stemphylium lycopersici* resistance gene, Sm, based on bulked segregant analysis in combination with genome resequencing. BMC Plant Biol 17(1):266

Yin XG, Wang XJ, Zhang Y, Pan GH, Yang QF (2005) Research progress on tomato bacterial wilt and resistance breeding in China. J Yunnan Agric Univ 20:163–167

Yordanov M, Stamova L, Stoyanova Z (1975) *Leveillula taurica* resistance in the tomato. Tomato Genet Coop Rep 25:24

Young ND, Tanksley SD (1989) RFLP analysis of the size of chromosomal segments retained around the tm-2 locus of tomato during backcross breeding. Theor Appl Genet 77:353–359

Yue SJ, Wu DH, Liang CY (1995) Studies on resistance heredity of bacterial wilt of tomato. J South China Agric Univ 16:91–95

Zamir D, Ekstein-Michelson I, Zakay Y, Navot N, Zeidan M, Sarfatti M et al (1994) Mapping and introgression of a tomato yellow leaf curl virus tolerance gene, ty-1. Theor Appl Genet 88:141–146. https://doi.org/10.1007/BF00225889

Zhang XM, Francis DM, Yang WC (2009) Evaluation of resistance to bacterial spot in varieties growing in China and marker-assisted selection. Acta Agric Boreali-Sin 24:183–187

Zhang C, Liu L, Zheng Z, Sun Y, Zhou L, Yang Y, Cheng F, Zhang Z, Wang X, Huang S, Xie B (2013) Fine mapping of the *Ph-3* gene conferring resistance to late blight (*Phytophthora infestans*) in tomato. Theor Appl Genet 126(10):2643–2653

Zhao TC, Sun FZ, Song WS (2001) Pathogen identification of bacterial speck of tomato. Acta Phytopathol Sin 31:37–42

Zheng Z, Nonomura T, Appiano M, Pavan S, Matsuda Y, Toyoda K et al (2013) Loss of function in Mlo orthologs reduces susceptibility of pepper and tomato to powdery mildew disease caused by *Leveillula taurica*. PLoS One 8(7):e70723. https://doi.org/10.1371/journal.pone.0070723

Chapter 3
Improvement of Onion Through Accelerated Approaches

Jiffinvir S. Khosa and Ajmer S. Dhatt

3.1 Introduction

Bulb onion (*Allium cepa* L.) is an ancient crop that is thought to have originated in Central Asia and has been cultivated for over 5000 years. Classical genetic and plant breeding approaches have been used to improve onion yield, quality and resistance against biotic and abiotic stresses. However, its biennial life cycle, cross-pollinated nature and high inbreeding depression have proved challenging for the characterization and breeding of improved traits. New technologies, notably next-generation sequencing, are providing researchers with the genomic resources and approaches to overcome these challenges. Using these genomic technologies, molecular markers are being rapidly developed and utilized for germplasm analysis and mapping in onion (Khosa et al. 2016). These new tools and knowledge are allowing the integration of molecular and conventional breeding to speed up onion improvement programmes. In this chapter, we outline recent progress in onion genomics and molecular genetics and prospects for enhancing onion yield and quality in the future.

Onion (*Allium cepa* L.) belongs to family Alliaceae and thought to have ancestors in Central-Asia, from where it spread all over the world (Brewster 2008). Onion is mainly grown for bulb and used almost daily in every home. Its main use is due to its aromatic and volatile oil allyl-propyl disulphide that impart cherish flavour to the food. Worldwide onion is grown on 49.6 lakh ha with 931.7 lakh MT production and 18.8 MT ha^{-1} productivity. In the last decade, 53.49% increase in area, 72.87% production and 12.63% productivity are the indicators of growth in onion all over the world. India ranked first in area (12.7 lakh ha) and second in production (215.64

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lakh MT) after the China. Onion earns foreign exchange more than Rs. 4000 crore annually, which is about 70% of the fresh vegetables and 50% of total vegetables and fruits (Kumar et al. 2015). Though onion production in India has increased by 300% in the last two decades, but the country requires 333.9 lakh MT onion by 2050 with present rate of consumption (6.7 kg capita^{-1}), export (9%), processing (6.75%) and losses (20%). This much production can be achieved by increasing the productivity to 28 MT ha^{-1} or by increasing the area under onion (Dhatt 2019). The horizontal expansion demands more land, which is a limiting factor. Therefore, focus on vertical increase through accelerated approaches of genetic improvement is needed in onions.

3.2 Shortening of Biennial Life Cycle

Onion has a biennial life cycle, in which bulb formation occurred in the first year followed by flowering in the second year for seed production. Onion breeders select desirable bulbs in first year based on their yield, shape, colour, quality and resistance against different diseases for seed production in the second year. As a result, onion breeding programmes is very slow and took 14–20 years to develop an improved cultivar (Brewster 2008; Khosa et al. 2016). Onion cultivars grown at low latitudes in tropical regions form bulbs at 10–12 h day length and have weak vernalization requirement as a result breeder can raise two crops in a year during rainy and winter season. Seed-to-seed approach can also be followed to complete the cycle in 1 year of tropical onion. The advancement in sowing of seeds by 2 months (in first week of September) resulted in induction of bolting in the overwinter crop under north Indian conditions (Dhatt, personal communication).

Long-day onion cultivars grown at high latitudes require 14–16 h day length to form bulb and show strong vernalization requirement to form flower (Brewster 2008). In order to accelerate onion breeding especially at high latitudes, the life cycle of bulb onion from biennial can be shorten by either accelerating bulb or seed production process. Onion responds to different environmental cues such as day length, temperature and light quality to form bulb and flowers (Brewster 2008). Recently, a speed breeding protocol has been developed for annual crop plants in which plant growth and development is accelerated by manipulating day length and light conditions to shorten the crop generation time (Watson et al. 2018). In speed breeding programme up to 6 generations per year can be achieved for spring wheat (*Triticum aestivum*), durum wheat (*T. durum*), barley (*Hordeum vulgare*), chickpea (*Cicer arietinum*) and pea (*Pisum sativum*), in comparison to two to three generations under normal glasshouse conditions (Watson et al. 2018).

Long days, lights with high far-red spectra and elevated temperature (25–30 ºC) can accelerate bulb formation in onions (Brewster 2008). Long-day onion cultivars are grown at 22 h photoperiod with high far-red light spectra that leads to bulb initiation within ~45 days, and mature bulbs can be harvested in ~80 days from date of sowing, whereas under field conditions, 5–6 months are needed for bulb formation

(Khosa and Havey, unpublished). This approach has a great potential to shorten the time for production of bulbs, and in future it would be interesting to induce flowering in these bulbs. Previous studies demonstrated that after harvesting onion bulbs undergo dormancy and require low temperature exposure for induction of flower stalk. The period of bulb dormancy depends upon genotype (~10 weeks) but in general can be reduced by storing bulbs at moderate temperatures (15–20 °C) or use of dormancy breaking chemicals (D'Angelo and Goldman 2018, 2019). Once dormancy is broken, bulbs should be store at low temperature 4–10 °C for 12 weeks to fulfil vernalization requirement and grow at 20 °C in long-day photoperiod to induce flowering in long-day onions (Brewster 2008; D'Angelo and Goldman 2019).

3.3 Production of Doubled Haploids

Development of true breeding lines is a daunting task in onion due to biennial nature, severe inbreeding depression and out-crossing behaviour. Under such situation, exploitation of doubled haploid (DH) approach has great potential in accelerating the basic and applied research in onion (Brewster 2008; Khosa et al. 2016). Plants with gametophytic (single set) chromosome number in their sporophyte are termed as haploids. Spontaneous or induced chromosome duplication of a haploids resulted in the development of doubled haploid (DH) plant. The first spontaneous haploid was observed in *Datura stramonium* L. (Blackslee et al. 1922), which paved the ways for many landmark studies on molecular, genetic and crop improvement. The first haploid generated through anther culture in *Datura innoxia* initiated extensive research on androgenesis (Guha and Maheshwari 1964), which follows haploid production through wide crossing in barley and tobacco (Kasha and Kao 1970; Burk et al. 1979). The androgenesis found highly successful in families, Solanaceae, Gramineae and Brassicaceae, but not in all the angiosperms (Forster et al. 2007). Haploids offer various advantages other than generating completely homozygous lines from a highly heterozygous parent in a single step. The genotypes with complete homozygosity have increased heritability of quantitative characters, which further increase the selection efficiency (Chen et al. 2010). The presence of single set of chromosomes eases the isolation of mutants with recessive gene (Hermsen and Ramana 1981). Double haploids can increase the efficiency of genome mapping by generating valuable information regarding the location of genes and QTLs that regulate economically important traits (Winzeler et al. 1987; Khush and Virmani 1996). In diploid, a genotype constitutes of three types of alleles ¼ AA, ½ Aa and ¼ aa for a trait, whereas in haploid the allelic frequency is ½ AA and ½ aa. The probability of achieving desirable trait (AA or aa) in a diploid is high in comparison to haploid counterpart. For 'n' segregating loci, the probability of obtaining desirable trait can be quantified as $(½\ n)^2$ and $(¼\ n)^2$ for haploid and diploid, respectively. The efficiency would increase as the number of segregating genes would increase in case of haploids.

3.3.1 Methods of DH Induction

Haploids are available spontaneous in nature or induced artificially and doubled haploid through duplication of haploid chromosome. Spontaneous occurrence of haploids in nature can be attributed to parthenogenesis or apogamy but occurs in very low frequency, therefore induced through artificial methods. It can be done in vivo (pollination with irradiated pollen, wide hybridization, chromosome elimination, use of inducer lines, pollination with pollen from a triploid plant, selection of twin seedlings) and in vitro (gametic embryogenesis). The gametic embryogenesis leads to formation of haploids due to meiotic segregation that leads to formation of gamete followed by its regeneration. In this, gametophyte experience irreversible switch from gametophytic phase of development to the sporophytic phase. The main techniques for initiation of gametic embryogenesis are in vitro gynogenesis (ovule and ovary culture) and androgenesis (anther or isolated microspore culture) (Forster and Thomas 2005; Germana 2010; Galazka and Niemirowicz-szczytt 2013).

3.3.1.1 Androgenesis

Androgenesis is the process in which microspores or young pollen grains are switched from their normal pollen development to an embryogenic pathway (Maraschin et al. 2005). The process of embryogenesis in pollen is induced through anther or isolated microspore culture. The microspore culture is advantageous to investigate cellular, biochemical, physiological and molecular processes implicated in pollen embryogenesis, but high expertise and better instruments are required (Nitsch 1977; Reinert and Bajaj 1977). The haploid production via anther culture is an in vitro process, in which microspore cells with haploid genomes develop into an embryo-like structure on culture medium, which further develops into haploid plantlet (Jauhar et al. 2009). Androgenesis has been exploited in cabbage, potato, brinjal and capsicum (Asakaviciute 2008; Başay and Ellialtıoğlu 2013; Olszewska et al. 2014), but in onion success was not achieved due to non-dehiscence of anthers and degeneration and failure in development of plantlets (Campion et al. 1984; Keller 1990; Martinez et al. 2000; Thakur 2016).

3.3.1.2 Gynogenesis

Gynogenesis is haploid regeneration from un-pollinated female gametophyte (ovule or ovary). It is potential alternative for haploid induction in species recalcitrant for androgenesis or in cases when high level of albino plants regenerated or in species characterized with male sterility or dioecy (Thomas et al. 2000; Bhat and Murthy 2007). Haploid induction through ovary or megaspore culture is a megaspore embryogenesis, where unfertilized eggs or other embryo sac cells developed into

haploid plantlets through parthenogenesis (Yang and Zhou 1982). It is the most efficient technique for haploid production in sugar beet, cucumber, zucchini, summer squash, melon and sweet potato (Kobayashi et al. 1993; Bohanec et al. 1995; Gemes et al. 1997; Ficcadenti et al. 1999; Gurel et al. 2000; Shalaby 2007; Moqbeli et al. 2013). In onion, investigations on gynogenesis were initiated upon failure of plantlet induction from the anthers (Campion et al. 1984; Keller 1990). Guha and Johri (1966) made first attempt to generate haploids from onion ovaries but were not successful due to plantlet emergence in control or pollinated ovaries only. The first report on haploid induction through gynogenesis utilizing un-pollinated ovules, ovaries and flower buds in onion was given by Keller (1990) and Campion and Alloni (1990). Subsequently, extensive work was done to develop and standardize the protocols for increasing the efficiency of gynogenesis in onion. Basic steps involved in haploid induction through gynogenesis have been described by Chen et al. (2010). The success of gynogenesis is influenced by genotype, geographic origin, explant, media composition and chromosome doubling in onion and discussed as below:

3.3.1.2.1 Genotype

Genetic makeup of the donor plant has been recognized as one of the most important factors that influences the haploid induction ability (Geoffriau et al. 1997; King et al. 1998; Bohanec and Jakse 1999; Martinez et al. 2000; Michalik et al. 2000; Ebrahimi and Zamani; 2009). Bohanec et al. (1995) observed genotypic response from 5.6% to 7.6% for ovary and 1% for ovule culture in onion. The regenerants from inbreds were more vigorous, but embryo quality was better with the synthetics. The genotypic effect on gynogenic performance during second cycle of gynogenesis was also observed (Javornik et al. 1998). The second cycle gynogenesis (SCG) line G126 (118.3%) showed significantly high induction frequency than G198 (2.35%), G3 (0.26%) and donor line (3.5–5.1%) and outperformed for embryo and plant yield also. It suggests that enhanced haploid induction frequency can be achieved with the selection for high gynogenic response among the genotypes. Based upon gynogenic response for haploid induction, Bohanec and Jakse (1999) divided 39 accessions into low (0–1%), medium (1–7.9%) and high (>8%) frequency groups, with share of 39, 46 and 15% accessions, respectively. The marked influence on haploid induction of individual donor plants within same accession indicated the presence of strong genetic effect for the induction rate (Bohanec et al. 1995). It was also observed that intermediate variety Torrentina has higher (9.5%) embryo generation capacity than long-day (2.8%) type Valcatorce INTA (Martinez et al. 2000). The embryos able to develop into whole plants including both haploid and diploid were reported 82% in Torrentina and 33% in Valcatorce INTA. The difference in the performance of two varieties for parameters under consideration indicated the strong role of genetic makeup of the genotype.

In an interspecific cross of *A. cepa* and *A. roylei*, low or no gynogenic response was observed in F_1 and F_2 generations (Alan et al. 2003), whereas it was better in

BC_1F_1 (0.1%) and BC_2F_2 (0.5%) generations. Enhanced performance of BC_1F_2 populations could be attributed to the segregation of *A. Cepa* alleles for better response to the culture. A substantial enhancement in performance of *A. roylei*-derived plants with the advanced generations indicated the dominance of genetic effects on development of gynogenic plants. Jakse et al. (2010) compared the performance of original donors (inbreds, populations and hybrids) and selfed progenies of 21 doubled haploid lines for various parameters, where 18 showed decrease (mean − 0.205 per flower plated) and 3 increase (mean 0.057 per flower plated) response than the donors. It indicates that gynogenic regeneration has polygenic control. Pollen viability of DH lines exhibited 0–96% variation and did not show correlation for high pollen viability and gynogenic response, as one of the lines has only 2% gynogenic response, but pollen viability was 83%.

3.3.1.2.2 Geographic Background of Genotype

The geographic adaptability of a genotype also influenced the gynogenic ability in onion. Geoffriau et al. (1997) investigated 22 varieties of different genetic structures from Northern Europe, Southern Europe, Eastern Europe and the USA. Among all, genotypes from the USA were significantly superior for regeneration and survival rates, whereas North European were better among the European group. However, sprouting time was not affected by the origin of varieties. Bohanec and Jakse (1999) also studied the gynogenic ability of individual donor plants collected from different geographical regions of Britain, Spain, France, Portugal, Slovenia, Macedonia, Turkey, Soviet Union, Japan and the USA. Accessions from the USA were most responsive (highest 22.6%, average 8%) followed by European (highest 3.8%, average 1.7%) and Japanese (highest 4.4%, average 0.9%). Indian germplasm also shows genetic variation for gynogenic response as it varies from 0.9% to 4.5% in 14 varieties (Anandhan et al. 2014).

3.3.1.2.3 Pretreatment

Stress treatments like cold or heat shock, dark period, media starvation, etc. affect the rate of embryogenesis by triggering the change from gametophytic path of development to the sporophytic phase. Pretreatment can be applied to various explants like intact flowers, inflorescence or isolated ovules. The optimization of such treatments in terms of explants, type of treatment, duration and finally the regeneration efficiency is prerequisite before its deployment (Chen et al. 2010). Use of flower buds from donor plants maintained at 15 °C showed tenfold increase in gynogenesis (Puddephat et al. 1999). However, performance of plants grown in growth chamber and field conditions for gynogenic parameters was found non-significant (Fayos et al. 2015). Surface sterilization of explants enhances the gynogenic efficiency in onion. Treatment of flower buds before anthesis with different concentrations of sodium hypochlorite (3–10%) along with few drops of Tween 20

for 10–15 min followed by rinsing in sterile water found promising (Campion and Alloni 1990; Keller 1990; Bohanec et al. 1995; Martinez et al. 2000; Ponce et al. 2006; Ebrahimi and Zamani 2009). Use of ethanol and dichloroisocyanuric acid disodium salt for sterilization has also been reported in onion (Geoffriau et al. 1997; Bohanec and Jakse 1999; Alan et al. 2003; Jakse et al. 2003, 2010).

3.3.1.2.4 Nature of Explant

In onion, haploid induction through androgenesis failed to generate results due to degeneration of anthers. Then increase in growth and size of ovaries encouraged the shift to gynogenesis (Guha and Johri 1966; Campion et al. 1984). Before anthesis, flower buds at maximum elongation correspond with early binucleate stage of microspores considered suitable for extraction of ovaries and ovules for inoculation (Campion and Alloni 1990; Keller 1990). Further, two-step culture procedure was followed, where pre-culture of flower was followed by ovary and ovule isolation (Bohanec et al. 1995). The frequency of regeneration found much higher from the ovaries (0.81%) than the ovules (0.02%). However, ovary culture was time and labour efficient than ovule culture. Geoffriau et al. (1997) suggested flower buds culture without the subculture as a practical and most efficient method for large number of accessions. The efficiency of single-step flower culture was three time higher than two-step ovary culture in onion (Bohanec and Jakse 1999). The whole flower bud culture leads to formation of undesirable callus in some cases; however this was not observed in ovary culture. The possible reason for the difference in behaviour of flower buds and ovaries could be the removal of nectaries during ovary excision and culture. Thus being labour and time efficient, implementation of flower bud culture method was suggested, whereas ovary culture can be employed, where flower bud cultures are accompanied with extreme callusing.

Though ideal size of flower bud is genotype specific, but medium and large size flower buds found to be most efficient for production of haploids via gynogenesis (Michalik et al. 2000). At the time of inoculation, small flower buds (2.3–3.0 mm) had 88% megaspore mother cells, medium (3.1–3.7 mm) 46% four-nucleate and 40% embryo sac, whereas large (3.8–4.4 mm) enclosed only mature embryo sac. After 14 days of culture, all the buds irrespective of size contained only mature embryo sacs. The frequency of embryos after 2 and 3 week of culture was 8.5 and 2.2%, respectively, though final embryo yield was similar (Musial et al. 2001, 2005). Culturing of flower buds 3–5 days before flowering corresponds with ideal bud size of 3–5 mm (Alan et al. 2004). For large-scale production of haploids through gynogenesis, follow-up of single-step culture of flower buds (without subculture) is considered as the most efficient approach in onion (Bohanec et al. 2003).

3.3.1.2.5 Media Composition

Haploid induction depends predominantly on genotype and media composition in onion. Regeneration of onion flower buds in one- or two-step culture approach on basal MS or BDS media in various combination of vitamins, growth regulators and macro- and microelements has been studied (Campion et al. 1992; Bohanec et al. 1995; Jakse et al. 1996; Martinez et al. 2000; Michalik et al. 2000). Use of growth regulators for fortification of various inductions and regeneration media in onion has been summarized in Table 3.1.

3.3.1.3 Chromosome Doubling

Spontaneous doubling in diploid species is of prime significance, as it offers more stability than haploids treated with antimitotic agents. However, spontaneous doubling that occurs in shoot apices (Jakse et al. 1996) and roots (Campion and Azzimonti 1988) is rare and with low rate in onion (Bohanec and Jakse 1999; Martinez et al. 2000; Bohanec 2002). Thus, to exploit the homozygosity of haploid lines, it is imperative to double their genome with duplication agents and restore the fertility (Jakse et al. 1996). The antimitotic agents can be applied to small onion bulbs (Campion et al. 1995), embryos (Grzebelus and Adamus 2004) and young plantlets during micropropagation (Avlan et al. 2004, 2007). The most commonly utilized antimitotic agents are colchicine, amiprophos-methyl (APM), oryzalin and trifluralin. The genetic structure of regenerants as haploid, diploid, triploid, tetraploid or mixoploid is considered authenticated with the flow cytometry analysis.

3.4 Marker-Assisted Selection

3.4.1 Development of Male Sterile Lines

Hybrid breeding in onion triggered after discovery of cytoplasmic male sterility by Jones and Emsweller (1936). Hybrids have the advantage of high yield, uniform bulb size, colour and maturity, longer storage and resistance to insect-pest and diseases (Brewster 2008). These traits made F_1 hybrids popular among growers and seed companies exploited it as an entrepreneurship. The market share of onion hybrids in the USA, Japan and Europe is 81, 71 and 50%, respectively. Hybrid development demands three three-line systems, viz. male sterile (A), maintainer (B) and pollinator (C) lines. Onion is a cross-pollinated, biennial and photo-thermosensitive crop; therefore development of parental lines is a daunting task. Male sterility is caused by interaction of cytoplasmic and nuclear alleles and is of two types in onion, viz. CMS-S and CMS-T. Male sterility due to CMS-S cytoplasm is maintained by single nuclear locus *Ms* (Jones and Clark 1943) and CMS-T by

Table 3.1 Growth regulators used for DH induction and regeneration in onion

Growth regulators	Concentration (mg/l)	References
Induction medium		
2,4-D + BAP	2 + 2	Muren (1989), Campion et al. (1992), Bohanec et al. (1995), Jakse et al. (1996), Geoffriau et al. (1997)
IBA + BAP	2.03 + 1.25	Keller (1990)
Tiba+aba	0.2 + 1	Campion and Alloni (1990)
2,4-D + BAP; NAA + BAP	2 + 0.12,1 + 0.12	Campion et al. (1992)
PAA + BAP	100 + 2	Jakse et al. (1996)
2,4-D + BAP	2 + 2	Jakseet al. (2010), Bohanec and Jakse (1999)
2,4D + BA+ putrescine + spermidine	0.01 mM + 0.01 mM + 0.2 mM + 0.1 mM	Ebrahimi and Zamani (2009)
BA+2,4-D	2 + 2	Puddephat et al. (1999)
Regeneration medium (mg/l)		
2iP+ IAA, 2iP+ NAA	2 + 1.5, 2 + 1	Puddephat et al. (1999)
IBA	1	Muren (1989)
NAA + 2,4D + IAA + BAP + 2iP	1 + 0.4 + 1,5 + 2 + 2	Campion and Alloni (1990)
TDZ	2, 2	Bohanec et al. (1995), Jakse et al. (1996)
NAA + GA3; NAA + BAP	1 + 1,1 + 2	Campion and Alloni (1990)
NAA+ 2iP	1 + 2	Campion and Alloni (1990), Bohanec et al. (1995)
IAA + BAP; IAA + 2iP;IAA + GA3;2,4D + BAP; 2,4D + 2iP; NAA + 2iP + ABA;NAA + BAP + GA3;BAP + GA3 + TIBA	1.5 + 2; 1.5 + 2; 1,5 + 1; 0,4 + 2; 0.4 + 2;1 + 2 + 1; 1 + 2 + 1;2 + 1 + 0.2	Campion and Alloni (1990)
IBA + BAP + GA3	2 + 0.1, 2 + 3.5	Keller (1990), Campion et al. (1992)

three independent loci (Schweisguth 1973). CMS-S system has simple inheritance, stable expression and used worldwide. Through conventional approach, 4–6 years are required to establish S or N cytoplasm and 10–12 years for transferring in desirable genetic backgrounds. A wide range of molecular markers have been developed to differentiate different cytotypes (N; S and T; Table 3.2); recently it has been demonstrated that HRM (high-resolution melting) assay can distinguish N, S and T cytoplasm; these markers will allow researchers to screen large number of plants as HRM is a sensitive, fast and cost-effective assay in comparison traditional PCR genotyping methods (Kim and Kim 2019). A maintainer line is required to sexually propagate male sterile lines and development of maintainer lines through conventional crossing took 8–10 years to overcome these limitation molecular markers linked with *Ms* locus which have been developed to follow marker-assisted selection for *Ms* locus followed by other PCR markers (Gökçe and Havey 2002; Bang et al. 2011, Havey 2013; Kim 2014, Huo et al. 2015; Khar and Saini 2016). Use of high-resolution melting markers for *Ms* locus can also be used to isolate restorer plants (Kim and Kim 2019). The development of male sterile and maintainer lines through conventional and molecular approaches has been depicted in Fig. 3.1a–c.

Using molecular markers we at Punjab Agricultural University, Ludhiana, have developed seven CMS lines from adapted populations of tropical onion (Dhatt 2016). These lines have been derived from cultivated varieties or advance populations, namely, Punjab Naroya (light red), Punjab Selection (light red), P-266 (dark red), Punjab White (white), PR-305 (red), PR-853 (red) and PYO-102 and named as D-97A&B, D-121 A&B, D-266A&B, D-30 A&B, D-305A&B, D-853 A&B and D-102 A&B, respectively. This approach has accelerated the heterosis breeding programme and enabled to develop CMS lines and release of first F_1 hybrid POH-1 in a time span of 7 years (Fig. 3.2). POH-1 gives comparable yield (554 q/ha) with exotic short day hybrids T-821 (Takki seeds) and Ceylon (Seminis) but has threefold longer storage life under naturally ventilated conditions (Dhatt, unpublished data).

Table 3.2 List of molecular markers developed to identify different cytoplasm in onion populations

Markers type	Cytotype differentiation	References
RFLPs	N and S	Courcel et al. (1989), Holford et al. (1991), Satoh et al. (1993), Havey (1993)
SCAR	N and S	Havey (1995)
PCR	N and S	Satoh (1998), Lily and Havey (2001)
PCR-RFLP	N, S and T	Cho et al. (2006)
PCR	N, S and T	Engelke et al. (2003), Kim et al. (2009), Kim and Yoon (2010)
SNPs	N and S	Kohn et al. (2013)
SNPs	N, S and T	Kim and Kim (2019)

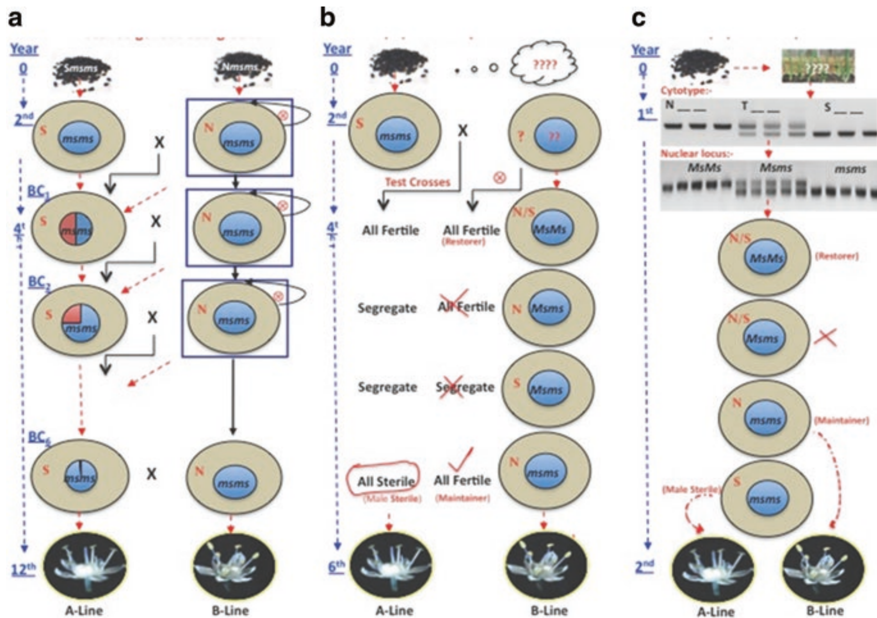

Fig. 3.1 Scheme for development of male sterile lines through conventional and molecular approach. (**a**) Transfer of male sterility in desired genetic background. (**b**) Development of male sterile lines from OP population through conventional approach. (**c**) Development of male sterile lines from OP population using molecular markers

3.4.2 Quality Traits

Quality in onion is determined from appearance (size, shape and colour), shelf life and biochemical compounds (pungency and soluble solids). Consumer preference for shape and colour varied all over the world. Bulbs may be white, red, brown or yellow; round, pyriform or flat; and soft or firm. Higher soluble solids are important for dehydrating industry to produce onion chips or powder. The amounts of s-alkyl cysteine sulfoxide precursors and the enzyme alliinase contribute to the content of volatile sulphur compounds for pungency. High pungency is preferred in Indian subcontinent and low in the USA and Europe. All these quality parameters have genetic base and can be improved through selection. Generally, bulb diameter and weight show low, and bulb height and shape index (polar: equator diameter) high heritability. Therefore, selection response is expected from bulb height than the size. Onion populations show marked differences in storage, firmness, pungency and dry matter content.

Bulb colour important to classify cultivars in onion is controlled by multiple loci (Reiman 1931; Clarke et al. 1944; El-Shafie and Davis 1967). The *I*-locus at which a dominant allele (*I*-) inhibits the colour development and bulb remains white. The expression of red, yellow or chartreuse pigments requires that the plant be

Fig. 3.2 Heterosis breeding in onion. (**a**) Male sterile flower. (**b**) Test crossing in pairs. (**c**) Maintenance of A&B lines. (**d**) F1 seed production. (**e**) F1 hybrid POH-1

homozygous recessive at *I*-locus. The *G*-locus conditions the production of coloured bulbs; all *iicc* bulbs are white. Plants with a dominant allele at the *G*-locus and homozygous recessive (*rr*) at the *R* locus are yellow to brown in colour. Red bulbs are conditioned by dominant alleles at both the '*G* and *R* loci (*iiG-R-*). Golden colour conditioned by a dominant and chartreuse by homozygous recessive allele at the *G*-locus. The complementary factors conditioning a light red bulb in progeny from a cross of two yellow bulbs have also been observed. An additional *L*-locus conditioned the red pigment, when the plant has the genotype *iiG- rrG- L-*. The epistatic interaction of these five loci implies close relation in pigment biosynthesis pathways, and red colour attributes in anthocyanin derivatives in onion (Fuleki 1971; Fossen et al. 1996). Anthocyanins are the largest subclass of flavonoids, particularly quercetin in red scales of onion (Terahara et al. 1994; Tsushida and Suzuki 1995; Fossen et al. 1996; Rhodes and Price 1996; Donner et al. 1997; Chu et al. 2000; Bahorun et al. 2004). Onion bulbs store fructans, fructose polysaccharides formed by the cumulative addition of a fructosyl group to a sucrose molecule. Fructans enhance the value of onion as a functional food by conferring prebiotic properties and lowering blood lipid and insulin levels (Ritsema and Smeekens 2003). Dry matter indirectly measured as soluble solid content (SSC) is associated with higher total fructan content, higher degree of polymerisation and lower content of fructose, glucose and sucrose (Darbyshire and Henry 1979). A high heritability

of SSC and carbohydrates has been observed but also showed significant correlation with bulb size and pungency (McCollum 1968; Kadams and Nwasike 1986; Lin et al. 1995; Simon 1995; Galmarini et al. 2001). However, substantial improvement through conventional approach has been made, but complex segregation of several genes for all the quality parameters makes the selection process slow and tedious. Molecular markers linked to bulb colour (Kim et al. 2004a, b, 2005a, b, 2006; Khar et al. 2008; Duangjit et al. 2014), flavonoids and soluble solids have been reported (Galmarini et al. 2001; Masuzaki et al. 2006; McCallum et al. 2006, 2007; Raines et al. 2009), which can be exploited to hasten the improvement programme of onion.

3.5 Genomic Resources for Marker Development and Gene Discovery

Bulb onion has very large (~16 Gbp) genome size and small research community, which restricts the usage of NGS to sequence whole genome. However, to aid marker development and gene discovery in bulb onion, different laboratories followed RNA sequencing approach and generated transcriptome datasets. The early transcriptome datasets have been developing using Sanger sequencing, but in recent years next-generation sequencing platforms have been extensively utilized (Khosa et al. 2016). In bulb onion transcriptome datasets have been used for marker development, linkage map construction and gene discovery (Khosa et al. 2016).

Bulked segregant RNA-seq (BSR-Seq) is a beneficial approach to identify polymorphic markers and differentially expressed genes in two contrasting bulks (Liu et al. 2012). In this approach next-generation sequencing combines with BSA (bulk segregant analysis) to identify candidate genes involved in different processes; in bulb onion BSR-Seq has been utilized to identify markers linked with fertility restoration and bulb colour (Michelmore et al. 1991; Liu et al. 2012; Kim et al. 2015; Baek et al. 2017). In order to discover genes responsible for fertility restoration, RNA-Seq was performed on bulk floral samples of male fertile and sterile plants. A total of 14 contigs containing homozygous SNPs showed perfect linkage disequilibrium (LD) with the *Ms* locus in diverse genetic backgrounds. However, based on the annotated function, high SNP frequency and presence of putative critical amino acid changes, *AcPMS1* (regulate DNA mismatch repair, MMR) regarded as candidate gene responsible for fertility restoration, but this study lacks functional data too, and the role of *AcPMS1* in fertility is not known (Kim et al. 2015).

3.6 Conclusion

Over the years, significant progress has been made in onion physiological and genetic studies to augment breeding programmes. The development of doubled haploids and molecular markers linked with trait of interest has allowed breeders to hasten onion breeding programme in a cost and time-effective manner. In coming years, it would be desirable to develop speed breeding protocol to annualize the life cycle of onion. Onion research community should focus on developing publically available reference genome assembly from doubled haploids to aid gene discovery and marker development. Recent advances in the CRISPR/Cas genome editing tools offer great potential for targeted modifications of genes to engineer valuable traits in commercially grown onion cultivars and inbred lines to overcome long generation time.

References

Alan AR, Brants A, Cobb ED, Goldschmied PA, Mutschler MA, Earle ED (2004) Fecund gynogenic lines from onion (*Allium cepa* L.) breeding materials. Plant Sci 167:1055–1066

Alan AR, Lim W, Mutschler MA, Earle ED (2007) Complementary strategies for ploidy manipulations in gynogenic onion (*Allium cepa* L.). Plant Sci 173:25–31

Anandhan S, Chavan AA, Gopal J, Mote SR, Shelke PV, Lawande KE (2014) Variation in gynogenic potential for haploid induction in Indian shortday onions. Ind J Genet 74(4):526–528

Asakaviciute R (2008) Androgenesis in anther culture of Lithuanian spring barley (*Hordeum vulgare* L.) and potato (*Solanum tuberosum* L.) cultivars. Turk J Biol 32:155–160

Baek G, Kim CW, Kim S (2017) Development of a molecular marker tightly linked to the C locus conferring a white bulb color in onion (*Allium cepa* L.) using bulked segregant analysis and RNA-Seq. Mol Breed 37:94

Bahorun T, Luximon RA, Crozier A, Aruoma OI (2004) Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. J Sci Food Agric 84:1553–1561

Bang H, Cho DY, Yoo KS, Yoon MK, Patil BS, Kim S (2011) Development of simple PCR-based markers linked to Ms locus, a restorer-of-fertility gene in onion (*Allium cepa* L.). Euphytica 179:439–449

Başay S, Ellialtıoğlu SS (2013) Effect of genotypical factors on the effectiveness of anther culture in eggplant (*Solanum melongena* L.). Turk J Biol 37:499–505

Bhat JG, Murthy HN (2007) Factors affecting in vitro gynogenic haploid production in Niger (*Guizotia abyssinica* (L. f.) Cass.). Plant Growth Regul 52:241–248

Blackslee AF, Belling J, Farnham ME, Bergner AD (1922) A haploid mutant in the jimson weed, Datura stramonium. Science 55:646–647

Bohanec B (2002) Doubled haploid onions. In: Rabinowitch H, Currah L (eds) *Allium* crop science: recent advances. CABI Publishing, Wallingford, pp 145–148

Bohanec B, Jakse M (1999) Variations in gynogenic response among long-day onion (*Allium cepa* L.) accessions. Plant Cell Rep 18:737–742

Bohanec B, Jakse M, Ihan A, Javornik B (1995) Studies of gynogenesis in onion (*Allium cepa* L.): induction procedures and genetic analysis of regenerants. Plant Sci 104:215–224

Bohanec B, Jakse M, Havey MJ (2003) Genetic analyses of gynogenetic haploid production in onion. J Am Soc Hortic Sci 128:571–574

Brewster JL (2008) Onions and other vegetable alliums. CABI, Oxfordshire
Burk LG, Gerstel DU, Wernsman EA (1979) Maternal haploids of *Nicotiana tabacum* L. from seed. Science 206:585
Campion B, Alloni C (1990) Induction of haploid plants in onion (*Allium cepa* L.) by in vitro culture of unpollinated ovules. Plant Cell Tissue Organ Cult 20:1–6
Campion B, Azzimonti MT (1988) Evolution of ploidy level in haploid plants of onion (*Allium cepa* L.) obtained through in vitro gynogenesis. In: Eucarpia 4th *Allium* symposium, Institute of Horticultural Research, Warwick, pp 85–89
Campion B, Falavigna A, Schiavi M (1984) Efforts for in vitro androgenesis in onion (*Allium cepa* L.). In: Proceedings of 3rd allium symposium eucarpia, p 110
Campion B, Azzimonti MT, Vicini E, Schiayi M, Falavigna A (1992) Advances in haploid induction in onion (*Allium cepa* L.) through in vitro gynogenesis. Plant Sci 86:97–104
Campion B, Perri E, Azzimonti MT, Vicini E, Schiavi M (1995) Spontaneous and induced chromosome doubling in gynogenic lines of onion (*Allium cepa* L.). Plant Breed 114:243–246
Chen JF, Cui L, Malik AA, Mbira KG (2010) In vitro haploid and dihaploid production via unfertilized ovule culture. Plant Cell Tissue Organ Cult 104:311–319
Cho K, Yang TJ, Hong SY, Kwon YS, Woo JG, Park HG (2006) Determination of cytoplasmic male sterile factors in onion plants (*Allium cepa* L.) using PCR-RFLP and SNP markers. Mol Cell 21(3):411–417
Chu YH, Chang CL, Hsu HF (2000) Flavonoid content of several vegetables and their antioxidant activity. J Sci Food Agr 80:561–566
Clarke AE, Jones HA, Little TM (1944) Inheritance of bulb color in the onion. Genetics 29:569–575
D'Angelo CJ, Goldman IL (2018) Temporal aspects of Vernalization and flowering in long-day storage onion. J Am Soc Hortic Sci 143(6):446–453
Darbyshire B, Henry RJ (1979) The association of fructans with high percentage dry matter weight in onion cultivars suitable for dehydrating. J Sci Food Agric 30:1035–1038
D'Angelo CJ, Goldman IL (2019) Breaking onion bulb Endodormancy with hydrogen peroxide. Hortic Sci 54(10):1694–1702
de Courcel AG, Veder F, Boussac JM (1989) DNA polymorphism in *Allium cepa* cytoplasms and its implications concerning the origin of onions. Theor Appl Genet 77:793
Dhatt AS (2016) Development of technologies for increasing productivity and availability of onion. In: Chadha KL et al (eds) doubling farmers income through horticulture. Astral International Pvt. Ltd., New Delhi, pp 154–163
Dhatt AS (2019) Potential and constraints of F_1 hybrids in tropical onion. In: Proceedings: international symposium on edible alliums: challenges and opportunities held during Feb 9–12 at YASHADA, Pune, pp 48–57
Donner H, Gao L, Mazza G (1997) Separation and characterization of simple malonylated anthocyanins in red onions *Allium cepa* L. Food Res Int 30:637–647
Duangjit J, Welsh K, Wise M, Bohanec B, Havey MJ (2014) Genetic analyses of anthocyanin concentrations and intensity of red bulb color among segregating haploid progenies of onion. Mol Breed 34(1):75–85
Ebrahimi R, Zamani Z (2009) Effect of polyamines on in vitro Gynogenesis of onion (*Allium cepa* L.). Am Eurasian J Sustain Agric 3:71–74
El-Shafie MW, Davis GN (1967) Inheritance of bulb color in the onion (*Allium cepa* L.). Hilgardia 38(17):607–622
Engelke T, Terefe D, Tatlioglu T (2003) A PCR-based marker system monitoring CMS (S), (T) and N cytoplasm in the onion (*Allium cepa* L). Theor Appl Genet 107:162–167
Fayos O, Valles MP, Garces-Claver A, Mallor C, Castillo AM (2015) Doubled haploid production from Spanish onion (*Allium cepa* L.) germplasm: embryogenesis induction, plant regeneration and chromosome doubling. Front Plant Sci 6:1–11
Ficcadenti N, Sestili S, Annibali S (1999) In vitro gynogenesis to induce haploid plants in melon (*Cucumis melo* L.). J Genet Breed 53:255–257

Forster BP, Thomas WTB (2005) Doubled haploids in genetics and plant breeding. Plant Breed Rev 25:57–88

Forster BP, Heberle-Bors E, Kasha KJ, Touraev A (2007) The resurgence of haploids in higher plants. Trends Plant Sci 12:368–375

Fossen T, Andersen OM, Ovstedal DO, Pedersen AT, Raknes A (1996) Characteristic anthocyanin pattern from onions and other *Allium* spp. J Food Sci 61:703–706

Fuleki A (1971) Anthocy anins in red onion, *Allium cepa*. J Food Sci 36(1):101–104

Galazka J, Niemirowicz-Szczytt K (2013) Review of research on haploid production in cucumber and other cucurbits. Folia Hortic 25:67–78

Galmarini CR, Goldman IL, Havey MJ (2001) Genetic analyses of correlated solids, flavor, and health-enhancing traits in onion (*Allium cepa* L.). Mol Gen Genomics 265:543–551

Gemes JA, Venczel G, Balogh P (1997) Haploid plant induction in zucchini (*Cucurbita pepo* L. convar. Giromontiina DUCH) and in cucumber (Cucumis sativus L.) lines through in vitro gynogenesis. Acta Hortic 447:623–624

Geoffriau E, Kahane R, Rancillac M (1997) Variation of gynogenesis ability in onion (*Allium cepa* L.). Euphytica 94:37–44

Germana MA (2010) Anther culture for haploid and doubled haploid production. Plant Cell Tissue Organ Cult 104:283–300

Gökçe AF, Havey MJ (2002) Linkage equilibrium among tightly linked RFLPs and the *Ms* locus in open-pollinated onion populations. J Am Soc Hortic Sci 127:944–946

Grzebelus E, Adamus A (2004) Effect of anti-mitotic agents on development and genome doubling of gynogenic onion (*Allium cepa* L.) embryos. Plant Sci 167:569–574

Guha S, Johri BM (1966) In vitro development of ovary and ovule of *Allium cepa* L. Phytomorphology 16:353–364

Guha S, Maheshwari SC (1964) In vitro production of embryos from anthers of Datura. Nature 204:497

Gurel S, Gurel E, Kaya Z (2000) Doubled haploid plant production from unpollinated ovules of sugar beet (*Beta vulgaris* L.). Plant Cell Rep 19:1155–1159

Havey MJ (1993) A putative donor of S-cytoplasm and its distribution among open-pollinated populations of onion. Theor Appl Genet 86:128–134

Havey MJ (1995) Identification of cytoplasms using the polymerase chain reaction to aid in the extraction of maintainer lines from open-pollinated populations of onion. Theor Appl Genet 90:263–268

Havey MJ (2013) Single nucleotide polymorphisms in linkage disequilibrium with the male-fertility restoration (*Ms*) locus in onion. J Am Soc Hortic Sci 119:90–93

Hermsen JG, Ramana MA (1981) Haploidy and plant breeding. Philos Trans R Soc Lond B 292:499–507

Holford P, Croft JH, Newbury HJ (1991) Differences between and possible origins of the cytoplasms found in fertile and male sterile onions (*Allium cepa* L). Theor Appl Genet 82:737–744

Huo YM, Liu BJ, Yang YY, Miao J, Gao LM, Kong SP, Wang ZB, Kitano H, Wu X (2015) *AcSKP1*, a multiplex PCR-based co-dominant marker in complete linkage disequilibrium with the male-fertility restoration (*Ms*) locus, and its application in open-pollinated populations of onion. Euphytica 204:711–722

Jakse M, Bohanec B, Ihan A (1996) Effect of media components on the gynogenic regeneration of onion (*Allium cepa* L.) cultivars and analysis of regenerants. Plant Cell Rep 15:934–938

Jakse M, Bohanec B, Havey MJ (2003) Chromosome doubling procedures of onion (*Allium cepa* L.) gynogenic embryos. Plant Cell Rep 21:905–910

Jakse M, Hirschegger P, Bohanec B, Havey MJ (2010) Evaluation of gynogenic responsiveness and pollen viability of selfed doubled haploid onion lines and chromosome doubling via somatic regeneration. J Am Soc Hortic Sci 135:67–73

Jauhar PP, Xu SS, Baenziger PS (2009) Haploidy in cultivated wheats: induction and utility in basic and applied research. Crop Sci 49:737–755

Javornik B, Bohanec B, Campion B (1998) Studies in the induction of a second cycle gynogenesis in onion (*Allium cepa* L.) and genetic analysis of the plant. Plant Breed 117:275–278

Jones HA, Clarke AE (1943) Inheritance of male sterility in onion and the production of hybrid seed. Proc Am Soc Hortic Sci 43:189–194

Jones HA, Emsweller SL (1936) A male sterile onion. Proc Am Soc Hortic Sci 34:582–585

Kadams AM, Nwasike CC (1986) Heritability and correlation studies of some vegetative traits in Nigerian local white onion. Plant Breed 97:232–236

Kasha KJ, Kao KN (1970) High frequency haploid production in barley (*Hordeum vulgare* L.). Nature 225:874–876

Keller ERJ (1990) Culture of unpollinated ovules, ovaries and flower buds in some species of the genus *Allium* and haploid induction via gynogenesis in onion (*Allium cepa* L.). Euphytica 47:241–247

Khar A, Jakse J, Havey MJ (2008) Segregations for onion bulb colors reveal that red is controlled by at least three loci. J Am Soc Hortic Sci 133(1):42–47

Khar A, Saini N (2016) Limitations of PCR-based molecular markers to identify male-sterile and maintainer plants from Indian onion (*Allium cepa* L.) populations. Plant Breeding 135(4):519–524

Khosa JS, Mc Callum J, Dhatt AS, Macknight RC (2016) Enhancing onion breeding using molecular tools. Plant Breed 135:9–20

Khush GS, Virmani SS (1996) Haploids in plant breeding. In: Jain SM, Sopory SK, Veilleux RE (eds) In vitro haploid production in higher plants, vol 1. Kluwer Academic Publisher, Dordrecht, pp 11–33

Kim S (2014) A codominant molecular marker in linkage disequilibrium with a restorer-of-fertility gene (Ms) and its application in reevaluation of inheritance of fertility restoration in onions. Mol Breed 34:769–778

Kim B, Kim S (2019) Identification of a variant of CMS-T cytoplasm and development of high resolution melting markers for distinguishing cytoplasm types and genotyping a restorer-of-fertility locus in onion (*Allium cepa* L.). Euphytica 215:164

Kim S, Binzel M, Yoo K, Park S, Pike LM (2004a) Pink (P). A new locus responsible for a pink trait in onions (*Allium cepa*) resulting from natural mutations of *Anthocyanidin synthase*. Mol Gen Genomics 272:18–27

Kim S, Binzel M, Yoo K, Park S, Pike LM (2004b) Inactivation of *DFR* (*Dihydroflavonol 4-reductase*) gene transcription results in blockage of anthocyanin production in yellow onions (*Allium cepa*). Mol Breed 14:253–263

Kim S, Yoo K, Pike LM (2005a) Development of a co-dominant PCR-based marker for an allelic selection of the pink trait in onions (*Allium cepa*) based on the insertion mutation in the promoter of the *Anthocyanidin synthase* gene. Theor Appl Genet 110:628–633

Kim S, Yoo K, Pike LM (2005b) Development of a PCR-based marker utilizing a deletion mutation in the *DFR* (*Dihydroflavonol 4-reductase*) gene responsible for the lack of anthocyanin production in yellow onions (*Allium cepa*). Theor Appl Genet 110:588–595

Kim S, Bang H, Yoo KS, Pike LM (2006) Identification of the fourth allele of the *ANS* (*Anthocyanidin synthase*) gene and its effect on red color intensity in onions (*Allium cepa*). Euphytica 149:45–51

Kim S, Lee ET, Cho DY, Han T, Bang H, Patil BS, Ahn YK, Yoon MK (2009) Identification of a novel chimeric gene, orf725, and its use in development of a molecular marker for distinguishing among three cytoplasm types in onion (*Allium cepa* L.). Theor Appl Genet 118:433–441

Kim S, Yoon M (2010) Comparison of mitochondrial and chloroplast genome seg- ments from three onion (*Allium cepa* L.) cytoplasm types and identification of a transsplicing intron of cox2. Curr Genet 56:177–188

Kim S, Kim CW, Park M, Choi D (2015) Identification of candidate genes associated with fertility restoration of cytoplasmic male-sterility in onion (*Allium cepa* L.) using a combination of bulked segregant analysis and RNA-seq. Theor Appl Genet 128(11):2289–2299

King JJ, Bradeen JM, Havey MJ (1998) Variability for RFLPs and relationships among elite commercial inbred an virtual hybrid populations of onion. J Am Soc Hortic Sci 123:1034–1037

Kobayashi RS, Sinden SL, Bouwkamp JC (1993) Ovule cultures of sweet potato (*Ipomoea batatas*) and closely related species. Plant Cell Tissue Organ Cult 32:77–82

Kohn V, Kielkowska A, Havey MJ (2013) Sequencing and annotation of the chloroplast DNAs of normal(N) male fertile and male sterile (S) cytoplasms of onion and single nucleotide polymorphisms distinguishing these cytoplasms. Genome 56:737–742

Kumar NKK, Gopal J, Parthasarthy (2015) The Onion. ICAR, New Delhi, India

Lilly JW, Havey MJ (2001) Sequence analysis of a chloroplast intergenic spacer for phylogenetic estimates in *Allium* section *Cepa* and a PCR-based polymorphism detecting mixtures of male-fertile and male-sterile cytoplasmic onion. Theor Appl Genet 102:78–82

Lin M, Watson JF, Baggett JR (1995) Inheritance of soluble solids and pyruvic acid content of bulb onion. J Am Soc Hortic Sci 120:119–122

Liu S, Yeh C, Tang HM, Nettleton D, Schnable PS (2012) Gene mapping via bulked segregant RNA-Seq (BSR-Seq). PLoS One 7:e36406

Maraschin SF, Priester W, Spaink HP, Wang M (2005) Androgenic switch: an example of plant embryogenesis from the male gametophyte perspective. J Exp Bot 56:1711–1726

Martinez LE, Augero CB, Lopez ME, Galmarini CR (2000) Improvement of in vitro gynogenesis induction in onion (*Allium cepa* L.) using polyamines. Plant Sci 156:221–226

Masuzaki S, Shigyo M, Yamauchi N (2006) Direct comparison between genomic constitution and flavonoid contents in *Allium* multiple alien addition lines reveals chromosomal locations of genes related to biosynthesis from dihydrokaempferol to quercetin glucosides in scaly leaf of shallot (*Allium cepa* L.). Theor Appl Genet 112:607–617

McCallum J, Clarke A, Pither-Joyce M, Shaw M, Butler R, Brash D, Scheffer SI, Heusden S, Shigyo M, Havey MJ (2006) Genetic mapping of a major gene affecting onion bulb fructan content. Theor Appl Genet 112:958–967

McCallum J, Pither-Joyce M, Shaw M, Kenel F, Davis S, Butler R, Scheffer J, Jakse J, Havey MJ (2007) Genetic mapping of sulfur assimilation genes reveals a QTL for onion bulb pungency. Theor Appl Genet 114:815–822

McCollum GD (1968) Heritability and genetic correlation of soluble solids, bulb size and shape in while sweet Spanish onion. Can J Genet Cylol 10:508–514

Michalik B, Adamus A, Nowak E (2000) Gynogenesis in polish onion cultivars. J Plant Physiol 156:211–216

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Moqbeli E, Peyvast G, Hamidoghli Y, Olfati JA (2013) In vitro cucumber haploid line generation in several new cultivars. J Mol Biol Biotechnol 21:18–25

Muren RC (1989) Haploid plant induction from unpollinated ovaries in onion. Hortic Sci 24:833–834

Musial K, Bohanec B, Przywara L (2001) Embryological study on gynogenesis in onion (*Allium cepa* L.). Sex Plant Reprod 13:335–341

Musial K, Bohanec B, Jakse M, Przywara L (2005) The development of onion (*Allium cepa* L.) embryo sacs in vitro and gynogenesis induction in relation to flower size. Vitro Cell Dev Biol Plant 41:446–452

Nitsch C (1977) Culture of isolate microspore. In: Reinert J, Bajaj YPS (eds) Applied and fundamental aspects of plant cell, tissue and organ culture. Springer, Berlin, pp 268–278

Olszewska D, Kisiala A, Niklas-nowak A, Nowaczyk P (2014) Study of in vitro anther culture in selected genotypes of genus Capsicum. Turk J Biol 38:118–124

Ponce M, Martinez L, Galmarini CR (2006) Influence of CCC, putrescine and gellam gum concentration on gynogenic embryo induction in Allium cepa. Biol Plant 50:425–428

Puddephat IJ, Robinson HT, Smith BM, Lynn J (1999) Influence of stock plant pretreatment on gynogenic embryo induction from flower buds of onion. Plant Cell Tissue Organ Cult 57:145–148

Raines S, Henson C, Havey MJ (2009) Genetic analyses of soluble carbohydrate concentrations in onion bulbs. J Am Soc Hortic Sci 134:618–623

Reiman GH (1931) Genetic factors for pigmentation in the onion and their relationship to disease resistance. J Agric Res 42:251–278

Reinert J, Bajaj YPS (1977) Anther culture: haploid production and its significance. In: Reinert J, Bajaj YPS (eds) Applied and fundamental aspects of plant cell, tissue and organ culture. Springer, Berlin, pp 251–267

Rhodes MJC, Price KR (1996) Analytical problems in the study of flavonoid compounds in onions. Food Chem 57:113–117

Ritsema T, Smeekens S (2003) Fructans: beneficial for plants and humans. Curr Opin Plant Biol 6:223–230

Sato Y (1998) PCR amplification of CMS-specific mitochondrial nucleotide sequences to identify cytoplasmic genotypes of onion (*Allium cepa* L.) sequences. Theor Appl Genet 96:367–370

Sato Y, Nagai M, Mikami T, Kinoshita T (1993) The use of mitochondrial DNA polymorphism in the classification of individual onion plants by cytoplasmic genotypes. Theor Appl Genet 86:345–348

Schweisguth B (1973) Etude d'un nouveau type de sterilite male chez l'oignon, *Allium cepa* L. Ann Amelior Plant 23:221–233

Shalaby TA (2007) Factors affecting haploid induction through in vitro gynogenesis in summer squash (*Cucurbita pepo* L.). Scientia Hortic 115:1–6

Simon PW (1995) Genetic analysis and total soluble solids in long storage onion. Euphytica 82:1–8

Terahara N, Yamaguch MA, Honda T (1994) Malonylated anthocyanins from bulbs of red onion *Allium cepa* L. Biosci Biotechnol Biochem 58:1324–1325

Thakur P (2016) In vitro production of double haploids (DH) in onion (*Allium cepa* L.). PhD Thesis, Punjab Agricultural University Ludhiana

Thomas WTB, Newton AC, Wilson A, Booth A, Macaulay M, Keith R (2000) Development of recombinant chromosome substitution lines: a barley resource. SCRI Annual Report, pp 99–100

Tsushida T, Suzuki M (1995) Isolation of flavonoid-glycosides in onion and identification by chemical synthesis of the glycosides. J Jpn Soc Food Sci Technol 42:100–108

Watson A, Ghosh S, Williams MJ, Cuddy WS et al (2018) Speed breeding is a powerful tool to accelerate crop research and breeding. Nat Plants 4(1):23–29

Winzeler H, Schmid J, Fried PM (1987) Field performance of androgenetic doubled haploid spring wheat line in comparison with line selected by the pedigree system. Plant Breed 99:41–48

Yang HY, Zhou C (1982) In vitro induction of haploid plants from unpollinated ovaries and ovules. Theor Appl Genet 63:97–104

Chapter 4
Rapid Methods for Onion Breeding

Anil Khar and Hira Singh

4.1 Introduction

Onion ($2n = 2x = 16$), known as king of vegetables, is an indispensable component of daily diet, worldwide. It is one of the most important vegetable crops grown throughout the world since ancient period. It is supposed to have originated in Southwestern Asia and has been under cultivation for bulb production for over 4700 years (Brewster 2008; Etana et al. 2019). Usually, onions are consumed as salads and for culinary purpose to augment flavor. Since primordial periods, onions were considered as key functional food. This has also been well documented in the oldest known "recipe book," a 4000-year-old Mesopotamian clay tablet (Bottero 1985; Khosa et al. 2016). Onion ranks second among the top ten vegetables grown in terms of global production and ranks third as exported fresh vegetable (Rabobank 2018). India is the second largest onion producer, after China, with an output of 19.40 million tons from an area of 1.2 million ha (FAOSTAT 2017). More than 50% of the vegetables exported from India are onion. In 2017, its foreign exchange earning value was estimated to be US $464 million as compared to cumulative export value of all other vegetables, amounting to US $420 million, including garlic and shallots (APEDA 2017). Although, India occupies 19% of the gross cultivated area and produces 17% of the production, yet the productivity is less (16.1 t/ha) compared to other high productivity countries, e.g., Republic of Korea (66.5 t/ha). Low productivity is due to lack of hybrids (5%) in the Indian market compared to 95% market share of hybrids in the USA, UK, Italy, etc. Research reports suggest that hybrids display higher heterosis over open pollinated varieties (Singh and Bhonde 2011). Being a highly cross-pollinated crop, onion is included in the category under

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very high inbreeding depression crops after carrot. The life cycle of onion from seed to seed is of 2 years. These two factors, viz., biennial generation time and high inbreeding depression, have hampered the breeding activities in onion. Further, onion is a photo-thermo-sensitive crop and has been classified into short-day, intermediate-day, and long-day varieties based on the day length requirement for bulb formation. Nevertheless, onion production has also been immensely influenced by changing climatic conditions, and demand of dry onion has been enhancing day by day. Thus, all such factors attract the attention of onion breeders to develop suitable and well-adapted cultivars having high-quality parameters and storability. For onion genetic improvement program, many conventional plant breeding strategies have been exploited to develop new cultivars having high yield potential and well adaptability. Being laborious, conventional approaches require prolonged time period to develop improved onion cultivars. To lessen the time period, we have to opt for rapid and speed breeding approaches in onion such as haploid induction, resistance breeding, shuttle breeding, integrated use of omics like molecular markers, marker-assisted breeding, genome editing, etc. This chapter is designed to focus on the rapid and speed breeding techniques for onion improvement. Main events which have helped in forwarding the knowledge toward understanding of genetics for rapid breeding are summarized in Table 4.1.

Table 4.1 Chronological development of onion breeding and genetic program

Breeding trait	Remarks	Country	Reference
Male sterility	First time identification of male sterility in onion (S-type cytoplasm) in 'Italian Red' cultivar at University of California	USA	Jones and Emsweller (1936)
	CMS-S restored by single nuclear gene	USA	Jones and Clarke (1943), Jones and Davis (1944)
	First report for determination of male sterility genotypes and cytotypes of onion by testing pollen in progeny	Auckland	Yen (2011)
	Identification of T-type cytoplasm in French onion cultivar 'Jaune paille des Vertus'	France	Berninger (1965)
	Report of *Galanthum* type cytoplasm	USA	McCollum (1971)
	CMS-T controlled by three independently segregating loci	France	Schweisguth (1973)
	S-CMS system stable under diverse environmental conditions	USA	Havey (2000)
	Use of PCR-based markers for S and T cytoplasm identification	Germany	Engelke et al. (2003)
	Development of PCR marker for distinguishing three cytoplasm types	South Korea	Kim et al. (2009)
	Report of new male sterile cytoplasm using *Allium roylei*	Japan	Vu et al. (2011)

(continued)

Table 4.1 (continued)

Breeding trait	Remarks	Country	Reference
	Development of PCR-based marker linked to *Ms* locus	South Korea	Bang et al. (2011)
	SNPs for *Ms* locus	USA	Havey (2013)
	First high-resolution linkage map of *Ms* locus	South Korea	Park et al. (2013)
	Codominant marker in linkage disequilibrium with *Ms* locus	South Korea	Kim (2014)
	Sequencing and annotation of chloroplast DNAs and identification of polymorphism of N and S cytoplasm	USA	Von Kohn et al. (2013)
	AcSKP1, a multiplex PCR-based codominant marker in complete LD with *Ms* locus	China	Huo et al. (2015)
	High-resolution tyramide-FISH mapping of markers tightly linked to *Ms* locus	USA	Khrustaleva et al. (2016/2015)
	Limitations of PCR-based markers in Indian onion population	India	Khar and Saini (2016)
	Identification of key genes related to male sterility using transcriptomics	China	Yuan et al. (2018)
	Identification of new cytoplasm "Y"' type (unstable male sterile flowers in Ethiopian and Egyptian onion populations)	South Korea	Kim et al. (2019)
Genetic transformation	Initiation of onion transformation work	New Zealand	Eady (1995)
	Herbicide-resistant onion	New Zealand	Eady et al. (2003)
	Development of "tearless onion" using RNAi technology	New Zealand	Eady et al. (2008)
	Transgenic onions for increased nutraceutical value	India	Naini et al. (2019)
Haploid induction	First report on in vitro gynogenesis	USA	Muren (1989)
	Influence of genotype, stock plant pretreatment, and genotype specificity	Italy	Campion and Alloni (1990)
		Slovenia	Bohanec et al. (1995)
		Slovenia	Jakše et al. (1996)
		France	Geoffriau et al. (1997a, b)
		UK	Puddephat et al. (1999)
		Poland	Michalik et al. (2000)
		Indonesia	Sulistyaningsih et al. (2002)
	Development of haploid interspecific hybrids	USA	Alan et al. (2003)
	Fecundity, chromosome doubling, and use of polyamines	Lithuania	Juokevieiene et al. (2005)

(continued)

Table 4.1 (continued)

Breeding trait	Remarks	Country	Reference
	Release of first haploid-based cultivar 'Onion Haploid-1'	USA	Havey and Bohanec (2007)
	Field performance of DH lines	South Korea	Kim et al. (2007)
		USA	Hyde et al. (2012)
Molecular markers	First molecular map based on RAPD markers	USA	Bradeen and Havey (1995)
	RFLP-based first genetic linkage map in the intraspecific cross BYG15-23 × AC43	USA	King et al. (1998)
	Use of AFLP markers	Netherlands	Van Heusden et al. (2000a, b)
	Genomic SSR markers	Germany	Fischer and Bachmann (2000)
	Developed genomic resources AlliumMap		McCallum et al. (2012)
	Genomic SSR markers		Baldwin et al. (2012)
	Transcriptome for SNP markers	USA	Duangjit et al. (2013)
	Linkage map using SNP markers in F2 population of cross between DehyA × B5351C	USA	Damon and Havey (2014)
	Development of SNP markers	Netherlands	Scholten et al. (2016)
	Use of genotype by sequencing (GBS) for genetic map	South Korea	Jo et al. (2017)
	200 SNP markers	USA	Havey and Ghavami (2018)
	SNP using ddRAD-seq		Lee et al. (2018)
	First report on onion transcriptome by using high-throughput sequencing	China	Yuan et al. (2018)
	KASP genotyping	Italy	Villano et al. (2019)

4.2 Haploid Induction

To initiate any crop improvement program, development of inbred homozygous lines is important, and this depends upon many factors such as crop nature, breeding system, flower structure, pollination behavior, etc. However, it is tedious and cumbersome process in cross-pollinated crops like onion due to its heterozygosity, predominant outbreeding nature, protandry genetic mechanism, biennial generation time, residual heterozygosity, and gigantic 16 Gbp (18X tomato) per 1C nucleus genome size (Aramuganathan and Earle 1991; Havey 1993; Scholten et al. 2016) leading to immense inbreeding depression (Muller 1883; Currah and Ockendon 1978; King et al. 1998; Villanueva-Mosqueda and Havey 2001). Cross-pollination nature causes significant reduction of bulb yield after one or two generations of

Fig. 4.1 Steps (**a**–**p**) in DH onion production. (**a**) Onion umbel with 25–30% flower buds open. (**b**) Selection of flower bud (3.5–4.5 mm) for inoculation. (**c**) Flower buds after 1.5 months of inoculation. (**d**) Induction of in vitro plantlet directly through flower bud. (**e**) Shifting the in vitro inducted plant to normal B5 medium. (**f**) Cytological determination of haploid (n = 8) status. (**g**) Flow cytometry for rapid identification of haploids. (**h**) Subculturing for multiple shoot induction and root formation. (**i**) Colchicine treatment under field conditions for DH induction. (**j**) Hardening of plants under field conditions. (**k**) Transfer of plants under field conditions. (**l**) Growing under field conditions. (**m**) DH onions harvested. (**n**) Flowering of haploid (H) onion bulbs. (**o**) Flower buds of haploid. (**p**) Flower buds of DH onion bulbs

inbreeding (Jones and Davis 1944; Benedek and Gaal 1972; Currah and Ockendon 1978). A schematic representation of steps involved in development of doubled haploids is given in Fig. 4.1.

Development of haploids is one of the techniques which can be used for speedy breeding program. Several factors are responsible for the successful development of onion haploid plants. These factors include genotype of donor plant, stage of flower/ovule development, pretreatment, culture medium, and agronomical/cultural practices. Among field and horticulture crops, anther culture is the most effective and

reliable method for the haploidy induction, because of high availability of microspores in anther. But anther culture has not been successful in onion (Keller and Korzun 1996). First report of haploid induction in onion through gynogenesis has been documented by Campion and Azzimonti (1988) followed by Muren (1989) and Campion and Alloni (1990). Muren (1989) induced haploids through in vitro gynogenesis and further categorized onion genotypes based on their induction efficiency response into high, medium, and low. Thereafter, several reports revealed that haploid induction is influenced by genotypic differences mainly due to day length, geographical origin, and genetic constitution (Bohanec et al. 1995; Campion et al. 1995a, b; Geoffriau et al. 1997a, b; Javornik et al. 1998; Martinez et al. 2000; Alan et al. 2004). Till today, no report on successful haploid induction through anther culture has been reported.

Genotype architecture of the donor plant and type of explant are the critical factors for successful in vitro gynogenesis (Jakše et al. 2010; Sivalingam et al. 2014; Fayos et al. 2015; Khar et al. 2018). To achieve successful haploid induction, the developmental stage of explants is one of the fundamental and key factors (Yang and Zhou 1982, Yang et al. 1990; Muren 1989; Mukhambetzhanov 1997; Bhojwani and Thomas 2001) for reprogramming the gametophytic to sporophytic pathway. In case of onion, ovules (un-pollinated), ovaries, or flower buds (as whole) have been exploited as an explant for haploid plant regeneration (Keller 1990; Campion et al. 1992; Bohanec et al. 1995; Geoffriau et al. 1997a, b; Michalik et al. 2000; Bohanec 2002). Among these explants, ovule culture elicited less in vitro response and is one of the laborious procedures compared to ovary or flower bud which displays efficient and similar haploid induction frequencies with respect to embryo induction (Campion and Schiavi 1994). According to Muren (1989), ovaries excised 3–5 days before anthesis showed high response, while the experiments of Musial et al. (2001) indicated that embryo sacs at early developmental stage have more ability for high parthenogenesis and might be more suitable for haploid induction than mature megagametophytes. The perfect stage for in vitro culture of flower bud is about 3–5 mm size which corresponds to 3–5 days before anthesis (Alan et al. 2004). Similarly, the studies conducted by Musial et al. (2005) revealed that the development of in vitro embryo sacs and induction of gynogenesis in onion depended upon the size of flower buds. This research group further investigated that the critical phase for onion gynogenesis is between 2 and 3 weeks of the culture when non-degenerated embryo sacs containing egg apparatus are available. The smaller flower buds showed less response compared to larger buds. Fayos et al. (2015) concluded that 3.5–4.5-mm-size flower buds are the perfect explants for the production of doubled haploids in onion. To achieve maximum gynogenic response from flower buds, stress treatments (Puddephat et al. 1999; Alan et al. 2004) play significant role.

Gynogenic development of the explant (flower bud) is greatly affected by the culture media formulations and its components such as organic nitrogen source, carbohydrates, fortification of synthetic growth regulators, polyamines, and gelling agent. There is a strong association between the type of explant (flower or ovary) and the different auxin used in the medium. Further, it is concluded that 2,4-dichlorophenoxy acetic acid (2,4-D) contributed well for both ovary and flower in vitro culture while naphthaleneacetic acid (NAA) was noticed superior for only

ovary culture (Campion et al. 1992). Earlier, various basal media, namely, B5 (Gamborg et al. 1968), MS (Murashige and Skoog 1962), and BDS (Dunstan and Short 1977), have been used for onion gynogenesis (Muren 1989; Campion and Alloni 1990; Keller 1990; Campion et al. 1992). Michalik et al. (2000) revealed that media composition is affected by genotypes and exhibited the maximum yield of gynogenic embryos on the B5/BDS media fortified with 2.0 mg/l 6-benzylaminopurine (BAP) and 2.0 mg/l 2,4-D followed by culturing in R regeneration medium (BDS supplemented with 1.0 mg/l NAA and 2.0 mg/l N6-2-isopentenyladenine). Furthermore, Geoffriau et al. (1997a, b) exploited B5 and MS medium for haploid induction and for regeneration, respectively. For onion gynogenesis, earlier two-step protocol was used, but currently a simplified and efficient one-step protocol comprising of the whole flower bud culturing in an induction medium until embryo stage developed (Bohanec and Jakše 1999; Jakše and Bohanec 2003) is prevalent. Composition of media is the critical factor for gynogenesis; addition of high sucrose (Muren 1989) and fortification of 2,4-D and BA have been reported to augment gynogenic responses (Campion et al. 1992, Bohanec and Jakše 1999). With the advancement, there are time to time manipulations in culture induction media composition for getting higher gynogenic response.

For gynogenic embryo induction and plantlet regeneration, polyamines play an immense role (Martinez et al. 2000; Geoffriau et al. 2006; Ponce et al. 2006; Ebrahimi and Zamani 2009; Forodi et al. 2009). Enhancing polyamine synthesis has been testified to precede or accompany callogenesis (Ponchet et al. 1982), organogenesis (Aribaud et al. 1994), and somatic embryogenesis (Liu et al. 1997). Use of various polyamines such as putrescine, spermidine, spermine, and cycocel (CCC) and their different combination has been helpful in reducing embryo regeneration time from 46–152 days to 60–90 days depending upon genotype and in enhancing gynogenic embryo development rate and regeneration of plantlets (Martinez et al. 2000; Geoffriau et al. 2006; Ponce et al. 2006; Ebrahimi and Zamani 2009; Forodi et al. 2009). Besides above media compositions, use of gelling agent is another important factor which significantly enhances the success of haploid induction. It has been reported that use of gellan gum enhanced number of regenerated embryos, compared to agar-solidified media (Jakše et al. 1996; Ponce et al. 2006).

4.2.1 Chromosome Doubling, Recovery, and Determination of Haploidy

Three types of explants have been used for treatment for chromosome doubling: intact plantlet (Jakše et al. 2003; Grzebelus and Adamus 2004), split basal (Campion et al. 1995a, b; Geoffriau et al. 1997a, b), and whole basal explant (Alan et al. 2004). The first two methods are of restricted utilization for the exploitation of onion DH lines. The last method is mainly used, and in this whole basal explants, from 2- to 4-month-old in vitro haploid plants, are treated with antimitotic chemicals for a specific duration (Alan et al. 2004). In onion, the plants of reduced ploidy level are very stable, and there is very low frequency of spontaneous diploidization (Jakše

et al. 2003) as compared to the other vegetable crops like *Brassica oleracea* (Bhatia et al. 2018). Different antimitotic agents like colchicine, dinitroaniline, oryzalin, trifluralin, and phosphoric amide herbicide amiprophos-methyl (APM) and their various combinations at various concentrations and for various periods of exposure are being used (Alan et al. 2004, 2007; Grzebelus and Adamus 2004). Recently, Fayos et al. (2015) recovered the highest number of doubled haploid plants through application of 25 μM APM in a solid medium for 24 h.

Ploidy determination of regenerated plants can be examined through chromosome study (root tip analysis), flow cytometry, and marker analysis. Karyotype analysis for determination of ploidy of onion regenerants has been reported (Campion and Alloni 1990; Martinez et al. 2000; Ebrahimi and Zamani 2009), while flow cytometry (Geoffriau et al. 1997a, b; Bohanec et al. 1995, 2003; Puddephat et al. 1999; Grzebelus and Adamus 2004; Alan et al. 2004, 2007; Ponce et al. 2006; Jakše et al. 2010; Fayos et al. 2015), morphological traits, esterase isozyme and RAPD markers (Campion et al. 1995a; Bohanec et al. 1995), and SSR markers (Sivalingam et al. 2014) have also been reported.

4.2.2 Genetics and Evaluation of DH Lines

The first study on genetics of gynogenic haploid production was conducted by Bohanec et al. (2003). They concluded that gynogenic efficiency is strongly affected by the genetic constitution of individual plant. Further, they revealed that gynogenic haploid induction in onion has a genetic basis with significant non-additive and environmental effect. To increase gynogenic haploid production, S_1 family selection method has been suggested which would result in a significant response to the selection for haploid induction in onion populations.

Time, space, and cost associated with onion inbred development are considerably higher than the DH production due to biennial nature and high inbreeding depression in consequence of accumulation of recessive lethal alleles. The produced DH lines show morphological uniformity and vigorous growth, which would help in inbred maintenance, potential use as parents for production of uniform and stable hybrids (Alan et al. 2004; Kim et al. 2007; Hyde et al. 2012). Commercially, Havey and Bohanec (2007) released 'Onion Haploid OH-1' which can be used as a responsive control for the extraction of gynogenic haploids of onion.

4.2.3 Application of DH Lines in Onion Genomic Research

'CUDH2150' a doubled haploid line from Cornell University (Alan et al. 2004) has been utilized for development of a linkage map to assign QTL for fructan content (*Frc*) and the genetic locus (R) conditioning red bulb color (Baldwin et al. 2012) and genetic analysis of bolting (Baldwin et al. 2014). Duangjit et al. (2013) exploited two onion DH lines (gynogenic haploids from the F_1 populations) for transcriptome sequencing to develop SNP-based genetic maps. Further, the same research group

identified significant QTLs on chromosomes 1, 4, and 8 using same population affecting anthocyanin concentration in onion bulbs (Duangjit et al. 2014). Kim et al. (2004) used haploid population derived from 'H6' to identify a new locus P, which is responsible for pink trait in onions. Same red male fertile doubled haploid line 'H6' has been reported by various authors (Bang et al. 2011, 2013; Kim et al. 2015a, b) to develop simple and efficient PCR markers linked to the restorer of fertility (*Ms* locus) gene. Abdelrahman et al. (2015) developed doubled haploid parents of onion, shallot, and their F_1 hybrid to study the transcriptome and associated metabolome variability in order to identify genes that would help in abiotic stress protection toward development of stress-tolerant variety.

4.3 Genetic Diversity

Molecular markers are being used extensively for determination of genetic diversity and population structure because of their neutral nature, reproducibility of results across laboratories, and no environmental effect on their expression. In spite of its economic significance as one of the major vegetable crops worldwide, genetic maps of onion (*Allium cepa* L.) remain relatively rudimentary. This is due to the enormous onion genome, 16.3 giga bases per 1C nucleus (Arumuganathan and Earle 1991), that makes the identification of some molecular markers technically difficult. Biennial generation time and severe inbreeding depression of onion also slow down the development of segregating families. Nevertheless, numerous classes of molecular markers have been developed and mapped in onion, including restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs), and single-nucleotide polymorphisms (SNPs) (King et al. 1998;Van Heusden et al. 2000a, b; Martin et al. 2005; Baldwin et al. 2012). Molecular markers, such as SSRs and SNPs, are especially useful for onion because they are codominant and efficiently revealed by the polymerase chain reaction. Onion SSRs and SNPs have been used for cultivar identification (Jakse et al. 2005), genetic diversity estimates (Baldwin et al. 2012), and tagging of chromosome regions affecting economically important traits such as fructan accumulation (McCallum et al. 2006; Raines et al. 2009), male sterility restoration (Gokce et al. 2002), and flavor (Galmarini et al. 2001; McCallum et al. 2007).

Marker-aided selection (MAS) has great potential in onion improvement because of the high cost of harvesting and vernalizing bulbs prior to flowering and completing crosses within sects. High-throughput platforms that allow for genotyping of a large number of markers across individuals would allow breeders to select plants at an early stage of development and advance to seed production only a fraction of the numbers of plants as compared to classical phenotypic selection. SNPs are the marker of choice for MAS in onion because of codominance, common occurrence among elite germplasms (Martin et al. 2005; Baldwin et al. 2012), and the availability of commercially available high-throughput genotyping platforms. Duangjit et al. (2013) identified 2285 SNPs amenable for genotyping using KASPar platform, whereas Havey and Ghavami (2018) identified 199 polymorphic SNPs distributed across 8 chromosomes of onion for diversity estimation.

4.4 Linkage Maps

Genetic map development in onion and other alliums has been limited by the difficulty in developing, maintaining, and exchanging genetic stocks, high degrees of heterozygosity, and a dearth of sequence data (McCallum 2007). The first published genetic map of an *Allium* species was developed by King et al. (1998) in the intraspecific onion cross 'BYG15-23 × AC43.' Constructed initially using RFLP markers, this map was subsequently augmented with SNP and SSR markers derived from EST sequencing (Kuhl et al. 2004; Martin et al. 2005). These more portable markers enabled partial map construction in other intraspecific onion crosses to enable map-based genetic analysis of fertility restoration (Gokce et al. 2002), color (Khar et al. 2008), and other bulb traits (McCallum et al. 2007, 2008). The breeding systems of *A. fistulosum* have facilitated development of several larger mapping pedigrees and detailed genetic maps based initially on SSR and AFLP markers (Tsukazaki et al. 2008). These maps were used to conduct QTL analysis for seedling vigor (Ohara et al. 2009). Further, Tsukazaki et al. (2010) reported a *A. fistulosum* map based on *A. fistulosum* genomic SSR markers and onion EST-derived SNP and SSR markers, providing further scope for comparative studies between onion and *A. fistulosum* genomes. The only *Allium* relative known to readily produce fertile hybrids with onion is *A. roylei* (Meer and Vries 1990), which has been used to develop an interspecific map (Van Heusden et al. 2000a, b) and backcross progenies with valuable disease resistance (Scholten et al. 2007). Since *A. roylei* also crosses with *A. fistulosum*, this has enabled development of bridge crosses containing all three genomes (Khrustaleva and Kik 1998), thus enabling a potential path for introgression of *A. fistulosum* genetics into onion. The key resource that has enabled alignment of *Allium* genetic maps to physical chromosomes and facilitated comparison among species is the sets of *A. fistulosum*-*A. cepa* alien monosomic addition lines (AMALs) developed by Shigyo et al. (1996). These were initially applied to anchor AFLP-based maps in the interspecific *A. cepa* × *A. roylei* cross (Heusden et al. 2000) and subsequently to anchor the 'BYG15-23 × AC43' map (Martin et al. 2005). Subsequently, these were used to anchor SSR-based maps in *A. fistulosum* (Tsukazaki et al. 2008) to physical chromosomes and to assign many more onion EST-derived anchor markers used in *A. fistulosum* maps (Tsukazaki et al. 2010).

4.5 Marker-Assisted Selection: Male Sterility

Male sterility is characterized by the appearance of non-viable pollen that is unable to participate in fertilization process. Development of onion hybrids became a reality when Jones and Emsweller (1936) discovered cytoplasmic male sterility induced by S cytoplasm in the onion cultivar 'Italian Red' with complete fertility restoration by a single nuclear restorer (*Ms*) gene (Jones and Clarke 1943). Later, Berninger (1965) identified another male sterile cytoplasm in French onion cultivar 'Jaune

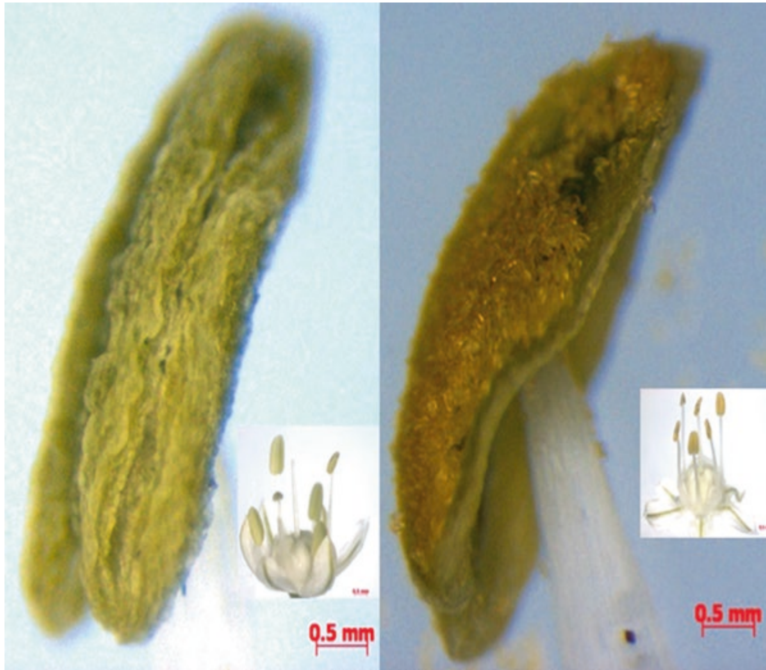

Fig. 4.2 Morphological difference in male sterile anther (8X) and male fertile (8X) anther (visualization under stereozoom microscope)

paille des Vertus', designated as CMS-T, where fertility restoration is controlled by three independently segregating loci (Schweisguth 1973). Apart from these cytoplasms, Kim et al. (2015b) suggested variants of onion cytoplasm as cytotype X and cytotype Y. Onion is a highly cross-pollinated crop taking 2 years life cycle from seed to seed. Being biennial in nature, 4–8 years are needed to identify cytoplasm types and *Ms* alleles using progeny tests (Havey 2000; Shigyo and Kik 2008). Basic difference between a male sterile and fertile anther can be visualized also (Fig. 4.2). Molecular markers capable of distinguishing mitotypes at the DNA level enable breeders to save time and effort. Many molecular markers to distinguish normal and CMS-S mitotypes have been developed based on the variation of relative copy numbers of mtDNA molecules (Sato 1998) and polymorphic sequences of chloroplast genomes (Havey 1995). Novel PCR markers for identification of CMS-T cytoplasm have also been identified (Engelke et al. 2003; Kim et al. 2009). These PCR markers allow breeders to rapidly identify the cytoplasm type of a single onion plant. A lot of research efforts toward development of PCR markers for identification of *Ms* locus have been done. Gokce et al. (2002) reported AOB272, closest restriction fragment length polymorphism (RFLP) marker to the *Ms* locus in the onion genome. Subsequently, easy and reproducible PCR markers OPT and PsaO, separately from AOB272 and AGF136, linked in opposite directions to the *Ms* locus, at distances of 1.5 and 6.4 cM were reported by Bang et al. (2011). Consequently, markers like

SCARs (Yang et al. 2013; Kim 2014), indels (Kim 2014; Kim et al. 2015a, b), PCR (Park et al. 2013; Huo et al. 2015; Kim et al. 2015a, b), and SNP (Havey 2013) linked to *Ms* locus have been reported. Till now, at least 10 markers for identification of cytoplasm and 30 markers for identification of *Ms* locus have been developed. Physical mapping of molecular markers linked tightly to *Ms* locus on onion chromosome 2 has revealed that markers are assigned to the proximal locations close to the centromere of the long arm of chromosome 2, a region of lower recombination. This explains why so many tightly linked markers, based on linkage mapping, are unable to detect in *Ms* locus in most of the populations (Khrustaleva et al. 2016/2015). The hypothesis was confirmed by the study of Khar and Saini (2016) who observed the limitations in using *Ms*-linked PCR markers for identification of *Ms* locus. Havey and Kohn (2017) postulated that limitation of molecular markers to predict linkage disequilibrium at *Ms* locus may be due to the rare recombination events or different male fertility restoration system.

4.6 Genetic Transformation

Genetic engineering to introduce new genes into plants has been achieved in many economically important crops, but a few reports exist for onion (*A. cepa* var. *cepa*) (Aswath et al. 2006). Successful plant transformation requires a plant regeneration system, a DNA delivery system, and a selection system to select transgenic cells. For onion, the characterization of these three aspects of transformation is still incomplete. In vitro culture and plant regeneration from *Allium* immature embryo and mature zygotic embryo explants has been widely reported (Eady et al. 1998; Eady and Lister 1998; Zheng et al. 1998, 1999). For the micropropagation of onion, suspension cultures are generally preferable to culture on solid medium because callus growth on solid medium is very slow. Regenerable suspension cells have been reported in *A. cepa* (Hansen et al. 1995; Karim and Adachi 1997; Zheng et al. 1999), *A. fistulosum* (Kim and Soh 1996; Lee and Ono 2000), and the interspecific hybrid *A. fistulosum* × *A. cepa* (Song and Peffley 1994). However, only a few of these reports describe regeneration protocols suitable for use in onion transformation studies (Eady 1995). Although genes of interest have been successfully introduced and expressed in onion cultures, transgenic plants were not regenerated, highlighting the need for further optimization of the culture system (Eady et al. 1996). Aswath et al. (2006) reported the *Agrobacterium* and biolistic-mediated transformation of onion through somatic embryogenesis. Efficient plant regeneration from mature embryo-derived suspension cultures of *A. cepa* has also been obtained (Zheng et al. 2004). Ramakrishnan et al. (2013) were able to develop an efficient and reliable system for somatic embryogenesis and regeneration in onion using shoot apex explants. There are only few published reports on successful onion transformation protocols (Eady et al. 2000, 2003; Zheng et al. 2001) with reporter genes. One of the selectable markers used, viz., bar gene, confers resistance to herbicides (Eady et al. 2003). Zheng et al. (2005) developed transgenic Bt-shallots

(*Allium cepa* L.) possessing resistance to army beet worm. These transformation protocols are *Agrobacterium* mediated although transformation following particle bombardment has also been demonstrated (Eady et al. 1996). Recently, Naini et al. (2019) reported the successful generation of transgenic onions that accumulated both resveratrol and its glycoside polydatin in leaves as well as bulbs. The transgenic production was found to increase the increased life span of haploid yeast. Hence, these transgenic onions can serve as a unique nutraceutical genetic resource.

4.7 Biotic Stress

Onions are affected by many insect pests and diseases. Major diseases affecting onions worldwide are purple blotch (*Alternaria porri*), *Stemphylium* blight (*Stemphylium vesicarium*), *Fusarium* basal rot (*Fusarium oxysporum* f. sp. *cepae*), downy mildew, etc. Most of research work has focussed on evaluation of resistant material through conventional techniques. One of the successful attempts on marker-assisted introgression of resistance is the transfer of downy mildew resistance gene from *Allium roylei* to *Allium cepa* (Kofoet et al. 1990). The resistant gene was named Pd1, and a RAPD (random amplified polymorphic DNA) marker located at 2.6 cM from the Pd1 locus was identified (De Vries et al. 1992). This RAPD marker was converted into a SCAR (sequence characterized amplified region marker) (Kik et al. 1997; Van Heusden et al. 2000a, b) marker and was mapped on the molecular linkage map, based on the *A. cepa-A. roylei* cross, assigned to chromosome 3 (Van Heusden et al. 2000a, b). However, the SCAR marker lost its discriminating power in advanced backcrossed populations (Scholten et al. 2007). Using the downy mildew-resistant cultivar 'Santero', Kim et al. (2016a, b) developed a robust PCR marker DMR1, linked to Pd1 downy mildew resistance gene, for detection of resistant plants.

Purple blotch is one of the devastating diseases in onion. Till date, no resistant source of onion has been used to develop resistant cultivars. Recently, molecular markers are being used to identify PCR markers which can hasten in breeding resistant cultivars. Ganesh and Veeregowda (2007) performed genetic linkage analysis in a F_2 population and inferred that a single dominant gene governs the resistance in a short-day onion genotype PBR287. Recently, Chand et al. (2018) has confirmed that resistance to purple blotch is controlled by a single dominant gene *ApR1* and identified SSR marker (AcSSR7) and an STS marker (ApR-40) for facilitating introgression of ApR1 into susceptible onion varieties for development of resistant genotypes.

Among insect pests affecting onion, thrips are by far the major pest affecting onion bulb and seed yield (Diaz-Montano et al. 2010) and are vector for iris yellow spot virus (Shock et al. 2008). Resistance work toward thrips started with Jones et al. (1934) reporting 'White Persian' as resistant because of light green foliage "glossy" (Jones 1944) and demonstrated it to be controlled by the recessive *gl* locus. Further reports have confirmed that glossy onions exhibit resistance to onion thrips (Pawar et al. 1975; Molenaar 1984; Alimousavi et al. 2007). Based on wax content,

onion leaves can be classified as glossy, semiglossy, and waxy. Damon et al. (2014) reported ketone hentriacontanone-16 as the most prevalent wax on leaves of waxy onion and suggested that semiglossy onion can be useful in managing thrips. Damon and Havey (2014) observed that region on chromosome 2 was associated with the acyl reduction pathway and the region on chromosome 5 affected the decarbonylation pathway of epicuticular wax biosynthesis and concluded that SNPs tagging regions on chromosomes 2 and 5 will be useful for marker-assisted breeding to develop cultivars resistant to onion thrips. Scholten et al. (2016) used SNP genotyping to detect the QTL for resistance to *Botrytis squamosa*, causal agent of *Botrytis* leaf blight in onion. A QTL region on chromosome 6, originating from *A. roylei*, was responsible for the resistance.

4.8 Shuttle Breeding

In India, onion is mostly grown as a short-day crop except in hilly regions of Himachal Pradesh, J&K, and Ladakh where it is grown under long-day conditions. The month of seed sowing, transplanting, and harvesting also varies from place to place (Table 4.2). In northern parts of India, onion is grown in two seasons, i.e., *kharif* (transplanting first fortnight of August) and *rabi* (transplanting between mid-December to mid of January). The bulbs for *kharif* season can be obtained in

Table 4.2 A schematic representation of seed sowing, transplanting, and harvesting of onions in India

Season	Month of seed sowing	Month of transplanting	Month of harvesting
Rajasthan, Haryana, Punjab, UP, Bihar			
Kharif Rabi	June–July Nov–Dec	July–Aug Dec–Jan	Nov–Dec May–June
Maharashtra and some parts of Gujarat			
Early kharif Kharif Late kharif Rabi	Feb–Mar May–June Aug–Sep Oct–Nov	April–May July–Aug Oct–Nov Dec–Jan	Aug–Sep Nov–Dec Jan–Mar April–May
West Bengal, Orissa			
Kharif Late *kharif* *Rabi*	June–July Aug–Sep Sep–Oct	Aug–Sep Oct–Nov Nov–Dec	Nov–Dec Feb–Mar Mar–Apr
Tamil Nadu, Karnataka, and Andhra Pradesh			
Early *kharif* *Kharif* *Rabi*	Feb–April May–June Sept–Oct	April–June July–Aug Nov–Dec	July–Sept Oct–Nov Mar–Apr
Long-day type			
Himachal Pradesh J&K Ladakh	Sep–Oct Nov–Dec March–April	Oct–Nov Feb–Mar April–May	June–July Sep–Oct Sep–Oct

November–December and of *rabi* season in May–June. Similarly, in central India (Maharashtra and some parts of Gujarat), transplanting can be done in early *kharif* (April–May), *kharif* (July–August), late *kharif* (October–November), and r*abi* (December–January), and harvesting can be done accordingly throughout the year. In case of higher hills of HP and J&K, onion seedlings are transplanted in October, remain under snow during December–January, and then grow, and bulbs are harvested in June–July. Similarly, in dry cold desert of Ladakh, onion is transplanted in April–May and harvested in October. Similarly, bulbs are planted in last week of April, and seeds are harvested in end of September. In southern parts of India, mostly multiplier onion (*Allium cepa* var. *aggregatum*) is grown.

Onion is a biennial crop since it takes 2 years to complete its life cycle. In order to breed new onion varieties, this is a major handicap as it takes longer time. To accelerate the breeding procedure, a new method of "seed to seed," which enables generation advancement in 1 year, has been proposed. In this method, under Northern Indian conditions, seed sowing is done in mid of August, and plants stay in vegetative stage until flowering is initiated during mid-March, and we can harvest seed by the end of May next year without actually harvesting and evaluating bulbs. It has been observed that the flower opening and seed formation takes time and the seed formation is not that encouraging. *Stemphylium* blight is also a limiting factor in plains. Transplanting in hills shows better seed setting.

One another method is to grow bulblets (February–May) and then store bulblets until first week of October. Bulblets can be transplanted in plains or in the hills based on bolting requirement of subject genotypes. The only drawback with these methods is that we don't get any opportunity to see bulbs; hence selection for bulb traits can't be achieved. It is useful during very early generations like F1 where our objective is just generation advancement. However, it could be useful for introgression of targeted traits, if we have marker support for specific traits.

There is a third method where seed sowing is done in June and transplanting in July–August and bulbs are harvested in November in Pune or Bangalore. The bulbs are then harvested and shifted to cooler locations of Solan or Kullu (HP) and Srinagar (J&K) for seed production. This method is known as "seed to bulb to seed," and using multilocations we can use this for breeding purpose. Here we get an opportunity to see bulb traits before transplanting in the field to get seed. The generation advancement is achieved in 1 year and can be useful for generation advancement beyond F_1.

To take advantage of the diverse climatic conditions of India, bulbs can also be produced in Northern/Central India during *kharif* season, and then the bulbs can be sent to higher hills of HP or J&K where they flower and can be used for breeding purpose. In this way life cycle of onion is completed in one season, and this helps in accelerated breeding of onion varieties. Most of the private companies and a few public institutes are taking advantage of these climatic conditions for accelerated breeding. For long-day conditions, D'Angelo and Goldman (2019) suggested that appropriate duration of vernalization and overcoming bulb dormancy are two factors for annualizing the long-day breeding program. Treatment of 20% hydrogen peroxide (2–4 h) and optimum chilling of 10 °C for 14 weeks was sufficient to initiate flowering in long-day onion.

To conclude, it can be said that rapid breeding methods of doubled haploid production for inbred development followed by use of PCR markers for identification of cytoplasm type and *Ms* locus will speed up hybrid development process in onion. Similarly, genomic and EST markers will aid in estimation of diversity, population structure, and heterotic combinations. With the advancements in genomics and next-generation sequencing, identification of markers, QTLs, linked to biotic and abiotic stress will benefit in rapid breeding program.

References

Abdelrahman M, Sawada Y, Nakabayashi R, Sato S, Hirakawa H, El-Sayed M, Hirai MY, Saito K, Yamauchi N, Shigyo M (2015) Integrating transcriptome and target metabolome variability in doubled haploids of *Allium cepa* for abiotic stress protection. Mol Breed 35:195

Alan AR, Mutschler MA, Brants A, Cobb E, Earle ED (2003) Production of gynogenic plants from hybrids of Allium cepa L. and A. roylei Stearn. Plant Sci 165(6):1201–1211

Alan AR, Brants A, Cobb E, Goldschmied P, Mutschler MA, Earle ED (2004) Fecund gynogenic lines from onion (*Allium cepa* L.) breeding materials. Plant Sci 167(5):1055–1066

Alan AR, Lim W, Mutschler MA, Earle ED (2007) Complementary strategies for ploidy manipulations in gynogenic onion (*Allium cepa* L.). Plant Sci 173(1):25–31

Alimousavi SA, Hassandokht MR, Moharramipour SA (2007) Evaluation of Iranian onion germplasms for resistance to Thrips. Int J Agric Biol 9:455–458

APEDA (2017) India export of agro food products. http://agriexchange.apeda.gov.in/indexp/Product_description_32head.aspx? gcode=0202

Aramuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Bio Rep 1991(9):208–218

Aribaud M, Carre M, Martin-Tanguy J (1994) Polyamine metabolism and *in vitro* cell multiplication and differentiation in leaf explants of *Chrysanthemum morifolium*Ramat. Plant Growth Regul 15:143–155

Aswath CR, Mo SY, Kim DH, Park SW (2006) Agrobacterium and biolistic transformation of onion using non-antibiotic selection marker phosphomannose isomerase. Plant Cell Rep 25(2):92–99

Baldwin S, Pither-Joyce M, Wright K, Chen L, McCallum J (2012) Development of robust genomic simple sequence repeat markers for estimation of genetic diversity within and among bulb onion (*Allium cepa* L.) populations. Mol Breed 30(3):1401–1411

Baldwin S, Revanna R, Pither-Joyce M, Shaw M, Wright K, Thomson S, Moya L, Lee R, Macknight R, McCallum J (2014) Genetic analyses of bolting in bulb onion (*Allium cepa* L.). Theor Appl Genet 127(3):535–547

Bang H, Cho DY, Yoo KS, Yoon MK, Patil BS, Kim S (2011) Development of simple PCR-based markers linked to the *Ms* locus: a restorer-of-fertility gene in onion (*Allium cepa* L.). Euphytica 179(3):439–449

Bang H, Kim S, Park SO, Yoo K, Patil BS (2013) Development of a codominant CAPS marker linked to the *Ms* locus controlling fertility restoration in onion (*Allium cepa* L.). Sci Hortic 153(4):42–49

Benedek P, Gaal E (1972) The effect of insect pollination on seed onion, with observations on the behaviour of honeybees on the crop. J Apic Res 11(3):175–180

Berninger E (1965) Contribution à l'e'tude de la stérilité mâle de l'oignon (*Allium cepa*L.). Ann Ame'lior Plant 15:183–199

Bhatia R, Dey SS, Parkash C, Sharma K, Sood S, Kumar R (2018) Modification of important factors for efficient microspore embryogenesis and doubled haploid production in field grown white cabbage (*Brassica oleracea* var. *capitata* L.) genotypes in India. Sci Hortic 233:178–187

Bhojwani SS, Thomas TD (2001) *In vitro* gynogenesis. In: Current trends in the embryology of angiosperms. Springer, Dordrecht, pp 489–507

Bohanec B (2002) Doubled haploid in onions. In: Rabinowitch HD, Currah L (eds) Allium crop science: recent advances. CABI Publishing, Wallingford, pp 145–158

Bohanec B, Jakše M (1999) Variations in gynogenic response among long-day onion (*Allium cepa* L.) accessions. Plant Cell Rep 18(9):737–742

Bohanec B, Jakše M, Ihan A, Javornik B (1995) Studies of gynogenesis in onion (*Allium cepa* L.): induction procedures and genetic analysis of regenerants. Plant Sci 104(2):215–224

Bohanec B, Jakše M, Havey MJ (2003) Genetic analyses of gynogenetic haploid production in onion. J Am Soc Hortic Sci 128(4):571–574

Bottéro J (1985) The cuisine of ancient Mesopotamia. Biblic Archaeol 48(1):36–47

Bradeen JM, Havey MJ (1995) Randomly amplified polymorphic DNA in bulb onion and its use to assess inbred integrity. J Am Soc Hortic Sci 120(5):752–758

Brewster JL (2008) Onions and other vegetable (No 15). CABI Int J Agric Biosci 6(5):265–271

Campion B, Alloni C (1990) Induction of haploid plants in onion (*Allium cepa* L.) by *in vitro* culture of unpollinated ovules. Plant Cell Tissue Organ Cult 20(1):1–6

Campion B, Azzimonti MT (1988) Evolution of ploidy level in haploid plants of onion (*Allium cepa*L.) obtained through *in vitro* gynogenesis. In: Proceeding of 4th eucarpia allium symposium. Institute of Horticultural Research Wellesbourne, Warwick, pp 6–9

Campion B, Schiavi M (1994) Production of doubled haploid lines of onion (*Allium cepa* L.): progress report and problems. In: International colloquium on impact of plant biotechnology on agriculture, Rogla, 5–7 June 1994

Campion B, Azzimonti MT, Vicini E, Schiavi M, Falavigna A (1992) Advances in haploid plant induction in onion (*Allium cepa* L.) through *in vitro* gynogenesis. Plant Sci 86(1):97–104

Campion B, Bohanec B, Javornik B (1995a) Gynogenic lines of onion (*Allium cepa* L.): evidence of their homozygosity. Theor Appl Genet 91(4):598–602

Campion B, Perri E, Azzimonti MT, Vicini E, Schiavi M (1995b) Spontaneous and induced chromosome doubling in gynogenic lines of onion (*Allium cepa* L.). Plant Breed 114(3):243–246

Chand SK, Nanda S, Joshi RK (2018) Genetics and molecular mapping of a novel purple blotch-resistant gene *ApR1* in onion (*Allium cepa* L.) using STS and SSR markers. Mol Breed 38(9):1–13. https://doi.org/10.1007/s11032-018-0864-4

Currah L, Ockendon D (1978) Protandry and the sequence of flower opening in the onion. New Phytol 81:419–428

D'Angelo CJ, Goldman IL (2019) Annualization of the long day onionbreeding cycle through threshold vernalization and dormancy disruption. Crop Breed Genet Genom 1:e190009

Damon S, Havey MJ (2014) Reduced epicuticular waxes are associated with insect tolerance. In: Conference of the American society of agronomy. Available at: https://scisoc.confex.com/crops/2013am/webprogram/Paper81114.html

Damon S, Groves R, Havey MJ (2014) Variation for epicuticular waxes and numbers of *Thripstabaci* on onion foliage. J Am Soc Hortic Sci 139:495–501

De Vries JN, Jongerius R, Sandbrink H, Lindhout P (1992) RAPD markers assist in resistance breeding. PRO 2:50–51

Diaz-Montano J, Fuchs M, Nault BA, Shelton AM (2010) Evaluation of onion cultivars for resistance to onion thrips (Thysanoptera: Thripidae) and iris yellow spot virus. J Econ Entomol 103:925–937

Duangjit J, Bohanec B, Chan AP, Town CD, Havey MJ (2013) Transcriptome sequencing to produce SNP-based genetic maps of onion. Theor Appl Genet 126(8):2093–2101

Duangjit J, Welsh K, Wise ML, Bohanec B, Havey MJ (2014) Genetic analyses of anthocyanin concentrations and intensity of red bulb color among segregating haploid progenies of onion. Mol Breed 34(1):75–85

Dunstan DI, Short KC (1977) Improved growth of tissue cultures of the onion (*Allium cepa* L.). Physiol Plant 41(1):70–72

Eady CC (1995) Towards the transformation of onions (*Allium cepa*). N Z J Crop Hortic Sci 23(3):239–250

Eady CC, Lister CE (1998) A comparison of four selective agents for use with *Allium cepa* L. immature embryos and immature embryo-derived cultures. Plant Cell Rep 18(1–2):117–121

Eady CC, Lister CE, Suo Y, Schaper D (1996) Transient expression of *uidA* constructs in *in vitro* onion (*Allium cepa* L.) cultures following particle bombardment and Agrobacterium-mediated DNA delivery. Plant Cell Rep 15(12):958–962

Eady CC, Butler RC, Suo Y (1998) Somatic embryogenesis and plant regeneration from immature embryo cultures of onion (*Allium cepa* L.). Plant Cell Rep 18(1–2):111–116

Eady CC, Weld RJ, Lister CE (2000) *Agrobacterium tumefaciens*-mediated transformation and transgenic-plant regeneration of onion (*Allium cepa* L.). Plant Cell Rep 19(4):376–381

Eady C, Davis S, Farrant J, Reader J, Kenel F (2003) Agrobacteriumtumefaciens-mediated transformation and regeneration of herbicideresistant onion (*Alliumcepa*) plants. Ann Appl Biol 142:213–217

Ebrahimi R, Zamani Z (2009) Effect of polyamines on *in vitro* gynogenesis of onion (*Allium cepa* L.). Am Eurasian J Sustain Agric 3(1):71–74

Engelke T, Terefe D, Tatlioglu T (2003) A PCR–521 based marker system monitoring CMS–S, CMS–T and –N cytoplasm in the onion (*Allium cepa*L.). Theor Appl Genet 107:162–167

Etana MB, Aga MC, Fufa BO (2019) Major onion (*Allium cepa* L.) production challenges in Ethiopia: a review. J Biol Agri Healthc 9(7). https://doi.org/10.7176/JBAH

FAOSTAT (2017) Onion production, area and productivity. http://faostat3.fao.org/browse/Q/QC/E

Fayos O, Vallés MP, Garcés-Claver A, Mallor C, Castillo AM (2015) Doubled haploid production from Spanish onion (*Allium cepa* L.) germplasm: embryogenesis induction, plant regeneration and chromosome doubling. Front Plant Sci 6:384

Fischer D, Bachmann K (2000) Onion microsatellites for germplasm analysis and their use in assessing intra- and interspecific relatedness within the subgenus Rhizirideum. Theor Appl Genet 101(1–2):153–164

Forodi BR, Hassandokht M, Kashi A, Sepahvand N (2009) Influence of spermidine on haploid plant production in Iranian onion (*Allium cepa* L.) populations through *in vitro* culture. Hortic Environ Biotechnol 50(5):461–466

Galmarini CR, Goldman IL, Havey MJ (2001) Genetic analyses of correlated solids, flavor, and health-enhancing traits in onion (*Allium cepa* L.). Mol Gen Genomics 265(3):543–551

Gamborg OL, Miller R, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 50(1):151–158

Ganesh CT, Veeregowda R (2007) Marker assisted selection for purple blotch disease resistance in onion (*Allium cepa* L.). In: Keshvachandran R, Nazeem PA, Girija D, John PS, Peter KV (eds) Recent trends in horticultural biotechnology. New India Publishing Agency, New Delhi, pp 505–515

Geoffriau E, Kahane R, Rancillac M (1997a) Variation of gynogenesis ability in onion (*Allium cepa* L.). Euphytica 94(1):37–44

Geoffriau E, Kahane R, Bellamy C, Rancillac M (1997b) Ploidy stability and *in vitro* chromosome doubling in gynogenic clones of onion (*Allium cepa* L.). Plant Sci 122(2):201–208

Geoffriau E, Kahane R, Martin-Tanguy J (2006) Polyamines are involved in the gynogenesis process in onion. Physiol Plant 127(1):119–129

Gokce AF, McCallum J, Sato Y, Havey MJ (2002) Molecular tagging of the *Ms*locus in onion. J Am Soc Hortic Sci 127:576–582

Grzebelus E, Adamus A (2004) Effect of anti-mitotic agents on development and genome doubling of gynogenic onion (*Allium cepa* L.) embryos. Plant Sci 167(3):569–574

Hansen EE, Hubstenberger JF, Phillips GC (1995) Regeneration of shoots from cell suspension-derived protoplasts of *Allium cepa*. Plant Cell Rep 15(1–2):8–11

Havey MJ (1993) A putative donor of S-cytoplasm and its distribution among open-pollinated populations of onion. Theor Appl Genet 86(1):128–134

Havey MJ (1995) Identification of cytoplasms using the polymerase chain reaction to aid in the extraction of maintainer lines from open–pollinated populations of onion. Theor Appl Genet 90:263–268

Havey MJ (2000) Diversity among male–sterility–inducing and male–fertile cytoplasms of onion. Theor Appl Genet 101:778–782

Havey MJ (2013) Single nucleotide polymorphisms in linkage disequilibrium with the male-fertility restoration (*Ms*) locus of onion. J Am Soc Hortic Sci 138:306–309

Havey MJ, Bohanec B (2007) Onion inbred line 'B8667 A&B' and Synthetic populations 'Sapporo-Ki-1 A&B' and 'Onion Haploid-1'. Hortic Sci 42(7):1731–1732

Havey MJ, Ghavami F (2018) Informativeness of single nucleotide polymorphisms and relationships among onion populations from important world production regions. J Am Soc Hortic Sci 143(1):34–44

Havey MJ, von Kohn C (2017) Efficacy of molecular markers *Jnurf13* and *AcPms1* for prediction of genotypes at the nuclear *Ms* locus in three open-pollinated populations of onion from North America. Hortic Sci 52(8):1052–1053

Huo YM, Liu BJ, Yang YY, Wu X (2015) *AcSKP1*, a multiplex PCR–based co-dominant marker in complete linkage disequilibrium with the male–fertility restoration (*Ms*) locus, and its application in open pollinated populations of onion. Euphytica 204(3):711–722

Hyde PT, Earle ED, Mutschler MA (2012) Doubled haploid onion (*Allium cepa* L.) lines and their impact on hybrid performance. Hortic Sci 47(12):1690–1695

Jakse J, Martin W, McCallum J, Havey MJ (2005) Single nucleotide polymorphisms, indels, and simple sequence repeats for onion cultivar identification. J Am Soc Hortic Sci 130(6):912–917

Jakše M, Bohanec B (2003) Haploid induction in onion via gynogenesis. In: Maluszynski M, Kasha KJ, Forster BP, Szarejko I (eds) Doubled haploid production in crop plants. Kluwer Academic Publishers, Dordrecht/Boston/London, pp 281–285

Jakše M, Bohanec B, Ihan A (1996) Effect of media components on the gynogenic regeneration of onion (*Allium cepa* L.) cultivars and analysis of regenerants. Plant Cell Rep 15(12):934–938

Jakše M, Havey MJ, Bohanec B (2003) Chromosome doubling procedures of onion (*Allium cepa* L.) gynogenic embryos. Plant Cell Rep 21(9):905–910

Jakše M, Hirschegger P, Bohanec B, Havey MJ (2010) Evaluation of gynogenic responsiveness and pollen viability of selfed doubled haploid onion lines and chromosome doubling via somatic regeneration. J Am Soc Hortic Sci 135(1):67–73

Javornik B, Bohanec B, Campion B (1998) Second cycle gynogenesis in onion (*Allium cepa* L.) and genetic analysis of the plants. Plant Breed 117(3):275–278

Jo J, Purushotham PM, Han K, Lee H-R, Nah G, Kang B-C (2017) Development of a genetic map for onion (*Allium cepa* L.) using reference-free genotyping-by-sequencing and SNP assays. Front Plant Sci 8(1606):1–8. https://doi.org/10.3389/fpls.2017.01606

Jones HA (1944) An autobiography. Herbertia 11:35–36

Jones HA, Clarke A (1943) Inheritance of male sterility in the onion and the production of hybrid seed. Proc Am Soc Hortic Sci 43:189–194

Jones HA, Davis G (1944) Inbreeding and heterosis and their relation to the development of new varieties of onions, vol 874. USDA Technical Bulletins, Washington

Jones HA, Emsweller SL (1936) A male–sterile onion. Proc Am Soc Hortic Sci 34:582–585

Jones HA, Bailey SF, Emsweller SL (1934) Thrips resistance in the onion. Hilgardia 8:215–232

Juokevieiene D, Stanys V, Bobinas E (2005) Gynogenesis pecularities of *Allium* L. vegetables grown in Lithuania. Biologija 3:6–9

Karim MA, Adachi T (1997) Cell suspension, isolation and culture of protoplasts of *Allium cepa*. Plant Cell Tissue Organ Cult 51(1):43–47

Keller J (1990) Results of anther and ovule culture in some species and hybrids in the genus *Allium* L. Arch Züchtungsforsch 20:189–197

Keller ERJ, Korzun L (1996) Haploidy in onion (*Allium cepa* L.) and other *Allium* species. In: Jain SM, Sopory SK, Veilleux RE (eds) *In vitro* haploid production in higher plants, vol 3. Kluwer Academic Publishers, Dordrecht, pp 51–75

Khar A, Saini N (2016) Limitations of PCR based molecular markers to identify male sterile and maintainer plants from Indian onion (*Allium cepa* L.) populations. Plant Breed 135:519–524

Khar A, Jakse J, Havey MJ (2008) Segregations for onion bulb colors reveal that red is controlled by at least three loci. J Am Soc Hortic Sci 133(1):42–47

Khar A, Kumar A, Islam S, Kumar A, Agarwal A (2018) Genotypic response towards haploid induction in short day tropical Indian onion (*Allium cepa* L.). Indian J Agric Sci 88(5):709–713

Khosa JS, McCallum J, Dhatt AS, Macknight RC (2016) Enhancing onion breeding using molecular tools. Plant Breed 135(1):9–20

Khrustaleva LI, Kik C (1998) Cytogenetical studies in the bridge cross *Allium cepa* × (*A. fistulosum* × *A. roylei*). Theor Appl Genet 96(1):8–14

Khrustaleva L, Jiang J, Havey MJ (2016/2015) High-resolution tyramide-FISH mapping of markers tightly linked to the male-fertility restoration (Ms) locus of onion. Theor Appl Genet 129:535–545

Kik C, Buiteveld J, Verbeek WHJ (1997) Biotechnological aspects of Allium breeding. Acta Hortic 433:291–297

Kim S (2014) A co-dominant molecular marker in linkage disequilibrium with a restorer of fertility gene (*Ms*) and its application in re–evaluation of inheritance of fertility restoration in onions. Mol Breed 34:769–778

Kim JW, Soh WY (1996) Plant regeneration through somatic embryogenesis from suspension cultures of *Allium fistulosum* L. Plant Sci 114(2):215–220

Kim S, Binzel ML, Yoo KS, Park S, Pike LM (2004) Pink (P), a new locus responsible for a pink trait in onions (*Allium cepa*) resulting from natural mutations of anthocyanidin synthase. Mol Gen Genomics 272(1):18–27

Kim S, Yoo KS, Pike LM (2007) Production of doubled haploid onions (*Allium cepa*) and evaluation of their field performance. Hortic Environ Biotechnol 48(3):143–147

Kim S, Lee ET, Cho DY, Han T, Bang H, Patil BS (2009) Identification of a novel chimeric gene, *orf*725, and its use in development of a molecular marker for distinguishing among three cytoplasm types in onion (*Allium cepa*L.). Theor Appl Genet 118:433–441

Kim S, Park J, Yang T (2015a) Comparative analysis of the complete chloroplast genome sequences of a normal male-fertile cytoplasm and two different cytoplasms conferring cytoplasmic male sterility in onion (*Allium cepa*L.). J Hortic Sci Biotechnol 90:459–468

Kim S, Kim CW, Park M, Choi D (2015b) Identification of candidate genes associated with fertility restoration of cytoplasmic male-sterility in onion (*Allium cepa* L.) using a combination of bulked segregant analysis and RNA-seq. Theor Appl Genet 128(11):2289–2299

Kim B, Kim K, Yang T, Kim S (2016a) Completion of the mitochondrial genome sequence of onion (*Allium cepa* L.) containing the CMS-S male-sterile cytoplasm and identification of an independent event of the *ccmFN* gene split. Curr Genet 62:873–885

Kim S, Kim CW, Choi MS, Kim SD (2016b) Development of a simple PCR marker tagging the *Allium roylei* fragment harboring resistance to downy mildew (*Peronospora destructor*) in onion (*Allium cepa* L.). Euphytica 208(3):561–569

Kim B, Yang T-J, Kim S (2019) Identification of a gene responsible for cytoplasmic male-sterility in onions (*Allium cepa* L.) using comparative analysis of mitochondrial genome sequences of two recently diverged cytoplasms. Theor Appl Genet 132(2):313–322

King JJ, Bradeen JM, Havey MJ (1998) Variability for restriction fragment-length polymorphisms (RFLPs) and relationships among elite commercial inbred and virtual hybrid onion populations. J Am Soc Hortic Sci 123(6):1034–1037

Kofoet A, Kik C, Wietsma WA, De Vries JN (1990) Inheritance of resistance to downy mildew (*Peronospora destructor* [Berk.] Casp.) from *Allium roylei*Stearn in the backcross *Allium cepa* L. (*A. roylei_ A. cepa*). Plant Breed 105:144–149

Kuhl JC, Cheung F, Yuan Q, Martin W, Zewdie Y, McCallum J, Catanach A, Rutherford P, Sink KC, Jenderek M, Prince JP (2004) A unique set of 11,008 onion expressed sequence tags reveals expressed sequence and genomic differences between the monocot orders Asparagales and Poales. Plant Cell 16(1):114–125

Lee KS, Ono K (2000) Somatic embryogenesis pathways in cultured *Allium fistulosum* cells. Cytologia 65(3):343–349

Lee J-H, Natarajan S, Biswas MK, Shirasawa K, Isobe S, Kim H-T, Park J-I, Seong C-N, Nou I-S, Min XJ (2018) SNP discovery of Korean short day onion inbred lines using double digest restriction site-associated DNA sequencing. PLOS ONE 13(8):e0201229

Liu ZH, Wang WC, Yan SY (1997) Effect of hormone treatment on callus formation and endogenous indole-acetic acid and polyamine contents of soybean hypocotyl cultivated in vitro. Bot Bull Acad Sin 38:171–176

Martin W, McCallum J, Shigyo M, Jakse J, Kuhl JC, Yamane N, Sink KC, Town CD, Havey MJ (2005) Genetic mapping of expressed sequences in onion and *in silico* comparisons show scant colinearity with rice. Mol Genet Genom 274:197–204

Martinez LE, Aguero CB, Lopez ME, Galmarini CR (2000) Improvement of *in vitro* gynogenesis induction in onion (*Allium cepa* L.) using polyamines. Plant Sci 156(2):221–226

McCallum J (2007) Onion. In: Kole C (ed) Vegetables. Springer, Berlin/Heidelberg, pp 331–347

McCallum J, Clarke A, Pither-Joyce M, Shaw M, Butler R, Brash D, Scheffer J, Sims I, van Heusden S, Shigyo M, Havey MJ (2006) Genetic mapping of a major gene affecting onion bulb fructan content. Theor Appl Genet 112(5):958–967

McCallum J, Pither-Joyce M, Shaw M, Kenel F, Davis S, Butler R, Scheffer J, Jakse J, Havey MJ (2007) Genetic mapping of sulfur assimilation genes reveals a QTL for onion bulb pungency. Theor Appl Genet 114(5):815–822

McCallum J, Thomson S, Pither-Joyce M, Kenel F, Clarke A, Havey MJ (2008) Genetic diversity analysis and single-nucleotide polymorphism marker development in cultivated bulb onion based on expressed sequence tag–simple sequence repeat markers. J Am Soc Hortic Sci 133(6):810–818

McCallum J, Baldwin S, Shigyo M, Deng Y, van Heusden S, Pither-Joyce M, Kenel F (2012) AlliumMap-A comparative genomics resource for cultivated Allium vegetables. BMC Genomics 13(1):168

McCollum G (1971) Sterility of some interspecific Allium hybrids. J Amer Soc Hort Sci 96:359–362

Michalik B, Adamus A, Nowak E (2000) Gynogenesis in polish onion cultivars. J Plant Physiol 156(2):211–216

Molenaar N (1984) Thrips (*Thripstabaci* L.) resistance and epicuticular wax characteristics of nonglossy and glossy onions (*Allium cepa* L.). PhD Dissertation, University of Wisconsin Madison

Mukhambetzhanov SK (1997) Culture of non-fertilized female gametophytes *in vitro*. Plant Cell Tissue Organ Cult 48(2):111–119

Muller H (1883) The fertilization of flowers (trans: Thompson DW). Macmillan and Company, London

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15(3):473–497

Muren RC (1989) Haploid plant induction from unpollinated ovaries in onion. Hortic Sci 24(5):833–834

Musial K, Bohanec B, Przywara L (2001) Embryological study on gynogenesis in onion (*Allium cepa* L.). Sex Plant Reprod 13(6):335–341

Musial K, Bohanec B, Jakše M, Przywara L (2005) The development of onion (*Allium cepa* L.) Embryo sacs in vitro and gynogenesis induction in relation to flower size. In Vitro Cell Dev Biol Plant 41(4):446–452

Naini R, Pavankumar P, Prabhakar S, Kancha RK, Rao KV, Reddy VD (2019) Evolvement of nutraceutical onion plants engineered for resveratrol biosynthetic pathway. Plant Cell Rep 38:1127. https://doi.org/10.1007/s00299-019-02432-y

Ohara T, Tsukazaki H, Song YS, Wako T, Yamashita KI, Kojima A (2009) Mapping of quantitative trait loci controlling seedling growth in bunching onion (*Allium fistulosum* L.). J Jpn Soc Hortic Sci 78(4):436–442

Park J, Bang H, Cho DY, Yoon M, Patil BS, Kim S (2013) Construction of high-resolution linkage map of the *Ms* locus, a restorer–of–fertility gene in onion (*Allium cepa* L.). Euphytica 192:267–278

Pawar BB, Patil AV, Sonone HN (1975) A thrips resistant glossy selection in white onions. Res J Mahatma Phule Agric Univ 6:152–153

Ponce M, Martinez L, Galmarini C (2006) Influence of CCC, putrescine and gellam gum concentration on gynogenic embryo induction in *Allium cepa*. Biol Plant 50(3):425–428

Ponchet M, Martin-Tanguy J, Marais A, Martin C (1982) Hydroxycinnamoyl acid amides and aromatic amines in the inflorescence of some Araceae species. Phytochemistry 21:2865–2869
Puddephat IJ, Robinson HT, Smith B, Lynn J (1999) Influence of stock plant pre-treatment on gynogenic embryo induction from flower buds of onion. Plant Cell Tissue Organ Cult 57(2):145–148
Rabobank (2018) The world vegetable map-Key global trends. https://www.rabobank.com/en/raboworld/articles/the-world-vegetable-map.html
Raines S, Henson C, Havey MJ (2009) Genetic analyses of soluble carbohydrate concentrations in onion bulbs. J Am Soc Hortic Sci 134(6):618–623
Ramakrishnan M, Ceasar SA, Duraipandiyan V, Daniel MA, Ignacimuthu S (2013) Efficacious somatic embryogenesis and fertile plant recovery from shoot apex explants of onion (*Allium cepa* L.). In Vitro Cell Dev Biol Plant 49(3):285–293
Sato Y (1998) PCR amplification of 671 CMS–specific mitochondrial nucleotide sequences to identify cytoplasmic genotypes of onion (*Allium cepa*L.). Theor Appl Genet 96:367–370
Scholten OE, Van Heusden AW, Khrustaleva LI, Burger-Meijer K, Mank RA, Antonise RGC, Harrewijn JL, Van Haecke W, Oocst EH, Peters RJ (2007) The long and winding road leading to the successful introgression of downy mildew resistance into onion. Euphytica 156:345–353
Scholten OE, van Kaauwen MP, Shahin A, Hendrickx PM, Keizer LP, Burger K, van Heusden AW, van der Linden CG, Vosman B (2016) SNP-markers in Allium species to facilitate introgression breeding in onion. BMC Plant Biol 16(1):187
Schweisguth B (1973) E'tuded'un nouveau type de stérilité male chez l'oignon, *Allium cepa*L. Ann Ame'lior Plant 23:221–233
Shigyo M, Kik C (2008) Onion. In: Vegetables II. Springer, New York, pp 121–159
Shigyo M, Tashiro Y, Isshiki S, Miyazaki S (1996) Establishment of a series of alien monosomic addition lines of Japanese bunching onion (*Allium fistulosum*L.) with extra chromosomes from shallot (*A. cepa* L. Aggregatum group). Genes Genet Syst 71(6):363–371
Shock CC, Feibert E, Jensen L, Mohan SK, Saunders LD (2008) Onion variety response to Iris yellow spot virus. Hortic Technol 18(3):539–544
Singh RK, Bhonde SR (2011) Performance studies of exotic onion (*Allium cepa* L.) hybrids in the Nashik region of Maharashtra. Ind J Hill Farm 24:29–31
Sivalingam A, Chavan AA, Gopal J, Mote SR, Shelke PV, Lawande KE (2014) Variation in gynogenic potential for haploid induction in Indian short-day onions. Ind J Genet 74(4):526–528
Song P, Peffley EB (1994) Plant regeneration from suspension cultures of *Allium fistulosum* and *A. fistulosum* × *A. cepa* interspecific hybrid. Plant Sci 98(1):63–68
Sulistyaningsih E, Yamashita K-i, Tashiro Y, Euphytica (2002) Haploid induction from F 1 hybrids between CMS shallot with *Allium galanthum* cytoplasm and common onion by unpollinated flower culture. Euphytica 125(1):139–144
Tsukazaki H, Yamashita KI, Yaguchi S, Masuzaki S, Fukuoka H, Yonemaru J, Kanamori H, Kono I, Hang TTM, Shigyo M, Kojima A (2008) Construction of SSR-based chromosome map in bunching onion (*Allium fistulosum*). Theor Appl Genet 117(8):1213
Tsukazaki H, Honjo M, Yamashita KI, Ohara T, Kojima A, Ohsawa R, Wako T (2010) Classification and identification of bunching onion (*Allium fistulosum*) varieties based on SSR markers. Breed Sci 60(2):139–152
Van der Meer QP, De Vries JN (1990) An interspecific cross between *Allium roylei*Stearn and *Allium cepa* L., and its backcross to *A. cepa*. Euphytica 47(1):29–31
Van Heusden AW, Shigyo M, Tashiro Y, Vrielink-van Ginkel R, Kik C (2000a) AFLP linkage group assignment to the chromosomes of *Allium cepa* L. via monosomic addition lines. Theor Appl Genet 100(3–4):480–486
Van Heusden AW, Van Ooijen JW, Vrielink-van Ginkel R, Verbeek WHJ, Wietsma WA, Kik C (2000b) A genetic map of an interspecific cross in Allium based on amplified fragment length polymorphism (AFLP™) markers. Theor Appl Genet 100(1):118–126
Villano C, Esposito S, Carucci F, Iorizzo M, Frusciante L, Carputo D, Aversano R (2019) High-throughput genotyping in onion reveals structure of genetic diversity and informative SNPs useful for molecular breeding. Mol Breed 39(5):1–11 https://doi.org/10.1007/s11032-018-0912-0

Villanueva-Mosqueda E, Havey MJ (2001) Genetic analyses of seed yield in onion. J Am Soc Hortic Sci 126(5):575–578

von Kohn C, Kiełkowska A, Havey MJ, Bonen L (2013) Sequencing and annotation of the chloroplast DNAs and identification of polymorphisms distinguishing normal male-fertile and male-sterile cytoplasms of onion. Genome 56(12):737–742

Vu HQ, Iwata M, Yamauchi N, Shigyo M (2011) Production of novel alloplasmic male sterile lines in *Allium cepa* harbouring the cytoplasm from Allium roylei. Plant Breed 130(4):469–475

Yang HY, Zhou C (1982) *In vitro* induction of haploid plants from unpollinated ovaries and ovules. Theor Appl Genet 63(2):97–104

Yang HY, Yan H, Zhou C (1990) *In vitro* production of haploids in Helianthus. In: Bajaj YPS (ed) Legumes and oilseed crops I. Springer, Berlin/Heidelberg, pp 472–484

Yang YY, Huo YM, Miao J, Liu BJ, Kong SP, Gao LM, Liu C, Wang ZB, Tahara Y, Kitano H, Wu X (2013) Identification of two SCAR markers co-segregated with the dominant *Ms*– and recessive *ms*–alleles in onion (*Allium cepa*L.). Euphytica 190:267–277

Yen DE (2011) Pollen sterility in pukekohe longkeeper onions. N Z J Agric Res 2(3):605–612

Yuan Q, Song C, Gao L, Zhang H, Yang C, Sheng J, Ren J, Chen D, Wang Y (2018) Transcriptome de novo assembly and analysis of differentially expressed genes related to cytoplasmic male sterility in onion. Plant Physiol Biochem 125:35–44

Zheng SJ, Henken B, Sofiari E, Jacobsen E, Krens FA, Kik C (1998) Factors influencing induction, propagation and regeneration of mature zygotic embryo-derived callus from *Allium cepa*. Plant Cell Tissue Organ Cult 53(2):99–105

Zheng S, Henken B, Sofiari E, Keizer P, Jacobsen E, Kik C, Krens F (1999) Effect of cytokinins and lines on plant regeneration from long-term callus and suspension cultures of *Allium cepa* L. Euphytica 108(2):83–90

Zheng SJ, Khrustaleva L, Henken B, Sofiari E, Jacobsen E, Kik C, Krens FA (2001) *Agrobacterium tumefaciens* - mediated transformation of *Allium cepa* L.: the production of transgenic onions and shallots. Mol Breed 7(2):101–115

Zheng SJ, Henken B, Ahn YK, Krens FA, Kik C (2004) The development of a reproducible *Agrobacterium tumefaciens* transformation system for garlic (*Allium sativum* L.) and the production of transgenic garlic resistant to beet armyworm (*Spodopteraexigua*Hübner). Mol Breed 14(3):293–307

Zheng SJ, Henken B, De Maagd RA, Purwito A, Krens FA, Kik C (2005) Two different *Bacillus thuringiensis* toxin genes confer resistance to beet armyworm (*Spodopteraexigua* Hübner) in transgenic Bt-shallots (*Allium cepa* L.). Transgenic Res 14(3):261–272

Chapter 5
Accelerated Improvement of Cole Vegetable Crops

Pritam Kalia and Shrawan Singh

5.1 Introduction

Cole vegetables are widely recognized as valuable sources of dietary fibre; minerals such as calcium and magnesium, vitamin C and provitamin A carotenoids; and certain glucosinolates that may confer a chemoprotective effect in human body. Cole vegetables are members of *Brassica oleracea* subspecies which include the cabbages, collards, cauliflower, broccoli, Brussels sprouts, the kales and kohlrabi (Table 5.1). These are predominant and hardy cool-season plants that are similar in cultural requirements, morphology, disease and insect pest susceptibilities. In the western hemisphere, including Europe, the predominance goes to cabbage, cauliflower and broccoli, while in Asia, cabbage and cauliflower are major Cole vegetables, and others like broccoli, knol khol, Brussels sprouts and kale are still grown at small scale. The centre of origin of *B. oleracea* is the Mediterranean region, and they are cultivated in Europe since ancient time from where they spread to other parts of the world. In India, introduction of Cole crops was initiated by Britishers in 1822 and continued to recent decades. Cole vegetables include many different morphotypes, which are well adapted to temperate climates, require quite simple cultivation techniques and produce abundant nutritious food for human beings.

Breeding strategy and targets are dependent on market trends. Successful breeders anticipate changes in the market by developing new varieties that are ready to be released to the growers when their demand increases. It is, therefore, interesting to see how breeding is reacting to eventual changes in *Brassica* consumption and to evaluate the potential influence that the *Brassica* market and growing systems may

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Table 5.1 Economically important Cole vegetable crops

Crop	Botanical name	Centre of origin/ evolution	Edible parts	Cultivation scale[a]	Breeding attempts[b]
Cauliflower	*B. oleracea* var. *botrytis* L.	Syria	Curd	Large	Large
Cabbage	*B. oleracea* var. *capitata* L.	Germany	Head	Large	Large
Broccoli	*B. oleracea* L. var. *italic* Plenck	Syria	Head	Medium	Fair
Brussels sprouts	*B. oleracea* L. var. *gemmifera* DC.	Belgium	Knobs	Medium	Limited
Kohlrabi	*B. oleracea* L. var. *gongylodes* DC.	Mediterranean area	Tubers	Small	Limited
Collards	*B. oleracea* var. *viridis* L.	Greece	Leaves	Small	Limited
Kale	*B. oleracea* L. var. *acephala* DC	Greece	Leaves	Small	Limited
Marrow-stem kale	*B. oleracea* L. var. *medullosa* Thell.	Southern Italy	Leaves	Small	Limited

[a]Based on cultivation area
[b]Based on varieties developed

have on the definition of breeding targets and priorities. The conventional breeding approaches take time and resources, hence, need to equip with innovative tools to enhance the efficiency, reduce time and increase accuracy of breeding methods. The innovative approaches such as genomics, transcriptomics, TILLING and EcoTILLING, single SNPs discovery and next-generation sequencing, genome-wide studies, association mapping, marker-assisted breeding and QTLs mapping and introgression and genomic selection have shown promise in breeding for complex traits. The genomics provides breeders with a new set of tools and techniques to facilitate study of the genotype of complex traits and their relationship with phenotype (Tester and Langridge 2010). They are leading towards genomics-based plant breeding which is better option to handle complex traits. Development of next-generation sequencing (NGS) methods provides genome-wide molecular tools for breeders such as large collections of molecular markers, high-throughput genotyping strategies, high-density genetic maps, new experimental populations, etc. that can be incorporated into existing breeding methods (Varshney and Tuberosa 2007; Tester and Langridge 2010; Lorenz et al. 2011). These high-throughput DNA sequencing technologies and bioinformatics facilitated new breeding approaches such as association mapping, marker-assisted selection, breeding by design, gene pyramiding and genomic selection (Collard and Mackill 2008). These approaches are useful for dealing with complex traits such as glucosinolates, heat tolerance and resistance to diseases and insect pests. Genomic tools are thus facilitating the detection of QTLs and the identification of existing favourable alleles of small effect, which have frequently remained unnoticed and have not been included in the gene pool used for breeding (Vaughan et al. 2007).

5.2 Breeding Objectives in Cole Vegetables

In Cole vegetables, the most important objective of breeding is crop uniformity for maturity, field appearance and shape and size of economic parts. New varieties/hybrids should have high commercial quality, including adequate size and shape, good colour, firmness and appearance to have the produces easily accepted by the trade. The breeding objectives and methodology earlier reviewed by Kalia (2009) have been further improved in this article. High level of uniformity is almost difficult to achieve in case of open-pollinated varieties and in F_1 hybrids using conventionally developed inbred lines due to the cross-pollination nature of the Cole vegetables. Production of F_1 hybrids with great extent of uniformity and in reduced time span could be possible by use of doubled haploid (DH) lines. Now, the cytoplasmic male sterility (CMS) system has been established in *B. oleracea* for use in hybrid breeding of Cole vegetables. This will help to overcome the problems of instability of SI level and complexities in maintenance of SI lines. Development of varieties/hybrids resistant to diseases and insect pests is an important objective both to reduce pesticide load in food and environment. Qualitative traits have relatively high importance in vegetable breeding. In *Brassica* Cole vegetables, selective approach for glucosinolates is mainly required because some have harmful health effects. New 'speciality traits' such as orange, green and purple curds in cauliflower, purple and yellow heads in broccoli, purple sprouts in Brussels sprouts and tuber in knol khol, etc. are gaining attraction. Further, in changing climatic scenario, development of resilient varieties/hybrids enabled with traits such as reduced crop period, extended reproductive phase, elongated root length, better blanching habit, etc. is desirable.

5.3 Understanding Evolutionary Process of Cole Vegetables

Crucifer species have a common evolutionary ancestry, and it is possible to generate interspecific crosses among the six *Brassica* species. Three diploid species *B. oleracea* (CC, $2n = 18$), *B. nigra* (BB, $2n = 16$) and *B. rapa* (AA, $2n = 20$) given rise to three amphidiploid species, namely, *B. carinata* (BBCC, $2n = 34$), *B. juncea* (AABB, $2n = 36$) and *B. napus* (AACC, $2n = 38$) (Hansen and Earle 1995). Accessions of these species can be difficult to discriminate morphologically and are frequently misidentified in germplasm collections. Chloroplast DNA (cpDNA) is highly conserved in plant kingdom, but cpDNA primers are useful to discriminate species type, along with the direction of the cross of the ancestral hybrids (Cunha et al. 2004). The modern *B. oleracea* crops are derived from the wild *B. oleracea* and not from the wild Mediterranean species (Hodgkin 1995), and the early cultivated forms of *B. oleracea* were brought from the Atlantic coast to the Mediterranean region where selection took place. However, Swarup and Brahmi (2005) refurbish the hypothesis of Mediterranean origin of these crops mainly through observing the

mutation and introgression from wild species. Liu et al. (2014) described new insights into genome duplication and gene divergence, imparting biochemical and morphological variation to *B. oleracea*. Maggioni et al. (2014) reported higher level of genetic diversity in leafy kales than in wild populations from Calabria and Sicily.

The distinct morphologies exhibited by *B. oleracea* subspecies represent one of the most spectacular illustrations of structural evolution in plants under domestication. *Brassica oleracea* is an extremely polymorphic species with at least six cultivated and one wild subspecies, all of which have extreme morphological divergence. Defining characteristics of *Brassica oleracea* are precociously large, undifferentiated inflorescence forming curd for *B. oleracea* var. *botrytis*; small flower buds for var. *italica*; leaves folded into compact heads and large terminal heads for var. *capitata*; loose or open foliage for var. *acephala*; small axillary heads for var. *gemmifera*; and much expanded to a bulbous stem for var. *gongylodes* (Tsunoda et al. 1980). In cauliflower, only ~10% of arrested inflorescence develops into floral primordia and normal flowers. The cauliflower 'curd' phenotype in mutants of *Arabidopsis thaliana* is due to a class of flower developmental regulatory genes, viz. *APETALA 1* (Mandel et al. 1992) and *CAULIFLOWER* (Gustafson-Brown et al. 1994) that specify the identity of the floral meristem in developing reproductive primordia. *Arabidopsis* individuals that are mutant for both *AP1* and *CAL* had arrested inflorescence and a dense mass similar to cauliflower curd. In *B. oleracea*, involvement of orthologous genes *BoCAL* has been proposed for altered inflorescence in var. *botrytis* (Kempin et al. 1995). The *BoCAL* allele has a premature termination codon at position 151 (E → stop) which appears to be of recent origin. Alleles carrying this nonsense mutation in exon 5 of *BoCAL* are nearly fixed in *B. oleracea* ssp. *botrytis* and *B. oleracea* ssp. *italica*, both of which show evolutionary modifications of inflorescence structures (Purugganan et al. 2000). Hence, specific alleles of *BoCAL* were selected at early stage of domestication. Based on molecular allelic variation, Smith and King (2000) suggested that curd type arose in southern Italy from a heading Calabrese broccoli via an intermediate Sicilian crop type. Close association of *BoAP1-a* and *BoAP1-c* with the self-incompatibility locus S may have reduced number of S-alleles within the gene pool. Duclos and Bjorkman (2008) investigated that the transcript abundance of *BoFUL* paralogues and *BoLFY* was highest at inflorescence meristem arrest, and maintenance of this arrest is a consequence of suppression of *BoCAL*, *BoAP1-a* or *BoLFY* or failure to suppress *BoTFL1* (a strong repressor of flowering in *Arabidopsis*). Hence, initiation of floral primordia and enlargement of floral buds in broccoli and cauliflower are not entirely controlled by homologues genes in *Arabidopsis*. Li et al. (2017) identified a novel homologous gene containing the Organ Size Related (OSR) domain *CDAG1* (Curd Development Associated Gene 1) in cauliflower. It has higher transcript levels in young tissues and promotes organ growth by increasing cell number which results into larger organ size and increased biomass. This gene inhibits transcriptional expression of endogenous OSR genes, *ARGOS* and *ARL*. Rosan et al. (2018) studied a genome-based model simulating the development of doubled haploid (DH) lines to time to curd induction and observed $R^2 = 0.40$ for the quantitative traits and $R^2 = 0.48$ for the GS model. The increase in *BoAP1-a* and *BoAP1-c*

transcript levels in cauliflower just before floral primordium initiation and the *BoLFY* expression are observed once plants are in the reproductive stage (Duclos and Bjorkman 2008). Application of GAs during reproductive development stage does not activate meristem identity genes or A-function genes (Yu et al. 2004). Hence, GAs (GA_3 and GA_{4+7}) can trigger the vegetative-to-reproductive transition in both cauliflower and broccoli resulting in early curd formation (Duclos and Bjorkman 2015); however, there is occurrence of bracting disorder.

5.4 Genetic Diversity and Exploring Wild Relatives

Pre-breeding is important activity in crop improvement, and it refers to all the activities designed to (i) identify desirable characteristics and/or genes from nonadapted (exotic or semi-exotic) materials and (ii) transfer these traits into an intermediate set of materials. In case of the diverse Cole vegetables, large numbers of species are being explored for CMS or other important traits. The wild or related species are canola (*B. napus* L. and hybrids with *B. campestris*); turnip (*Brassica rapa* subsp. *rapa* L.) and Chinese cabbage (*B. campestris* L.); rutabaga or Swede (*B. napobrassica* L.); black mustard (*B. nigra* Koch), brown mustard [*B. juncea* (L.) Czern] and Ethiopian mustard (*B. carinata*); and *B. macrocarpa, B. villosa, B. rupestris* and *B. incana* for *B. oleracea*. Tonguc and Griffiths (2004) produced interspecific hybrid plants and BC_1 progeny through embryo rescue between *Brassica carinata* 'BI 360883' and *B. oleracea* cultivars 'Titleist' and 'Cecile' to transfer resistance to powdery mildew into *B. oleracea.* All interspecific hybrids and eight of the BC_1 plants were resistant to powdery mildew. Introgression of black rot resistance (for both race 1 and race 4) from *B. carinata* was attempted into snowball cauliflower using embryo rescue by Dey et al. (2015) and in Indian cauliflower genotype for Xcc race 1 by Sharma et al. (2017). Successful introgression of genes by sexual hybridization for club root resistance from *B. napus* was performed in cabbage (Chiang et al. 1977) and from *Raphanus sativus* into *B. oleracea* var. *botrytis* (Hagimori et al. 1992). Sarikamis et al. (2006) introgressed genomic segment (having Myb28 allele) from *B. villosa* into broccoli which had enhanced levels of 4-methylsulphinylbutyl glucosinolate. Gu et al. (2014) attempted cross-breeding with these introgressed inbred lines and doubled haploids and reported significantly higher glucoraphanin in 31 of the 61 F_1 hybrids than commercial cultivar Youxiu. Sharma et al. (2016) initiated introgression of black rot resistance *Xca1bc* locus (on B7 chromosome) from *B. carinata* to *B. oleracea* var. *botrytis* using ILPAt1g70610 marker and embryo rescue. Understanding the syntenic relationships between plant species can be useful for rapid transfer of knowledges from one to other. In case of *Brassicas*, *A. thaliana* is a model plant for molecular studies, and Cheng et al. (2012) investigated syntenic gene analysis between *Brassica rapa* and other Brassicaceae species *Arabidopsis lyrata* and *Thellungiella parvula* and reported that only 5851 genes in *B. rapa* had no syntenic counterparts in any of the other three species.

Pre- and post-fertilization barriers limited the diversity of different genomic combinations in *Brassica*. Haploid genomes of each parent are combined in sexual hybrids, which usually results in problems of pairing of non-homologous chromosomes during meiosis. Combinations of other *Brassica* genomes into somatic hybrids combinations have been produced to improve disease resistance in *B. oleracea.* Black rot resistance was transferred from *B. napus* to *B. oleracea* (Hansen and Earle 1995). The primary hybrids have been utilized as bridges for transferring the genes for resistance to the cultivated crop by backcrossing. In several cases, for example, club root, black spot and black rot, BC_1 progenies have been obtained displaying disease resistance confirming, thereby, that transfer and inheritance of the resistance genes can be established via fusion. Arumugam et al. (2000) developed somatic hybrids by fusing protoplasts of CMS *B. juncea* (AABB) with '*oxy*' cytoplasm and normal *B. oleracea* (CC) and corrected chlorosis problem. Ishikawa et al. (2003) produced somatic hybrids between greenish protoplasts isolated from the mesophyll of *Moricandia arvensis* (MaMa, 2*n* = 28) and colourless ones from hypocotyls of *Brassica oleracea* (CC, 2*n* = 18) through cell fusion. In leaf anatomy, the somatic hybrid showed the C_3–C_4 intermediate trait as in *M. arvensis*. The somatic hybrids are expected to be used as bridging plant material to introduce the C_3–C_4 intermediate trait into *Brassica* crop species. Somatic hybrids were also generated between *Arabidopsis thaliana* and cabbage (Yamagishi et al. 2008). Wang et al. (2016) studied 28 putative introgression lines (ILs) from asymmetric somatic hybridization of cauliflower (2*n* = 18, CC) and *Brassica nigra* (2*n* = 16, BB) and generated novel germplasm resource for cauliflower improvement. Protoplast fusion has been used to develop disease-resistant lines. Ren et al. (2000) selected protoplasts from *B. rapa* and *B. oleracea* genotypes for resistance to *Erwinia* soft rot and reported that the offspring populations of the somatic hybrids (F_1–S_1 and F_1–BC_1) had resistance. The isolated protoplast mass fusion from different sources of tissues makes it possible to combine genetic material independent of species and sexual compatibility.

Liu et al. (2014) published whole genome sequences of cabbage (line 02–12) and revealed a multilayered asymmetrical evolution of the *Brassica* genomes and mechanisms of polyploid genome evolution underlying speciation. Afterwards, the factors for route of genome evolution were decided by asymmetrical gene loss, asymmetrical amplification of transposable elements and tandem duplications, preferential enrichment of genes for certain pathways and divergence in DNA sequence and expression, including alternative splicing among a large number of paralogous and orthologous genes. Genome-wide survey of genetic diversity is useful to elucidate the causative genetic differences that give rise to observed phenotypic variation providing a foundation for dissecting complex traits by genome-wide association studies. Genome-wide association relies on the natural patterns of linkage disequilibrium (LD) in the target population, and it helps in narrowing down the mapping resolution from a large genomic portion where the LD level is relatively high to a single marker when the LD level is very low. The 'omics' disciplines, like proteomics and metabolomics, are useful to understand how the changes in the genotype lead to differences in the final phenotype. Phenomics is a new addition in

high-throughput technologies to characterize germplasm (Imaging 2009). El-Esawi et al. (2016) reported 27.1% genetic variation among accessions while 72.9% within the accessions in cabbage, cauliflower, Brussels sprouts and kale using SSR markers from Ireland. Yousef et al. (2018) characterized 192 cauliflower accessions from the USDA and IPK genebanks with genotyping by sequencing (GBS). The analysis of genetic diversity revealed that accessions formed two major groups that represented the two genebanks and were not related to the country of origin, indicating that the composition and type of accessions have a strong effect on the germplasm structure, even though, regeneration procedures and local adaptation to regeneration conditions too influence.

5.5 Genetic Mechanisms for Hybrid Breeding

5.5.1 Self-Incompatibility

Self-incompatibility (SI) is an elaborated breeding system for securing outcrossing and maximum recombination in the angiosperms. In the Brassicaceae, it is classified into the homomorphic sporophytic type. The SI locus (S-locus) is very polymorphic, and cross-compatibility between plants depends on the allelic forms (S-haplotypes) of genes located on it. Among these genes, SLG (S-locus glycoprotein gene) is specifically expressed in the stigma. Two classes (class I and class II) of SLG genes have been described, and antibodies allowing a specific identification of class I or class II SLG are available. Edh et al. (2009) investigated evolutionary relationships among *S* haplotypes in the Brassicaceae family using DNA sequences from the *SRK* and *SCR* genes of the *Brassica cretica* with available sequence data in other Brassicaceae species in public domain. They could find that wild and cultivated *Brassica* species have similar levels of *SRK* diversity, indicating that domestication has had but a minor effect on *S*-locus diversity in *Brassica*. The SI system is a genetically controlled mechanism which favours cross-pollination and commonly used in hybrid seed production of Cole crops. All *Brassica* vegetables have sporophytic SI system being strongest in kale and weakest in summer (European) cauliflower. A detailed investigation in Indian cauliflower for SI level revealed that inbred lines of maturity group I have strongest SI followed by maturity group II and group III showed weak SI (Chatterjee and Swarup 1984; Sharma et al. 2005). Singh et al. (2002) reported high level of SI in 13 genotypes from different groups such as in group I (IIVR-I, IIVR-50 and Kuwari-I); group II (Kataki Early-29, Hazipur-4 BP and Kataki 12); group III (Agahani JBT-23/60, Agahani-8, Agahani Long Leaf and Agahani Small Leaf); and group IV (Pusi Hazipur and Pusi-4). The Agahani-31 was found self-compatible. Bud pollination and spraying with 3% NaCl solution are used to break SI for maintaining SI lines. In cauliflower, Pusa Kartik Sankar and Pusa Hybrid-2 and in cabbage Pusa Cabbage Hybrid-1 have been developed using SI lines and released for commercial cultivation in India (Sharma et al. 2005). Verma et al. (2017) characterized SI lines of early- and mid-maturity Indian cauliflower using quantitative and

molecular analyses and reported higher diversity in mid-maturity group. However, the SI system also has problems, i.e., firstly, parental material may have low yields due to inbreeding depression, and, secondly, the procedure of breeding an inbred parent may result in considerable inadvertent selection in favour of self-fertile material within that inbred line. This results in selfing and forms higher proportion of selfed seeds in the F_1 which considerably reduces its value.

5.5.2 Male Sterility

The male sterile plants have mainly arisen as spontaneous mutants. In the Brassicaceae family, the first example of CMS was described by *Ogura* (Dunemann and Grunewaldt 1991) in a Japanese population of radish. Now there are different CMS sources available for *Brassica* crops (Yamagishi and Bhat 2014), and Shu et al. (2016) used mitochondrial markers for detection of CMS sources. The Ogura CMS system is controlled by a mitochondrial *orf138* locus that consists of two co-transcribed open reading frames: orf138 and orfB (also called *atp8*, encoding ATP synthase subunit 8) (Grelon et al. 1994). In *B. rapa*, the chlorosis of *Ogura*-CMS is due to downregulation of genes for chloroplast proteins (Jeong et al. 2017b). The commercial production of triple-cross hybrids started in 1989 with the first variety Serrida, registered on the official catalogue in France. At the same time extensive work was done to transfer this *Ogura* cytoplasm to various *Brassica* crop species by interspecific crosses (Bannerot et al. 1977).

The CMS is a maternally inherited trait encoded in the mitochondrial genome, and the male sterile phenotype arises as a result of interaction of a mitochondrial CMS gene and a nuclear fertility restoring (*Rf*) gene. It is fact that the CMS does not exist in cauliflower or its related Cole crops but *Ogura* CMS system was introduced to broccoli through backcross breeding which was later transferred from broccoli to cauliflower. Later on, it was transferred into heat-tolerant Indian cauliflower from kale and broccoli. Four lines, namely, MS-91, MS-51, MS-11 and MS-110, received 'Ogura CMS' via kale, whereas five lines (MS-01, MS-04, MS-05, MS-09 and MS-10) had this CMS system from broccoli. Use of male sterile lines not only extended the range of heterosis but also improved the quality and efficiency of hybrid seed production. Ruffio-Chable et al. (1993) reported influence of the temperature on the male sterile phenotype wherein it was revealed that at low temperature, some male sterile plants developed partial to complete male fertility, whereas at high temperature, male fertile plants became male sterile. Kaminski et al. (2012) observed presence of untypically developed plants with chimeral generative stacks or partially fertile flowers among segregating test-cross progeny of improved maintainers of *Brassica nigra* cytoplasm (CMS)-based cauliflower CMS lines suggesting the presence of some non-allelic genetic factors modifying the fertility/sterility character for a part of the plants. In *Brassicas*, several other CMS systems (e.g. oxyrrhina, polima, tournefortii and moricandia) are being investigated, but so far these could not be successfully used for hybrid seed production due to various limitations, viz. breakdown of male sterility, chlorosis, abnormalities in petals, poor nectar function and lack of appropriate restorer lines which need adequate attention.

Many cytoplasmic male sterility (CMS) systems have been elaborated in the *Brassica oleracea*. Protoplast fusion between more or less related species was used by Kameya et al. (1989) in *B. oleracea* to acquire new CMS systems. Protoplast fusion was used by Yarrow et al. (1990) to transfer the 'Polima' cytoplasm from *B. napus* to *B. oleracea* and by Cardi and Earle (1997) to transfer the *B. tournefortii* cytoplasm from *B. rapa* to *B. oleracea.* Protoplast fusion was also extensively used to induce new combinations of cytoplasmic traits. Male sterility has often been obtained by introducing the genome of one species into an alien cytoplasm and results from mitochondria/nucleus interactions, but at the same time some defects may appear from chloroplast/nucleus or mitochondria/nucleus incompatibilities. When *B. oleracea* lines were converted into *Ogura*-based male sterile lines, they showed severe chlorophyll deficiency, low nectar secretion and low heterosis under low temperature. These undesirable effects are due to the incompatibility between (*Ogura*) chloroplast and (*B. oleracea*) nucleus, and the problem could be overcome by chloroplast substitution by somatic hybridization and repeated backcrossing to cabbage (Dey et al. 2013; Walters and Earle 1993). Improved '*Ogura'* cytoplasms were then transferred to vegetable *B. rapa* (Heath et al. 1994) and cabbage (Sigareva and Earle 1997). Protoplast fusion was also efficient in combining atrazine-resistant chloroplasts in *B. rapa* with the CMS trait of *B. nigra* cytoplasm in *B. oleracea* (Christey et al. 1991).

Bhatia et al. (2015) developed protocol for in vitro maintenance of *Ogura* CMS lines of cauliflower using hypocotyls and curds as explants using MS medium supplemented with 2.5 mg/l kinetin, 0.2 mg/l naphthaleneacetic acid and 0.2 mg/l gibberellic acid. Nahm et al. (2005) described a novel male sterility system in radish as NWB-CMS with higher degree of male sterility than *Ogura* CMS from the Cruciferae family. It is different from *Ogura* system for (i) the degree of sterility of the NWB CMS was more profound than any of the other CMS types, including Ogura CMS; (ii) the NWB-CMS line was not determined to contain the *Ogura* CMS specific factor, *orf138*; (iii) there is a polymorphism of mitochondrial genome between NWB CMS and *Ogura* CMS with RFLP analysis; (iv) the genomic region between the *atp6* and *nad3* genes in the NWB CMS-specific PCR-based DNA marker; and (v) the *Ogura* CMS line was restored to male fertility when crossed with several breeding lines, whereas the NWB CMS line was not restored to male fertility, and all the progenies became male sterile. In comparison to the *Ogura* CMS lines which exhibit rudimentary, gloomy, shrunken anthers, with no visible pollen, the anthers of the NWB CMS lines were more yellowish, with some visible pollen hence attracting insect pollinators. The amount of pollen per anther in the NWB CMS line was approximately half that of male fertile lines, but all the pollen were unviable. It has been transferred to cabbage through protoplast fusion.

Conversion of elite breeding lines into CMS lines is an important requirement for use in hybrid breeding. Dey et al. (2011b) developed *Ogura*-based improved cytoplasmic male sterile (CMS) lines of snowball cauliflower, viz. Ogu1A, Ogu2A and Ogu3A, through conventional backcrossing. Chamola et al. (2013) transferred cytoplasmic male sterility from alloplasmic *B. juncea* and *B. napus* to cauliflower through interspecific hybridization and embryo culture. The CMS system has been used in commercial F_1 hybrid production in *B. oleracea* using an improved '*Ogura'* cytoplasm (Pelletier et al. 1989). Introgression of *Ogura* cytoplasm also altered

important quality traits in *Ogura* cybrid cytoplasm-based cauliflower CMS lines (Dey et al. 2017a). Dey et al. (2014b) did not observe significant difference among A and B lines for most of the vegetative traits, but they varied for curd maturity, leaf number, leaf size and plant height. They also investigated 25 CMS lines for different agronomic and floral traits along with combining ability and SSR marker analyses (Dey et al. 2017b). Other improved '*Ogura*' cytoplasms are available in *B. oleracea* as well as in vegetable *B. rapa*. In cabbage breeding, where the F_1 hybrid is harvested as a vegetative produce and its production is not dependent on fertility restoration, the ogu-CMS is well accepted and used as a valuable hybridizing system (de Melo and de Giordano 1994).

5.5.3 Combining Ability Studies

In cauliflower, a number of investigations have been done on identification of inbreds or varieties for use in heterosis breeding through specific combining ability (SCA). Locus-specific and/or genome-wide differential heterozygosity, including epistasis, plays an important role in the generation of the observed heterosis. Dixit et al. (2004) reported sufficient heterosis for early maturity, net curd weight, curd size index and curd yield. They observed Pusa Synthetic × Pusa Sharad, Pusa Synthetic × RCjob-1, RCJob-1 × Pant Shubhra, Pant Shubhra × Pusa synthetic and Pant Shubhra × Pusa Sharad as most promising crosses for yield attributes in Indian cauliflower. Earliness is an important trait of tropical cauliflower which has sufficient heterosis. Dey et al. (2014a, b) reported that the CMS line Ogu12A of cauliflower was good general combiner (GCA effect) for most of the important vitamins and antioxidant pigments. The proportions of gca/σ2sca were <1 in 40 hybrids and indicated presence of nonadditive gene action for the traits. The study suggested that the very high heterosis for ascorbic acid, anthocyanin and carotenoids in cauliflower indicated the scope for development of F_1 hybrids rich in phytonutrients. Appreciable heterosis over better parent and standard check (Punjab Giant-26) exists for curd compactness, and best cross was D-2 × PG-35 (Thakur et al. 2004). Saha et al. (2015) reported that overdominance had predominant role for marketable curd weight, curd diameter and curd depth. For marketable curd weight, dominance (h) and dominance × dominance (l) components with duplicate type of epistasis were present. Curd colour was determined by single gene with white being recessive. In Snowball group, Lawyana was the best general combiner for curd weight, curd size and leaf size. Sel.12 and Pyramis were best general combiners for early maturity and gross weight, respectively. Line IHR3, IHR4, IHR9 and IHR36 were good combiners for most of the characters. Additive genetic effect is more important than nonadditive effects in the expression of resistance to diseases, average curd mass, curd colour and hollow stalk incidence in Brazilian cauliflower materials (Arashida et al. 2017).

Varalakshmi (2009) performed line × tester analysis involving four lines and five testers in early cauliflower and reported predominance of nonadditive gene action

for days taken for 50% curd initiation, days taken for 50% curd maturity, leaf number, leaf weight, stalk weight, curd size and curd weight. In snowball cauliflower, Ram et al. (2017) revealed wide range of heterosis for important dietary minerals and identified CMS lines with good combining ability. Verma and Kalia (2017) analysed genetic component of variance, and variance due to specific combining ability (σ^2 sca) revealed preponderance of dominant variance and nonadditive gene action for leaf area (cm^2), plant height (cm), marketable curd weight (kg), net curd weight (kg), gross plant weight (kg), harvest index and curd compactness. In hybrids, contribution of lines was higher over the testers for all the traits.

5.5.4 Heterosis Breeding

Three principal requirements need to be fulfilled for breeding of hybrid cultivars: viz. (i) there must be a sufficient amount of heterosis; (ii) a system for large production of hybrid seed must be available; and (iii) there must be an efficient method to identify hybrids with high combining ability. In Cole vegetables, three different genetic mechanisms exist to produce hybrid seed: (i) self-incompatibility (SI), (ii) nuclear male sterility (NMS) and (iii) cytoplasmic male sterility (CMS). Inbreds may be obtained from in vitro anther or microspore culture as doubled haploids (DHs) or by selfing for five generations. In the latter case, stigmas are hand pollinated either by 2–5 days prior to anthesis when the SI barrier in the stigma has not yet been developed or spraying 3% NaCl solution (Monteiro et al. 1988). Habib et al. (2017) identified crosses BI-7042-24 × SH-BR-4, Calaberese Amule × SH-BR-4, SH-BR-1 × SH-BR-4, SH-BR-2 × Palam Vichitra and Palam Samridhi × Green Head as superior for total head yield and yield-related traits in broccoli.

In some of the *Brassica* vegetables, hybrid development is limited due to (i) selfing of parental plants or sib-mating within the parental lines giving rise to 'off type', (ii) less effective SI than other *Brassicas*, (iii) nonsynchronous flowering of male and female parents leading to increased proportion of sibs, (iv) inflorescence of cauliflower tends to be cymose which results in a shorter period of flowering flush leading to nonsynchrony of flowering of parent inbreds and (v) minor heterosis for curd size in contrast to substantial heterosis in other *Brassicas*. In the case of cauliflower, the extent of heterosis was reported for days to curd maturity (−3.92 to −16.3%), plant height (−10.40 to 31.33%), plant spread (−10.68 to −29.52%) and number of leaves/plant (−10.44 to −39.27%) (Garg and Lal 2005). Heterotic combinations have better performance for quality traits such as curd compactness (−36.37 to 30.58%) and colour. High heterosis was recorded for yield traits which ranged from −51.77 to 24.25%. The variation in response to stresses and in performance of hybrids could be due to changes in the salicylic acid- and auxin-regulated pathways that correlate with differences in the enhanced leaf growth (Groszmann et al. 2015). Similarly, sufficient heterosis has been reported in cabbage (Kibar et al. 2015), broccoli (Hale et al. 2007) and kohlrabi (Yumei et al. 1998). Jeong et al. (2017a, b) identified genes associated with the mechanism of heterosis of *B.*

oleracea hybrid and useful to reveal the complexity of regulatory gene networks associated with genetic mechanism of heterosis in the plant life cycle.

Prakash et al. (2015) reported highest heterosis for head weight was recorded in the hybrid S-621 × GA-121 (37.9%) followed by S-624 × GA-111 (34.1%). Pathak et al. (Pathak et al. 2007) identified 'Golden Acre' and 'Pride of Asia' as the best general combiners for marketable yield, and 'Golden Acre' × 'Pride of India', 'Golden Acre' × 'Pride of Asia' and 'Pride of India' × 'Pride of Asia' were promising specific combiners. Heterosis was reported for superoxide dismutase, peroxidase and catalase enzymes and for mineral elements in cabbage (Singh et al. 2010). Kanwar and Karla (2004) reported that dominance variance was more pronounced than additive genetic variance for all the traits except net curd weight indicating the importance of dominant gene effects. Thakur and Vidyasagar (2016) compared the SI and CMS systems for performance of hybrids in cabbage and reported that the CMS-based hybrids excelled in their performance for majority of the characters whereas for few characters, SI system-based hybrids were better. In kohlrabi, Kachroo (1984) reported medium order heterosis for most of the economic characters like net tuber weight, tuber leaf ratio dry matter content and tuber yield, and additive gene action was preponderant.

Dey et al. (2011a, b) performed line × tester analysis by taking three CMS lines (Ogu1A, Ogu2A and Ogu3A) free from floral deformities as female parent with nine diverse lines of snowball cauliflower as tester. The parent Ogu2A exhibited highest GCA effect for curd yield (4.51) and harvest index (1.97) and Ogu1A for earliness (−2.73). They further determined significant variability and heritability for CUPRAC, FRAP and total phenolics content in cauliflower. Verma and Kalia (2016) observed significant improvements in mean of the hybrids over the parents for horticultural traits in early- and mid-maturity groups. Mean of these traits were higher in mid-maturity group over early maturity. In early-maturity group, six lines (cc-13, cc-14, 14-4-17, 351aa, Pusa Deepali and SL-7) had least divergence. The combination of heterosis and diversity analysis suggested that moderate genetic diversity is most desirable to produce highly heterotic hybrids in Indian cauliflower.

5.5.4.1 Hybrids/Varieties Developed in Cole Vegetables in India

Cabbage and cauliflower are fourth and fifth important vegetable crops in India. Cauliflower has been grouped into four maturity groups depending upon temperature required for curd initiation and curd development. These are early (20–27 °C), mid-early (16–20 °C), mid-late (12–16 °C) and late or Snowball (10–16 °C) groups and also named according to months of crop maturity as September–October, November–December, December–January and February–March, respectively (Singh and Sharma 2003). Presently, each of the maturity group has improved varieties which enabled grower for year-round cultivation and earn benefits. The details of hybrids/improved varieties are given in Table 5.2 (Fig. 5.1).

Cabbage is one among Cole crops where F_1 hybrids have become very popular. Public sector has also come forward, and the first indigenous hybrid 'Pusa Cabbage Hybrid-1' (KGMR-1) was developed by utilizing SI line (Table 5.3). Recently, a CMS system-based F_1 hybrid 'KTCBH-81' of cabbage (Fig. 5.2) has been identified

Table 5.2 Varieties of Indian cauliflower developed and released in India in different maturity groups

Variety	Source	Year	Maturity	Yield (t/ha)	Remark
September maturity group (sowing, mid-May; transplanting, June end; temperature for curding, 20–30 °C)					
Pusa Meghna	IARI, New Delhi	2004	September mid	12	Compact, cream white curds
October maturity group (sowing, May end–mid-June; transplanting, mid-July; temperature for curding, 20–25 °C)					
Pusa Kartiki	IARI, New Delhi	2015	October end	22	Compact, white curds
Pusa Ashwini	IARI, New Delhi	2015	October mid	18	Compact, white curds
Sabour Agrim	BAU, Sabour	2013	2nd October	15	Compact white curds
Kashi Kunwari	IIVR, Varanasi	2005	October	16	White, compact
Pusa Kartik Sankar (F_1)	IARI, New Delhi	2004	2nd October	16	Compact, white curd
Pant Gobhi 3	GBPAUT, Pantnagar	1993	October	10	Cream, compact curds
Pusa Deepali	IARI, New Delhi	1975	2nd October	12	White, self-blanching
November maturity group (sowing, July end; transplanting, August end; temperature for curding, 15–20 °C)					
Kashi Agahani	IIVR, Varanasi	2008	December end	22	White, compact curd
Pusa Sharad	IARI, New Delhi	2004	November	24	White, compact curds
Pusa hybrid 2 (F_1)	IARI, New Delhi	1994	December mid	23	White, compact
Pant Gobhi 2	GBPAUT, Pantnagar	1993	November	12	Creamy white compact
Hisar 1	HAU, Hisar	NA	November end	12	Cream, compact curds
December maturity group (sowing, August end; transplanting, September end to mid-October; temperature for curding, 12–15 °C)					
Pusa Shukti	IARI, New Delhi	2011	January	35	White, compact
Pusa Paushja	IARI, New Delhi	2008	December	30	White, compact
Late maturity group (sowing, September end to mid-October; transplanting, October beginning; temperature for curding, 16–20 °C)					
Pusa snowball KT-25	IARI RS, Katrain		February	34	White, compact
Pusa snowball K-1	IARI RS, Katrain		February	30	White compact
Pusa snowball Hybrid-1	IARI RS, Katrain		February	35	White compact

NA data not available

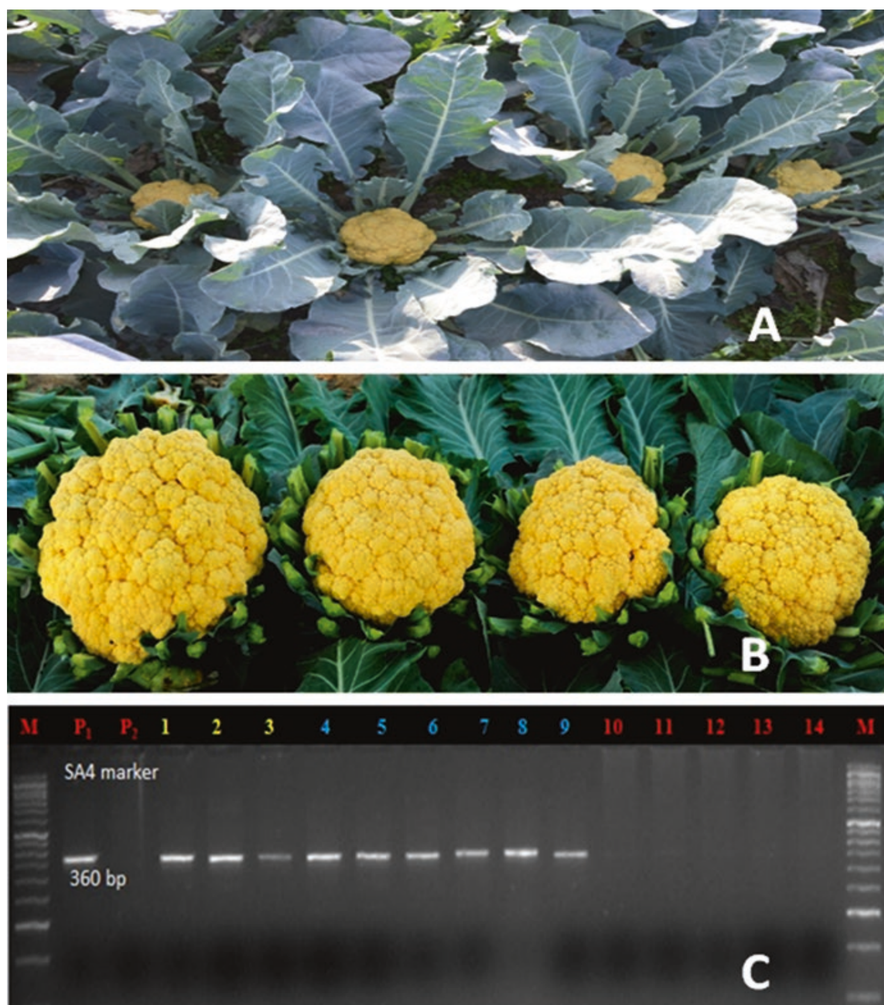

Fig. 5.1 Pusa Kesari VitA-1: First ever indigenously bred biofortified β-carotene-rich cauliflower. (**a**) and (**b**) Curds at different developmental stages. (**c**) Identified marker for foreground selection of *Or* gene. M, Marker ladder; 50 bp, P_1 1227 homozygous *Or* inbred line, P_2 DC 309 homozygous white, F_1 1–4 Dark orange, 5–9 BC_2F_1 Dark orange individuals, 10–14 BC_2F_1 white individuals. The fragments were separated on 3.0% metaphor agarose gel

for release under the AICRP (vegetable crops). Cytoplasmic male sterility (CMS) system was transferred successfully from EC 173419 to cabbage (Prakash et al. 2018), broccoli and knol khol at ICAR-IARI Regional Station, Katrain, India. Presently, *Ogura* CMS lines along with their respective maintainers and stable self-incompatible lines of cabbage are being utilized for the development of F_1 hybrids at the IARI Regional Station, Katrain.

Table 5.3 The details of the important varieties of cabbage

Variety	Year	Source	Days for maturity	Weight of head (kg)	Yield per ha	Head shape	Remark
Golden acre	1976	IARI RS, Katrain	60–65 days	1–1.5	25	Round, compact	Early, interior white colour
Pusa Mukta	1985	IARI RS, Katrain	70–75 days	1.5–2.0	20–30 t	Flattish round, solid	Moderate resistant to black rot, poor field stand
Pusa Drum Head (Japan)	1970	IARI RS, Katrain	90 days	3.0–4.0	50–54	Flat, less compact	Field resistant to black leg
Pride of India	NA	YSP UHF, Solan	70–75 days	1–1.5	20–28	Small round compact	–
Kinner red	NA	YSP UHF, Solan	70–80 days	0.5–0.06		Oval, compact	Red cabbage
September (Germany)	NA	TNAU, Coimbatore	110 days	4.0–6.0	40–50	Oblong, very compact	For Nilgiri Hills Long stalk
Pusa Ageti	2000	IARI, new Delhi	70–90 days	0.6–1.2	11–33	Flattish round	1st tropical variety, produce seeds in plains
Pusa cabbage Hybrid-1	2012	IARI RS, Katrain	60–65	0.6–1.0	30–40	Round, compact	1st public sector hybrid; black rot resistant

NA data not available

Fig. 5.2 Pusa cabbage Hybrid 81

Broccoli is an emerging Cole crop in Indian market for its taste and health benefiting properties. It is an exotic crop, and introduced genetic material was evaluated in different centres, i.e. IARI RS, Katrain, and CSK Himachal Pradesh Agricultural University (HPAU), Palampur, which resulted into promising varieties for Indian condition as given in Table 5.4 (Fig. 5.3). The CMS system has been transferred in

Table 5.4 Improved varieties of broccoli

Variety	Source	Days for maturity	Head weight (g)	Yield per ha	Head colour	Remark
Pusa KTS-1	IARI RS, Katrain	85–95	350–450	16 (hills) 6.4(plains)	Solid green	Sprouting type
Palam Samridhi	HPKV, Palampur	80–90	300–400	15–20	Green	Sprouting type
Palam Vichitra	HPKV, Palampur	115–120	350–450	20–22	Purple	Heading type
Palam Kanchan	HPKV, Palampur	140–145	350–400	25–27	Yellowish green	Heading type
Palam Haritika	HPKV, Palampur	145–150	300–400	17–22	Green	Sprouting type
Punjab broccoli 1	PAU, Ludhiana	65–70 days	300–400	16–17	Bluish dark green	Sprouting type

Fig. 5.3 Cole crops. (**a**) Heading broccoli, Palam Vichitra. (**b**) Knol khol: Palam Tender Knob

broccoli lines at IARI RS, Katrain, for use in hybrid breeding. Also, new lines of green sprouting and purple heading broccoli are in advance stages at IARI, New Delhi.

Breeding programme in knol khol is aimed for earliness, higher productivity, resistance to bolting, slow fibre development and better field staying capacity. Self-incompatibility is available for hybrid breeding, but most of the commercial varieties in public sector are open-pollinated such as White Vienna (early, dwarf, globular knobs with creamy white flesh, 55–60 days), Purple Vienna (early, 55–65 days, globular knobs), King of North (early, 60–65 days, flat round) and Large Green (late, 75 days, flat round). Recently, Pusa Virat (knobs 800–900 g, diameter 13–14 cm, better field stand, yield 19 t/ha) has been released from IARI RS, Katrain, and Palam Tender Knob from HPAU, Palampur. Palam Tender Knob is an early variety, light green knobs, yields 25–27 t/ha and has better shelf life. In Brussels sprouts, the recommended variety for India are Hilds Ideal (80 days, 55–60 cm; 45–50 sprouts, 7–8 g; green compact; 10–15 t/ha). Kale is under cultivation in Jammu and Kashmir as Karam Sag. Central Kale Pusa 64 is developed by IARI RS, Katrain. It has highly serrated, purplish green leaves, 40–50 cm in length and 15–20 cm in width, plant height is 50–60 cm, and average leaf yield over locations is 35.0 t/ha. It has high tolerance to cold and frost conditions.

5.6 Resistance Breeding

Development of resistant varieties is most acceptable and environment friendly option for disease management. Resistance breeding has resulted into various successful examples in different vegetable crops. The development of resistant varieties, however, requires thorough understanding of evolutionary interrelationship of host and pathogen. The success of resistance breeding lies in understanding of the genetic sources of resistance, racial composition of pathogen and genetic basis of host-pathogen interaction. It is also essential to have the knowledge of scope of manipulation of host-pathogen interaction. Important insect pests in Cole vegetables are diamondback moth (*Plutella xylostella* L.), cabbage stem borer (*Hellula undalis* Fab.), cabbage caterpillar (*Pieris brassicae* L.), cabbage semi-loopers (*Plusia orichalcea* Fab. and *P. nigrisigna* Walker), painted bug (*Bagrada cruciferarum* Krik) and aphids (*Brevicoryne brassicae* L.). No stable resistance reported for these insects, except a moderate-resistant line PI 234599 for diamondback moth in cauliflower. Diseases deteriorate quality and affect yield; hence, breeding for disease resistance is one of the top priority in Cole vegetables. Important bacterial diseases are black rot [*Xanthomonas campestris* pv. *campestris* (Pammel) Dowson] and bacterial soft rot [*Erwinia carotovora* pv. *carotovora* (L.R. Jones) *Hollander*]; fungal diseases are leaf spot or curd blight [*Alternaria brassicicola* (Schweinitz, Wiltshire)], downy mildew [*Hyaloperonospora* Constant. *parasitica* (Pers.:Fr) Fr.], club root (*Plasmodiophora brassicae* Wor.), stalk rot and watery soft rot [*Sclerotinia sclerotiorum* (Lib.) de Bary] and fusarium yellows (*Fusarium oxysporum* f.

conglutinans). Resistance sources against these diseases have been identified in cultivated as well as wild species (Sharma et al. 1995; Singh et al. 2013; Saha et al. 2016); some of them are in use in resistance breeding. Resistance is a relative term reflecting hereditary capability of the host to reduce the development of pathogen after its infection so that severity of diseases is minimized. The strategy for resistance breeding depends on knowledge of gene-for-gene relationship and host-pathogen interaction for efficient deployment of resistance genes in alternate forms. Fehr (1991) categorized three alternate strategies such as (i) developing cultivars with single major gene against the prevalent pest; (ii) combining genes controlling prevalent and minor races of pests in the form of mixture of different genotypes, especially as multiline varieties; and (iii) placing genes controlling prevalent and minor races into a single cultivar, i.e. pyramiding of resistance genes. In cauliflower, the resistance mechanism to most of the diseases is governed by the respective single dominant gene; hence, their manipulation is easy. Hybrid breeding, backcross breeding and recurrent breeding are common methods which have been employed in cauliflower resistance breeding. In the case of black rot, four races have been reported, and deployment of resistance genes for each race in a cultivated variety can be done through gene pyramiding. The steps in resistance breeding are (i) collection and maintenance of resistance genes for use in breeding programme. The sources of R gene may be advance breeding lines or new genetic stocks developed through pre-breeding, commercial varieties, landraces or primitive cultivars and wild relatives in the form of original progenitors or related species, and (ii) incorporation of one of the resistance gene can be done by incorporation of a resistance parent in hybridization programme. Further, the backcross is the most commonly used breeding method to incorporate resistance into existing adapted varieties. This method does not disturb the overall genetic constitution of the recipient commercial variety. The monogenic dominant resistance to downy mildew and black rot can be transferred into cultivated varieties by backcross method. Further, use of one R parent having desirable horticultural traits in hybrid breeding can result into R hybrids against these pathogens. Gene pyramiding approach can also be employed for development of varieties having resistance to both the diseases.

5.7 Breeding for Heat Tolerance

Heat stress resistance is an ability of some genotypes to perform better than others when they are subjected to the same level of heat stress. The mechanisms of heat stress are grouped into (i) heat avoidance and (ii) heat tolerance. Heat avoidance indicates ability of a genotype to dissipate the radiation energy and, thereby, to avoid a rise in plant architecture to a stress level. Temperature higher than the specified range for a cultivar delays onset of curd initiation in cauliflower, while lower than this range causes formation of small curd known as ‘button’. Similarly, temperature affects transformative stages and quality of edible parts in broccoli, cabbage and knol khol. In Cole crops, conventional breeding has resulted in several

varieties which are tolerant to heat and various other weather vagaries such as Pusa Ageti, tropical type in cabbage; Pusa Meghna; Pusa Ashwini and Pusa Kartiki early-maturing heat-tolerant cauliflowers; and Pusa Virat frost-tolerant knol khol. But, to handle this complex trait, molecular breeding approaches appear to be better alternative. In broccoli, Barnham et al. (2017) performed multiple quantitative trait loci (QTL) mapping of 1423 single-nucleotide polymorphisms developed through genotyping-by-sequencing and identified five QTL and one positive epistatic interaction that explained 62.1% of variation in heat tolerance. Pang et al. (2015a) detected six QTLs conferring resistance to head splitting in chromosome 2, 4 and 6. Two QTLs, SPL-2-1 and SPL-4-1, are located on chromosomes 2 and 4, respectively. Markers BRPGM0676 and BRMS137 were tightly linked with head-splitting resistance and reported to be conserved in QTL SPL-2-1 region. In cauliflower, Matschegewski et al. (2015) performed transcriptional profiling of flowering genes *FLOWERING LOCUS C* (*BoFLC*) and *VERNALIZATION 2* (*BoVRN2*) and reported increased expression levels of *BoVRN2* in genotypes with faster curding. The functional relevance of both the genes, however, was not consistent indicating that there are some independent mechanisms in temperature-regulated floral transition.

5.8 Breeding for Quality Traits

5.8.1 Breeding for Selective Increase of Glucosinolates

Cole vegetables have anti-carcinogenic glucosinolates which get hydrolysed to bioactive compounds such as isothiocyanates (ITC) and indoles by plant-based enzyme myrosinase or less efficiently by colonic microflora. Isothiocyanates are potent inducers of Phase II enzymes and stimulate apoptosis but inhibit mitosis in human tumour cells, in vitro and in vivo. Importantly, not all glucosinolates are good enough for health; hence, selective approach is more appropriate as followed in breeding of broccoli variety ‘Beneforte® (Seminis Vegetable Seeds, Inc.)’ (Mithen et al. 1987; Sarikamis et al. 2006). Kushad et al. (1999) have shown that the total glucosinolate content in five groups of *Brassica oleracea* (broccoli, Brussels sprouts, cabbage, cauliflower and kale) ranges from 10.9 to 25.1 μmol/g dry weight basis. Natural variability for total glucosinolates content in leaves and edible portion has been reviewed by Verkerk et al. (2009).

The wild species *Brassica villosa* was explored to enhance glucosinolates in broccoli, and Faulkner et al. (1998) developed F_1 crosses with broccoli, and F_1 plants reported to have >1010-fold increase in 3-methysulphinylpropyl and 4-methylsulphinylbutyl suggesting to develop lines for breeding use. Hanschen and Schreiner (2017) reported that broccoli and red cabbage were mainly rich in 4-methylsulfinyl butyl glucosinolate (glucoraphanin), whereas cauliflower, savoy cabbage and white cabbage contained mainly 2-propenyl (sinigrin) and 3-(methylsulfinyl)propyl glucosinolate (glucoiberin). They also showed the effect of ontogeny (developmental stages) during the head development on the formation of

glucosinolates and their respective hydrolysis products. Wide variation in total glucosinolates is also reported in other *Brassica* vegetables such as Chinese cabbage (4.48–31.58 µmol/g of dw) in 24 varieties by Kim et al. (2010) and Chinese *B. campestris* vegetables (0.34–3.0 µmol/g fw) by Chen et al. (2008b) in China. Padilla et al. (2007) observed variation in glucosinolates in *B. rapa* varieties grown on two sites, i.e. Salcedo (11.8–74.0 µmol/g dw) and Forzanes (7.5–74.0 µmol/g dw). This was attributed to plant age, temperature, water stress and soil type (Rosa et al. 1997). The *GS-AOP*, *GS-ELONG, GS-ALK, GS-OHP, GS-OH* and *GS-OX* genes have been reported for glucosinolates pathway in *Arabidopsis* and Bro-GS-elong and Bro-myro in broccoli, besides details of the linked molecular markers to glucosinolate content in Chinese cabbage (Gong et al. 2014) and *Brassica rapa* (Hirani et al. 2012). These sources and available information can be effectively used to develop hybrids/varieties selectively rich in glucosinolates. Vanlalneihi et al. (2019a) investigated sinigrin content in 16 genotypes of early-maturity group of Indian cauliflower and reported it in range of 3.29–16.37 µmol 100 g^{-1} FW with maximum in DC 41–5 in curd portion. They also analysed sinigrin in 14 genotypes of mid-early maturity group, and it was reported to be highest in leaves of DC306 (39.50 µmol 100 g^{-1} FW) and curds of DC326 (36.93 µmol 100 g^{-1} FW) (Vanlalneihi et al. 2019b).

5.8.2 Beta-carotene Biofortification

Vitamin A deficiency is one of the most noticeable nutritional problems in many parts of the world, and the World Health Organization (WHO) estimate (1995–2005) states that 250 million preschool-age children and approximately 7.2 million pregnant women in developing countries are vitamin A deficient. The vitamin A deficiency leads to loss of night blindness and retarded physical growth. In India, about 87 million preschool children are deficient in vitamin A. Human body is unable to synthesize vitamin A de novo from endogenous isoprenoid precursors; hence, plant carotenoids provide the primary dietary sources of provitamin A. Further, carotenoids act as antioxidant which has been implicated in reducing the risk of cancer and cardiovascular diseases. Hence, new nutrient-rich biofortified food sources are required to meet the challenge of micronutrient malnutrition. In the wake of extensive cultivation and consumption of cauliflower across the country, its enrichment for beta-carotene by introgressing '*Or*' gene is a promising attempt to tackle beta-carotene-deficiency-related malnutrition problem in India and the world.

A spontaneous '*Or'* mutant gene in cauliflower showed that the predominant carotenoid that accumulated in the affected tissues is β-carotene, which can reach levels of several hundred folds higher than those found in the comparable tissues of wild-type cauliflower (Li et al. 2001). The '*Or'* gene, which encodes a DnaJ cysteine-rich domain-containing protein, confers orange curd with high levels of β-carotene accumulation. Rather than directly regulating carotenoid biosynthesis, the '*Or'* gene appears to mediate the differentiation of plastids and/or noncoloured plastids in apical shoot and inflorescence meristematic tissues of curd into

chromoplasts for the associated carotenoid accumulation (Lu et al. 2006). The '*Or*' gene was introgressed into white colour elite inbred line of Indian cauliflower through marker-assisted backcross breeding at ICAR-IARI, New Delhi (Muthukumar et al. 2017; Kalia et al. 2018), which enhanced beta-carotene content up to 12 ppm. The programme has resulted into development of a beta-carotene-rich cauliflower variety 'Pusa KesriVitA-1' in mid-maturity group. It is the first ever indigenously bred biofortified beta-carotene (800–1000 μg/100g fresh weight basis)-rich cauliflower variety. It has been identified by the ICAR-IARI Variety Identification Committee during 2015 for the National Capital Territory and has potential to grow in other cauliflower-growing regions in the country (Annual Report 2015–2016). Normally, the white curd cauliflower does not contain any beta-carotene content; however, in some cases, there may be negligible presence of 0–6 μg/100g. Its curds are orange coloured, compact and very attractive with semi-self-blanching growth habit. It is suitable for September–January growing period in north Indian conditions. Average marketable curd weight is about 1.25 kg with an approximate marketable yield of 35.0–40.0 t/ha. Soon such genotypes are also expected in early and late groups. Besides, hybrids in all the maturity groups are in pipeline which are expected to have even better performance. The enrichment of Indian cauliflower with beta-carotene content has been carried out by employing a natural '*Or*' mutant in cauliflower which does not involve any transgenic issue. This '*Or*' gene encodes a plastid-associated protein containing a cys-rich domain found in DnaJ-like molecular chaperones. A function role of '*Or*' involves the differentiation of noncoloured plastids into chromoplasts which provide the deposition sink for carotenoid accumulation which is otherwise not present in white cauliflower. The cauliflower is grown and consumed abundantly by all the sections of the society across the regions and available at nominal prices in the main season. It has immense potential as an option in fight against vitamin A deficiency in Indian population.

5.8.3 Anthocyanin Biofortification

Among the members of Cole crops, anthocyanin accumulation occurred due to natural mutations. It has been genetically investigated for curd (pre-floral apical meristem) in purple cauliflower variety 'Graffiti' as a single semi-dominant gene *Pr* (Chiu et al. 2010), in ornamental kale as a single dominant gene *BoPr* for purple leaf (Liu et al. 2017), *BrPur* gene in Chinese cabbage (Wang et al. 2014) and similarly red leaf trait by a single dominant *Re* gene in ornamental kale (Ren et al. 2015). Genetic regulation of red leaf colour in cabbage was indicated by Yuan et al. (2009), but nature of genetic has not been proposed yet. Anthocyanin biosynthesis is a conserved pathway in plants, and transcriptional regulation of three structural genes, namely, R2R3MYB transcription factors, basic helix loop-helix (bHLH) and WD40 proteins, appears to be the major mechanism to control this pathway (Broun 2005). In *Arabidopsis*, accumulation of anthocyanin in vegetative tissues is mediated by four MYB proteins, namely, PAP1 (production of anthocyanin pigment 1), PAP2, MYB113 and MYB114,

and three bHLH proteins (transparent test 8, Glabra3 and enhancer of Glabra3) (Nesi et al. 2000; Gonalez et al. 2008). The *Pr* gene is a natural mutant in cauliflower itself and encodes for R2R3MYB transcription factor that exhibits tissue-specific expression (Chiu et al. 2010). They indicated that Harbinger DNA transposon insertion in the upstream regulatory region of *Pr-D* gene governs the changes in anthocyanin accumulation in cauliflower 'Griffiti'. Yuan et al. (2009) designed 17 gene-specific primers using highly homologous sequences in *Brassica oleracea* genome with *Arabidopsis* anthocyanin genomic information, all of them distinguished purple and green types. These markers were used by Chiu et al. (2010), and they isolated the *Pr* gene from cultivar 'Graffiti' through candidate gene-fine mapping analysis and developed a high-resolution genetic map with three PCR-based markers, namely, BoMYB2, BoMYB3 and BoMYB4 (<0.2 cM) linked to *Pr* gene.

5.9 Innovative Techniques in Cole Vegetables

5.9.1 Marker-Assisted Breeding

Marker-assisted selection (MAS) is an indirect process where selection is carried out on the basis of a marker instead of the trait itself. The successful application of MAS relies on the tight association between the marker and the major gene or QTL responsible for the trait. The new genomic tools accelerate the identification of markers tightly linked to target genomic regions. The accumulation of genes from different sources which confer resistance against the same disease is an example and is indeed one of the most widespread applications of gene pyramiding. Molecular markers ensure a reasonable likelihood that the genotype combining favourable alleles is present in the population (Ishii and Yonezawa 2007). The advent of modern molecular techniques is playing an important role in understanding the organization and relationships of the *Brassica* genomes. Results from these studies not only confirmed the origin of the amphidiploid species but also suggested that the A and C genomes originated from a single lineage, whereas the B genome is genetically distant to both A and C genomes forming a separate lineage (Warwick and Black 1991). Cole genome has been sequenced by Liu et al. (2014), and a number of linked molecular markers for important traits have been identified in different *Brassica oleracea* crops (Table 5.5). The genomic sequences are useful tools for development of robust DNA markers. The genetic maps in *Brassica* also serve the purpose of (a) understanding the relationship among the genomes of the *Brassica*-cultivated diploid species and (b) utilization in applied genetics and breeding of the numerous *Brassica* crops. In cauliflower, Kalia et al. (2017) identified two closely linked (1.6 cM) markers (RAPD-OPO-04833 and ISSR-11635) to black rot resistance locus (*Xca1bo)* and converted them into sequence characterized amplified region (SCAR) markers. These two SCAR markers, namely, ScOPO-04833 and ScPKPS-11635, were found to be linked to the black rot resistance locus *Xca1bo*. The RAPD and ISSR markers were mapped with downy mildew resistance gene (Ppa3) in cauliflower by Singh et al. (2012).

Table 5.5 Linked molecular markers in vegetable crops

Crop	Trait	Gene	Marker	Reference
Cauliflower	High carotene	*Or*	SSR	Li and Garvin (2003)
	High anthocyanin	*Pr*	BoMYB3 BoMYB4	Chiu et al. (2010)
	Black rot	*Xca1bo (in B. oleracea)*	RAPD-OPO-04_{833} and ISSR-11_{635}	Saha et al. (2014)
		Xca1bo (in B. oleracea)	ScOPO-04_{833} and ScPKPS-11_{635}	Kalia et al. (2017)
		Xca1bc (in B. carinata)	SSR, ILP	Sharma et al. (2016)
	Downy mildew	*Ppa3*	RAPD, ISSR	Singh et al. (2012)
Broccoli	Flowering time	QTL	RFLP	Camargo and Osborn (1996)
	Downy mildew	Pp523	OPK17_980, AT.CTA_133/134	Farinho et al. (2004)
	Heat stress	QTLs	SSR	Barnham et al. (2017)
	High glucoraphanin and glucoiberin	QTLs	SSR	Sarikamis et al. (2006)
Cabbage	Black rot	BRQTL-C3, BRQTL-C6	SNP	Lee et al. (2015)
	Genic male sterility	*CDMs399–3*	EST-SSR	Chen et al. (2013)
	Head shape	QTLs (*Htd* 3.1, *Htd* 8.1)	SSR, InDel	Pang et al. (2015a, b)
	Head splitting	*QTLs* (SPL-2-1, SPL-4-1)	SSR	Pang et al. (2015a, b)
	Yellow-green leaf	*Ygl-1*	InDel	Liu et al. (2016)
	Plant height, leaf length, head transverse diameter	QTLs Ph 3.1, Ll 3.2, Htd 3.2	InDel	Lv et al. (2016)
	Sclerotinia rot resistance	QTLs (6 for stem and 12 for leaf resistance)	SSR	Mei et al. (2013)
	Fusarium wilt resistance	*FOC1*	InDel	Lv et al. (2014)
	Petal colour	*Cpc-1*	InDel	Han et al. (2015)
Kale	Purple leaf gene	*BoPr*	InDel	Liu et al. (2017)

5.9.2 *Transgenics in Cole Crops*

Lu et al. (2006) transformed cauliflower with *Or* transgenesis associated with acellular process that triggers the differentiation of proplastids or other noncoloured plastids into chromoplasts for carotenoids accumulation. The expression of *'Or'* transgene leads to the formation of large membranous chromoplasts in the

cauliflower curd cells of the *'Or'* transformants. Transformation of the *'Or'* gene into wild-type cauliflower converts the white colour of curd tissue into distinct orange colour with increased level of β-carotene. A successful protocol for genetic transformation of cauliflower employing the process of agroinfection was proposed by Kowalczyk et al. (2018) with variety Pionier transformation via *Rhizobium rhizogenes* (ATCC 18534, A4) with higher (72%) transformation efficiency GUS assay (55%). Transgenic approach was used by Jin et al. (2000) to target resistance to diamondback moth larvae in cabbage through *Agrobacterium tumefaciens*-mediated transformation with *Bacillus thuringiensis* (Bt) *cry* genes. Cabbage plants transformed with a synthetic *Bt* gene, *cry1Ab3*, were all resistant to larvae of the pest. Kalia et al. (2020) investigated insecticidal efficacy of *Cry1B/Cry1C* genes in transformed lines of cauliflower, and cabbage was assessed by feeding neonates of DBM on detached leaves. From a large number of transformed lines analysed, it is obvious that the *Cry1B/Cry1C* genes potentially exhibited insecticidal activity. In broccoli, postharvest yellowing of heads (made up of unopened flower buds) due to chlorophyll degradation by chlorophyllase genes (putative genes *BoCLH1*, *BoCLH2* and *BoCLH3*) affect market quality. Paul et al. (2005) transformed a tropical cabbage breeding line 'DTC 507' by a synthetic fusion gene of *Bacillus thuringiensis* encoding a translational fusion product of *Cry1B* and *Cry1Ab* δ-endotoxins by *Agrobacterium*-mediated transformation. Chen et al. (2008a, b) cloned three putative chlorophyllase genes, of them only *BoCLH1* transcribed during postharvest senescence and antisense *BoCLH1* transcripts showed positive correlations with slower postharvest yellowing. Russell et al. (2017) reviewed progress on deployment of pyramided *Bt* genes *cry1B* and *cry1C* for the control of *Plutella xylostella*, *Crocidolomia pavonana*, *Hellula undalis* and *Pieris spp*. in cabbage and cauliflower. A reengineered *Bt* construct has proved effective in trials with *Arabidopsis* and canola in Australia. However, it is currently under test in transformed cauliflower in India.

5.9.3 Genome and Transcriptome Sequencing

The NGS technologies have been changed with reduced cost and time for sequencing compared to Sanger technology and making possible to perform millions of sequencing reactions in parallel. Second-generation sequencing (massively paralleled sequencing) technologies such as Roche/454 pyrosequencing and Illumina/Solexa sequencing are already profusely used to sequence plant species, and third-generation sequencing (also known as long-read sequencing) are currently under active development and incorporated to sequencing projects PacBio RS (Pacific Biosciences), Helicos (Helicos) or Ion Torrent (Life Technologies). Nowadays, it is feasible to sequence most crop genomes (excluding those with a very large and complex genome) with a relatively modest budget, by combining Sanger with NGS technologies. However, as comparative to whole genome sequencing, transcriptome sequencing has been a cheaper alternative. The RNA-seq is independent of a priori

knowledge on the sequence under investigation, thereby also allowing analysis of poorly characterized species. It helps to study gene expression and identify novel RNA species and directly reveals sequence identity, crucial for analysis of unknown genes and novel transcript isoforms.

Liu et al. (2014) generated draft genome sequence of cabbage covering 98% of 630 Mb genome and compared it with *B. rapa* which revealed numerous chromosome rearrangements and asymmetrical gene loss in duplicated genomic blocks, asymmetrical amplification of transposable elements, differential gene co-retention for specific pathways and variation in gene expression. Glucosinolates biosynthesis related genes were illustrated due to consequence of genome duplication and gene divergence. Later on, Sun et al. (2019) sequenced and assembled cauliflower genome (584.60 Mb) with a contig N50 of 2.11 Mb and contained 47,772 genes, of that 56.65% of the genome showed repetitive sequences. Among these sequences, long terminal repeats (LTRs) were the most abundant (32.71% of the genome), followed by transposable elements (TEs) (12.62%). Comparative genomic analysis confirmed that the present-day cauliflower diverged from the ancestral *B. oleracea* species ~3.0 million years ago (Mya) and its speciation (~2.0 Mya) was later than that of cabbage (approx. 2.6 Mya) and other *Brassicas* (over 2.0 Mya). According to them, chromosome no. 03 of cauliflower shared the most syntenic blocks with the A, B (*Brassica* species) and C (*B. oleracea*) genomes indicating this as the most ancient one in the cauliflower genome. Yang et al. (2018) assembled the cabbage mtDNA by whole genome sequencing (WGS) and assembled sequence reads in a circular structure of 219,975 bp. They observed differences in the arrangement of bases at different regions, genome realignment of the plastid genome and mtDNAs and associated presence of heteroplasmy and reverse arrangement of the heteroplasmic blocks within the other mtDNAs which might be one of the causal factors for its diversity. They confirmed existence of different mtDNAs in diverse *B. oleracea* subspecies.

5.9.4 TILLING and EcoTILLING

TILLING and EcoTILLING plant breeding requires genetic variability to be selected in order to increase the frequencies of favourable alleles and genetic combinations. Sources of natural genetic variability can be found within the crop, mostly in the form of landraces, and also in the wild relatives. Although many conventional genebanks are reservoir of natural variability, artificial mutant collections are source of novel traits. These are created by radiation, chemical mutagenesis or transgenic, insertion and RNA interference methods for use in plant breeding (Parry et al. 2009) as reported in *Arabidopsis* (http://www.arabidopsis.org) which is a member of Brassicaceae family. The artificial mutant collections frequently contain novel variability which may be useful to study and develop new traits. Targeting Induced Local Lesions in Genomes (TILLING) (Till et al. 2003) is able to identify all allelic variants of a DNA region present in an artificial mutant collection. A similar

procedure called ecotype TILLING (EcoTILLING) (Comai et al. 2004) is another way to identify allelic variants for targeting genes in natural collections. These two methods are based on the use of endonucleases, such as CEL I or Endo I, that recognize and cut mismatches in the double helix of DNA (Till et al. 2004; Triques et al. 2008). Himelblau et al. (2009) showed effectiveness of mutagenesis and demonstrated TILLING as an efficient reverse genetic technique in *B. oleracea*. In *Brassica rapa,* Stephenson et al. (2010) developed the ethyl methanesulfonate (EMS) TILLING resource with mutation density of approximately 1 per 60 kb in population. Wang et al. (2010) used EcoTILLING to generate genetic resources with low erucic acid by targeting *FAE1* (*fatty acid elongase1*), a key gene for erucic acid synthesis in seeds of *Brassica* species by extending the fatty acid chain lengths from C18 to C20 and C22 and identified polymorphisms in *Bn.FAE1-A8* and *Bn. FAE1-C3*. According to Zeng et al. (2012), ORG-EcoTILLING is a powerful and cost-effective alternative method for high-throughput genome-wide assessment of inter- and intraspecific chloroplast and mitochondrial DNA polymorphisms.

5.9.5 SNPs Discovery and Use in Genotyping Platforms

The NGS is very helpful for plant breeders to discover genetic variation even with limited technical expertise and at minimal cost. Parallel development of computational pipeline tools accelerated accurate mining of genomic sequences for genetic variants that can be converted into robust genetic markers such as simple sequence repeats (SSRs) and single-nucleotide polymorphism (SNPs) (Deschamps and Campbell 2010). Nowadays, the SSRs and SNPs are predominant and mostly used markers in plant genetic analysis. The SNPs have become choice markers in modern breeding programme due to their abundance, stability, amenability to automation and cost-effectiveness (Ganal et al. 2009). The genotyping-by-sequencing (GBS) is also in use for generating genome-wide markers. It is one of the low-cost and high-throughput sequencing-based genotyping approaches that utilize a relatively simple library construction protocol efficiently (Elshire et al. 2011). The SNP discovery was performed by genotyping-by-sequencing (GBS) in cauliflower and broccoli by Stansell et al. (2018) while investigating phylogenetic patterns, population structure and domestication footprints with and without reliance upon a reference genome and produced 141,317 and 20,815 filtered SNPs, respectively. Lee et al. (2016) used GBS technique to construct a high-resolution genetic map (879.9 cM) and identified SNPs along with two and single major QTLs for Race 2 and Race 9 of clubroot pathogen *Plasmodiophora brassicae*, respectively. Whole-genome resequencing is another for SNP discovery, and Lee et al. (2015) followed this approach in cabbage-identified dCAPs markers for one major QTL and three minor QTLs for black rot resistance. Further, Zhang et al. (2019) also used whole-genome sequence and identified 30 BoSWEET genes in cabbage which act as sugar transporters and influence plants response to chilling and clubroot disease.

5.9.6 CRISPR/Cas$_9$ in Cole Crops

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) is a recent tool for genome editing (Cong et al. 2013). It consists of a nuclease (Cas9) and two short single-strand RNAs (crRNA and tracrRNA) which are fused to form single-guide RNA (sgRNA) for genome editing. Cas9 and a gRNA form a ribonucleoprotein complex and bind to genomic DNA. In Cole vegetables, Jansson (2018) was the first to describe the gene edited using CRISPR-Cas9 (a *Brassica* deletion mutant) using cabbage as model plant and PsbS as target gene. Murovec et al. (2018) introduced preassembled ribonucleoprotein complexes (RNPs) into cabbage protoplasts with PEG 4000. Four sgRNAs targeting two endogenous genes (the *FRI* and *PDS* genes, two sgRNAs per gene) were introduced and detected 1.15–24.51% mutation. Ma et al. (2019) targeted phytoene desaturase gene (*BoPDS*), S-receptor kinase gene (*BoSRK*) and male-sterility-associated gene *BoMS1* genes in cabbage by CRISPR/Cas9 gene editing using a construct with tandemly arrayed tRNA-sgRNA architecture. They reported that *BoSRK3* gene mutation suppressed self-incompatibility completely, and BoMS1 gene mutation produced a completely male-sterile mutant. Lawrenson et al. (2019) described an *Agrobacterium*-mediated delivery approach to deliver Cas9 and dual sgRNAs into 4-day-old cotyledonary petioles of *Brassica oleracea* and detected nearly 10% of primary transgenic plants. In subsequent T_1 and T_2 generations, it segregated away from the T-DNA and enabled the recovery of nontransgenic, genome-edited plants carrying a variety of mutations at the target locus.

References

Annual Report (2015–2016) Indian Agricultural Research Institute, New Delhi – 110012, p 25

Arashida FM, Maluf WR, Carvalho RC (2017) General and specific combining ability in tropical winter cauliflower. Hortic Bras 35(2):167–173

Arumugam N, Mukhopadhyay A, Gupta V, Sodhi YS, Verma JK, Pental D, Pradhan AK (2000) Somatic cell hybridization of 'oxy' CMS *Brassica juncea* (AABB) with *B. oleracea* (CC) for correction of chlorosis and transfer of novel organelle combinations to allotetraploid *Brassicas*. Theor Appl Genet 100:1043–1049

Bannerot H, Boulidard L, Chupeau Y (1977) Unexpected difficulties met with the radish cytoplasm. Cruciferae Newsl 2:16

Bhatia R, Dey SS, Sharma K, Prakash C, Kuamr R (2015) *In vitro* maintenance of CMS lines of Indian cauliflower: an alternative for conventional CMS-based hybrid seed production. J Hortic Sci Biotechnol 90(2):171–179

Branham SE, Stansell ZJ, Couillard DM, Farnham MW (2017) Quantitative trait loci mapping of heat tolerance in broccoli (*Brassica oleracea* var. *italica*) using genotyping-by-sequencing. Theor Appl Genet 130(3):529–538

Broun P (2005) Transcriptional control of flavonoid biosynthesis: a complex network of conserved regulators involved in multiple aspects of differentiation in *Arabidopsis*. Curr Opin Plant Biol 8:272–279

Camargo LE, Osborn TC (1996) Mapping loci controlling flowering time in *Brassica oleracea*. Theor Appl Genet 92(5):610–616

Cardi T, Earle ED (1997) Production of new CMS *Brassica oleracea* by transfer of 'Anand' cytoplasm from *B. rapa* through protoplast fusion. Theor Appl Genet 94:202–212

Chamola R, Balyan HS, Bhat SR (2013) Transfer of cytoplasmic male sterility from alloplasmic *Brassica juncea* and *B. napus* to cauliflower (*B. oleracea* var. *botrytis*) through interspecific hybridization and embryo culture. Indian J Genet 73(2):203–210

Chatterjee SS, Swarup V (1984) Self-incompatibility in Indian cauliflower. Cruciferae Newsl 9:25–27

Chen LFO, Lin CH, Kelkar SM, Chang YM, Shaw JF (2008a) Transgenic broccoli (*Brassica oleracea* var. *italica*) with antisense chlorophyllase (*BoCLH1*) delays postharvest yellowing. Plant Sci 174(1):25–31

Chen X, Zhu Z, Gerendas J, Zimmermann N (2008b) Glucosinolates in Chinese *Brassica campestris* vegetables: chinese cabbage, purple cai-tai, choysum, pakchoi, and turnip. Hortic Sci 43(2):571–574

Chen C, Mu Z, Zhi-yuan F, Qing-biao W, Yang-yong Z, Yu-mei L, Li-mei Y, Fei C (2013) A co-dominant marker BoE332 applied to marker-assisted selection of homozygous male-sterile plants in cabbage (*Brassica oleracea* var. *capitata* L.). J Integr Agric 12(4):596–602

Cheng F, Wu J, Fang L, Wang X (2012) Syntenic gene analysis between *Brassica rapa* and other Brassicaceae species. Front Plant Sci 3:198. https://doi.org/10.3389/fpls.2012.00198

Chiang MS, Chiang BY, Grant WF (1977) Transfer of resistance to race 2 of *Plasmodiophora brassicae* from *Brassica napus* to cabbage (*B. oleracea* var. *capitata*). I. Interspecific hybridization between *B. napus* and *B. oleracea* var. *capitata*. Euphytica 26:319–326

Chiu LW, Zhou X, Burke S, Wu X, Prior RL, Li L (2010) The purple cauliflower arises from activation of a MYB transcription factor. Plant Physiol 154(3):1470–1480

Christey MC, Makaroff CA, Earle ED (1991) Atrazine resistant cytoplasmic male sterile-nigra-broccoli obtained by protoplast fusion between cytoplasmic male sterile *Brassica oleracea* and attrazine resistant *Brassica campestris*. Theor Appl Genet 83:201–208

Collard BCY, Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philos Trans R Soc B Biol Sci 363:557–572

Comai L, Young K, Till BJ, Reynolds SH, Greene EA, Codomo CA et al (2004) Efficient discovery of DNA polymorphisms in natural populations by EcoTILLING. Plant J 37:778–786

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N et al (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 339:819–823

Cunha C, Tonguc M, Griffiths PD (2004) Discrimination of diploid crucifer species using PCR-RFLP of chloroplast DNA. Hortic Sci 39(7):1575–1577

de Melo PE, de Giordano LB (1994) Effect of Ogura male-sterile cytoplasm on the performance of cabbage hybrid variety. II. Commercial characteristics. *Euphytica* 78(1–2):149–154

Deschamps S, Campbell MA (2010) Utilization of next-generation sequencing platforms in plant genomics and genetic variant discovery. Mol Breed 25:553–570

Dey SS, Sharma SR, Bhatia R, Prakash C, Barwal RN (2011a) Superior CMS (Ogura) lines with better combining ability improve yield and maturity in cauliflower (*Brassica oleracea* var. *botrytis*). Euphytica 182:187–197

Dey SS, Sharma SR, Kumar PR, Prakash C (2011b) Development and characterization of "Ogura" based improved CMS lines of cauliflower (*Brassica oleracea* var. *botrytis* L.). Indian J Genet Plant Breed 71:37–42

Dey SS, Bhatia R, Sharma SR, Parkash C, Sureja AK (2013) Effects of chloroplast substituted *Ogura* male sterile cytoplasm on the performance of cauliflower (*Brassica oleracea* var. *botrytis* L.) F_1 hybrids. Sci Hortic 157:45–51

Dey SS, Singh N, Bhatia R, Parkash C, Chandel C (2014a) Genetic combining ability and heterosis for important vitamins and antioxidant pigments in cauliflower (*Brassica oleracea* var. *botrytis* L.). Euphytica 195(2):169–181

Dey SS, Bhatia R, Parkash C, Kalia P, Barwal RN (2014b) Evaluation of cauliflower (*Brassica oleracea* var. *botrytis*) CMS (*Ogura*) lines for agronomic and floral traits. Indian J Hortic 71(3):424–427

Dey SS, Sharma K, Dey RB, Kumar GS, Singh D, Kumar R, Parkash C (2015) Inter specific hybridization (*Brassica carinata*× *Brassica oleracea*) for introgression of black rot resistance genes into Indian cauliflower (*B. oleracea* var. *botrytis* L.). Euphytica 204(1):149–162

Dey SS, Bhata R, Prakash C, Sharma S, Dabral M, Mishra V et al (2017a) Alteration in important quality traits and antioxidant activities in *Brassica oleracea* with *Ogura* cybrid cytoplasm. Plant Breed 136(3):400–409

Dey SS, Bhatia R, Bhardwaj I, Mishra V, Sharma K, Prakash C et al (2017b) Molecular-agronomic characterization and genetic study reveals usefulness of refined Ogura cytoplasm based CMS lines in hybrid breeding of cauliflower (*Brassica oleracea* var. *botrytis* L.). Sci Hortic 224:27–36

Dixit SK, Singh BP, Ram HH (2004) Heterosis and combining ability in Indian cauliflower (*Brassica oleracea* var. *botrytis* L.). Veg Sci 31(2):164–167

Duclos DV, Bjorkman T (2008) Meristem identity gene expression during curd proliferation and flower initiation in *Brassica oleracea*. J Exp Bot 59(2):421–433

Duclos V, Bjorkman T (2015) Gibberellin control of reproductive transitions in *Brassica oleracea* curd development. J Am Soc Hortic Sci 140(1):57–67

Dunemann F, Grunewaldt J (1991) Identification of a monogenic dominant male sterility mutant in broccoli (*Brassica oleracea* L. var. *italica* Plenck). Plant Breed 106:161–163

Edh K, Widen B, Ceplitis A (2009) The evolution and diversification of *s*-locus haplotypes in the *Brassicaceae* family. Genetics 181(3):977–984

El-Esawi MA, Germaine K, Bourke P, Malone R (2016) Genetic diversity and population structure of *Brassica oleracea* germplasm in Ireland using SSR markers. C R Biol 339(3–4):133–140

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES et al (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 6:e19379. https://doi.org/10.1371/journal.pone.0019379

Farinho M, Coelho P, Carlier J, Svetleva D, Monteiro A, Leitao J (2004) Mapping of a locus for adult plant resistance to downy mildew in broccoli (*Brassica oleracea* convar. *italica*). Theor Appl Genet 109(7):1392–1398

Faulkner K, Mithen R, Williamson G (1998) Selective increase of the potential anticarcinogen 4-methylsulphinylbutyl glucosinolate in broccoli. Carcinogenesis 19:605–609

Fehr W (1991) Principles of cultivar development: theory and technique. Agronomy books, vol 1. https://lib.dr.iastate.edu/agron_books/1

Ganal MW, Altmann T, Roder MS (2009) SNP identification in crop plants. Curr Opin Plant Biol 12:211–217

Garg N, Lal T (2005) Components of variation and genetic parameters in cauliflower. Haryana J Hortic Sci 34(1/2):113–115

Gong HY, Luo SX, Gu AX, Wang YH, Jun ZJ, Xing SS (2014) Development of SSR markers linked to glucosinolate synthesis genes in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). J Agric Biotechnol 22(10):1232–1241

Gonzalez A, Zhao M, Leavitt JM, Lloyd AM (2008) Regulation of the anthocyanin biosynthetic pathway by the TTG1/bHLH/Myb transcriptional complex in *Arabidopsis* seedlings. Plant J 53(5):814–827

Grelon M, Budar F, Bonhomme S, Pelletier G (1994) *Ogura* cytoplasmic male-sterility (CNS)-associated orf138 is translated into a mitochondrial membrane polypeptide in male-sterile *Brassica* cybrids. Mol Gen Genomics 243:540–547

Groszmann M, Gonzalez-Bayon R, Lyons RL, Greaves IK, Kazan K et al (2015) Hormone-regulated defense and stress response networks contribute to heterosis in *Arabidopsis* F_1 hybrids. Proc Natl Acad Sci USA 112(46):E6397–E6406

Gu H, Wang J, Yu H, Zhao Z, Sheng X, Chen J, Xu Y (2014) Development and validation of high-glucoraphanin broccoli F1 hybrids and parental lines. J Am Soc Hortic Sci 139(4):460–468

Gustafson-Brown C, Savidge B, Yanofsky MF (1994) Regulation of the *Arabidopsis floral* homeotic gene *APETALA1*. Cell 76:131–143

Habib K, Ahmed N, Khan SH, Mehdi M (2017) Heterosis in sprouting broccoli (*Brassica oleracea* var. *italica* Plenck.). Green Farm 8(5):1018–1023

Hagimori M, Nagaoka M, Kato N, Yoshikawa H (1992) Production and characterization of somatic hybrids between the Japanese radish and cauliflower. Theor Appl Genet 84:819–824

Hale AL, Farnham MW, Nzaramba MN, Kimbeng CA (2007) Heterosis for horticultural traits in broccoli. Theor Appl Genet 115(3):351–360

Han F-G, Yang C, Fang Z-Y, Yang L-M, Zhunag M et al (2015) Inheritance and InDel markers closely linked to petal color gene (*cpc-1*) in *Brassica oleracea*. Mol Breed 35:160. https://doi.org/10.1007/s1103

Hanschen FS, Schreiner M (2017) Isothiocyanates, nitriles, and epithionitriles from glucosinolates are affected by genotype and developmental stage in *Brassica oleracea* Varieties. Front Plant Sci 8:1095. https://doi.org/10.3389/fpls.2017.01095

Hansen LN, Earle ED (1995) Transfer of resistance to *Xanthomonas campestris* pv *campestris* in to *Brassica oleracea* L. by protoplast fusion. Theor Appl Genet 91:1293–1300

Heath DW, Earle ED, Dickson MH (1994) Introgressing cold tolerant *Ogura* cytoplasms from rapeseed into pak choi and Chinese cabbage. Hortic Sci 29:202–203

Himelblau E, Gilchrist EJ, Buono K, Bizzell C, Mentzer L et al (2009) Forward and reverse genetics of rapid-cycling *Brassica oleracea*. Theor Appl Genet 118(5):953–961

Hirani AH, Li G, Zelmer CD, McVetty PBE, Asif M, Goyal A (2012) Molecular genetics of glucosinolate biosynthesis in *Brassicas*: genetic manipulation and application aspects. In: Goyal A (ed) Crop plant. https://doi.org/10.5772/45646

Hodgkin T (1995) Cabbages, kales etc. *Brassica oleracea* (Cruciferae). In: Smartt J, Simmonds NW (eds) Evolution of crop plants. Longman, London, pp 76–82

Imaging FE (2009) With 'phenomics,' plant scientists hope to shift breeding into overdrive. Science 325:380–381

Ishii T, Yonezawa K (2007) Optimization of the marker-based procedures for pyramiding genes from multiple donor lines: II. Strategies for selecting the objective homozygous plant. Crop Sci 47:1878–1886

Ishikawa S, Bang SW, Kaneko Y, Matsuzawa Y (2003) Production and characterization of intergeneric somatic hybrids between *Moricandia arvensis* and *Brassica oleracea*. Plant Breed 122:233–238

Jansson S (2018) Gene-edited plants on the plate: the 'CRISPR cabbage story'. Physiol Plant 164(4):396–405

Jeong S-Y, Ahmed NU, Jung H-J, Kim H-T, Park J-I, Nou I-S (2017a) Discovery of candidate genes for heterosis breeding in *Brassica oleracea* L. Acta Physiol Plant 39:180

Jeong S-W, Yi H, Song H, Lee S-S, Park Y, Hur Y (2017b) Chlorosis of Ogura-CMS *Brassica rapa* is due to down-regulation of genes for chloroplast proteins. J Plant Biotechnol 44:115–124

Jin RG, Liu YB, Tabashnik BE, Borthakur D (2000) Development of transgenic cabbage (Brassica oleracea var. capitata) for insect resistance by agrobacterium tumefaciens-mediated transformation. In Vitro Cell Dev Biol Plant 36(4):231–237

Kachroo UJ (1984) Heterosis and combining ability in kohlrabi (*Brassica oleracea* var. *gongylodes* L). Masters dissertation, CSKHPKV Solan

Kalia P (2009) Genetic improvement in vegetable crucifers. In: Gupta SK (ed) Biology and breeding of crucifers. CRC Press Taylor & Francis Group, Boca Raton, pp 309–342

Kalia P, Saha P, Roy S (2017) Development of RAPD and ISSR derived SCAR *markers* linked to *Xca1Bo* gene conferring resistance to black rot disease in cauliflower (*Brassica oleracea* var. *botrytis* L.). Euphytica 213:232. https://doi.org/10.1007/s10681-017-2025-y

Kalia P, Muthukumar P, Soi S (2018) Marker-assisted introgression of the *Or* gene for enhancing β-carotene content in Indian cauliflower. Acta Hortic 1203:121–128

Kalia P, Aminedi R, Golz J, Russel D, Choudhary P et al (2020) Development of diamondback moth resistant transgenic cabbage and cauliflower by stacking *Cry1B* & *Cry1C Bt* genes. Acta Hortic. (In Press)

Kameya Y, Kanzaki H, Toki S, Abe T (1989) Transfer of radish (*Raphanus sativus* L.) chloroplasts into cabbage (*Brassica oleracea* L.) by protoplast fusion. Jpn J Genet 64:27–34

Kaminski P, Dyki B, Stepowska AA (2012) Improvement of cauliflower male sterile lines with brassica nigra cytoplasm, phenotypic expression and possibility of practical application. J Agric Sci 4(4):190–200

Kanwar MS, Karla BN (2004) Genetic analysis in late cauliflower (*Brassica oleracea* var. *botrytis* L.) subjected to North Carolina mating design-I. Indian J Genet Plant Breed 64(3):225–227

Kempin S, Savidge S, Yanofsky MF (1995) Molecular basis of the cauliflower phenotype in *Arabidopsis*. Science 267:522–525

Kibar B, Karaagac O, Kar H (2015) Heterosis for yield contributing head traits in cabbage (*Brassica oleracea* var. *capitata*). Cienc Investig Agrar 42(2):205–216

Kim JKC, Sang M, Sun JL, Dong J et al (2010) Variation of glucosinolates in vegetable crops of *Brassica rapa* L. *ssp. pekinensis*. Food Chem 119(1):423–428

Kowalczyk T, Gerszberg A, Durańska P, Biłas R, Hnatuszko-Konka K (2018) High efficiency transformation of *Brassica oleracea* var. *botrytis* plants by *Rhizobium rhizogenes*. AMB Express 8(1):125. https://doi.org/10.1186/s13568-018-0656-6

Kushad MM, Brown AF, Kurilich AC, Juvik JA, Klein BP, Wallig MA, Jeffery EH (1999) Variation of glucosinolates in vegetable crops of *Brassica oleracea*. J Agric Food Chem 47(4):1541–1548

Lawrenson T, Hundleby P, Harwood W (2019) Creating targeted gene knockouts in *Brassica oleracea* Using CRISPR/Cas9. In: Qi Y (ed) Plant genome editing with CRISPR systems. Methods in molecular biology, 1917. Humana Press, New York

Lee J, Izzah NK, Jayakodi M, Perumal S et al (2015) Genome-wide SNP identification and QTL mapping for black rot resistance in cabbage. BMC Plant Biol 15(1):32. https://doi.org/10.1186/s12870-015-0424-6

Lee J, Izzah NK, Choi B-S, Joh HJ, Lee S-C, Perumal S et al (2016) Genotyping-by-sequencing map permits identification of clubroot resistance QTLs and revision of the reference genome assembly in cabbage (*Brassica oleracea* L.). DNA Res 23:29–41

Li L, Garvin DF (2003) Molecular mapping of *Or*, a gene inducing beta-carotene accumulation in cauliflower (*Brassica oleracea* L. var. *botrytis*). Genome 46(4):588–594

Li L, Paolillo DJ, Parthasarathy MV, DiMuzio EM, Garvin DF (2001) A novel gene mutation that confers abnormal patterns of β-carotene accumulation in cauliflower (*Brassica oleracea* var. *botrytis*). Plant J 26(1):59–67

Li H, Liu Q, Zhang Q, Qin E, Jin C, Wang Y, Wu M et al (2017) Curd development associated gene (*CDAG1*) in cauliflower (*Brassica oleracea* L. var. *botrytis*) could result in enlarged organ size and increased biomass. Plant Sci 254:82–94

Liu S, Liu Y, Yang X et al (2014) The *Brassica oleracea* genome reveals the asymmetrical evolution of polyploidy genomes. Nat Commun 5:3930. https://doi.org/10.1038/ncomms4930

Liu XP, Yang C, Han FQ, Fang ZY, Yang LM, Zhuang M et al (2016) Genetics and fine mapping of a yellow-green leaf gene (*ygl-1*) in cabbage (*Brassica oleracea* var. *capitata* L.). Mol Breed 36:1–8. https://doi.org/10.1007/s11032-015-0425-z

Liu X, Gao B, Han F, Fang Z, Yang L, Zhuang M et al (2017) Genetics and fine mapping of a purple leaf gene *BoPr* in ornamental kale (*Brassica oleracea* L. var. *acephala*). BMC Genomics 18:230. https://doi.org/10.1186/s12864-017-3613-x

Lorenz AJ, Chao S, Asoro FG, Heffner EL, Hayashi T et al (2011) Genomic selection in plant breeding: knowledge and prospects. Adv Agron 110:77–123

Lu S, Eck JV, Zhou X, Lopez AB, Halloran DM et al (2006) The cauliflower *Or* gene encodes a DnaJ cysteine-rich domain containing protein that mediates high levels of β-carotene accumulation. Plant Cell 18:3594–3605

Lv HH, Fang ZY, Yang LM, Zhang YY, Wang QB, Liu YM et al (2014) Mapping and analysis of a novel candidate *Fusarium* wilt resistance gene *FOC1* in *Brassica oleracea*. BMC Genomics 15:1094. https://doi.org/10.1186/1471-2164-15-1094

Lv H, Wang Q, Liu X, Han F, Fang Z, Yang L et al (2016) Whole-genome mapping reveals novel QTL clusters associated with main agronomic traits of cabbage (*Brassica oleracea* var. *capitata* L.). Front Plant Sci 7:989. https://doi.org/10.3389/fpls.2016.00989

Ma C, Zhu C, Zheng M, Liu M, Zhang D, Liu B, Li Q, Si J, Ren X, Song H (2019) CRISPR/Cas9-mediated multiple gene editing in *Brassica oleracea* var. *capitata* using the endogenous tRNA-processing system. Hortic Res 6(1):20

Maggioni L, Bothmer RV, Poulsen G, Branca F, Bagger JR (2014) Genetic diversity and population structure of leafy kale and Brassica rupestris Raf. In South Italy. Hereditas 151:145–158

Mandel MA, Gustafson-Brown C, Savidge B, Yanofsky MF (1992) Molecular characterization of the Arabidopsis floral homeotic gene APETALA1. Nature 360:273–277

Matschegewski C, Zetzsche H, Hasan Y, Leibeguth L, Briggs W, Ordon F, Uptmoor R (2015) Genetic variation of temperature-regulated curd induction in cauliflower: elucidation of floral transition by genome-wide association mapping and gene expression analysis. Front Plant Sci 10(6):720

Mei J, Ding Y, Lu K, Wei D, Liu Y, Disi O et al (2013) Identification of genomic regions involved in resistance against Sclerotinia sclerotiorum from wild Brassica oleracea. Theor Appl Genet 126:549–556

Mithen RF, Lewis BG, Heaney RK, Fenwick GR (1987) Glucosinolates of wild and cultivated *Brassica* species. Phytochemistry 26(7):1969–1973

Monteiro AA, Gabelman WH, Williams PH (1988) The use of sodium chloride solution to overcome self incompatibility in *Brassica campestris*. Cruciferae Newsl 13:122–123

Murovec J, Gucek K, Bohanec B, Avbelj M, Jerala R (2018) DNA-free genome editing of *Brassica oleracea* and *B. rapa* protoplasts using CRISPR-Cas9 ribonucleoprotein complexes. Front Plant Sci 9:1594

Muthukumar P, Kalia P, Sharma M, Vashisht S (2017) Study of β-carotene enhancing '*Or*' gene effects on yield and contributing traits in mid-season Indian cauliflower (*Brassica oleracea* var. *botrytis* L.). Indian J Hortic 74(4):520–525

Nahm SH, Lee HJ, Lee SW, Joo GY, Harn CH, Yang SG, Min BW (2005) Development of a molecular marker specific to a novel CMS line in radish (*Raphanus sativus* L.). Theor Appl Genet 111(6):1191–1200

Nesi N, Debeaujon I, Jond C, Pelletier G, Caboche M, Lepiniec L (2000) The TT8 gene encodes a basic helix-loop-helix domain protein required for expression of DFR and BAN genes in *Arabidopsis* siliques. Plant Cell 12:1863–1878

Padilla G, Cartea ME, Velasco P, de Haro A, Ordas A (2007) Variation of glucosinolates in vegetable crops of *Brassica rapa*. Phytochemistry 68(4):536–545

Pang W, Li X, Choi SR et al (2015a) Mapping QTLs of resistance to head splitting in cabbage (*Brassica oleracea* L.var. *capitata* L.). Mol Breed 35:126. https://doi.org/10.1007/s11032-015-0318-1

Pang W, Kim YY, Li X, Choi SR, Wang Y, Sung CK, Im S et al (2015b) Anatomic characteristics associated with head splitting in cabbage (*Brassica oleracea* var. *capitata* L.). PLoS One 10(11):e0142202. https://doi.org/10.1371/journal.pone.0142202

Parry MAJ, Madgwick PJ, Bayon C, Tearall K, Hernandez-Lopez A et al (2009) Mutation discovery for crop improvement. J Exp Bot 60:2817–2825

Pathak S, Kumar J, Vidyasagar VS (2007) Heterosis and combining ability for marketable yield and component traits in cabbage (*Brassica oleracea* var *capitata*). Indian J Agric Sci 77(2):97–100

Paul A, Sharma SR, Sresty TVS, Devi S, Bala S, Kumar PS et al (2005) Transgenic cabbage (*Brassica oleracea* var. *capitata*) resistant to Diamondback moth (*Plutella xylostella*). Indian J Biotechnol 4:72–77

Pelletier G, Ferrault M, Lancelin D, Boulidard L (1989) CMS *Brassica oleracea* cybrids and their potential for hybrid seed production. 12th Eucarpia Congress, Gottingen 11(7):15

Prakash C, Dey SS, Bhatia R, Dhiman MR (2015) Indigenously developed SI and CMS lines in hybrid breeding of cabbage. Indian J Hortic 72(2):212–217

Prakash C, Kumar S, Singh R, Kumar A et al (2018) Ogura-based CMS lines with different nuclear backgrounds of cabbage revealed substantial diversity at morphological and molecular levels. 3 Biotech 8(1):27. https://doi.org/10.1007/s13205-017-1047-4

Purugganan MD, Boyles AL, Suddith JI (2000) Variation and selection at the *CAULIFLOWER* floral homeotic gene accompanying the evolution of domesticated *Brassica oleracea*. Genetics 155:855–862

Ram H, Dey SS, Krishnan SG, Kar A, Bhardwaj R et al (2017) Heterosis and combining ability for mineral nutrients in snowball cauliflower (*Brassica oleracea* var. *botrytis* L.) using ogura cytoplasmic male sterile lines. In: Proceedings of the national academy of sciences, India – section B: biological sciences. https://doi.org/10.1007/s40011-017-0874-8

Ren JP, Dickson MH, Earle ED (2000) Improved resistance to bacterial soft rot by protoplast fusion between *Brassica rapa* and *B. oleracea*. Theor Appl Genet 100:810–819

Ren J, Liu ZY, Niu RQ, Feng H (2015) Mapping of *Re*, a gene conferring the red leaf trait in ornamental kale (*Brassica oleracea*. L. var. *acephala*). Plant Breed 134:494–500

Rosa EAS, Heaney RK, Fenwick GR, Portas CAM (1997) Glucosinolates in crop plants. Hortic Rev 19:99–215

Rosan A, Hasan Y, Briggs W, Uptmoor R (2018) Genome-based prediction of time to curd induction in cauliflower. Front Plant Sci 9:78. https://doi.org/10.3389/fpls.2018.00078

Ruffio-Chable V, Bellis H, Herve Y (1993) A dominant gene for male sterility in cauliflower (*Brassica oleracea* var. *botrytis*). Phenotype expression, inheritance and use in F_1 hybrid production. Euphytica 67:9–17

Russell DA, Huang D, Bhalla P, Singh M, Robin C et al (2017) Progress in the development of transgenic cabbage, cauliflower and canola expressing stacked bts for caterpillar control and RNAi for aphid suppression. Mysore J Agric Sci (Special Issue) 51A:159–167

Saha P, Kalia P, Sonah H, Sharma TR (2014) Molecular mapping of black rot resistance locus *Xca1bo* on chromosome 3 in Indian cauliflower (*Brassica oleracea* var. *botrytis* L.). Plant Breed 133(2):268–274

Saha P, Kalia P, Joshi S, Vinod (2015) Genetic analysis of yield components and curd color of midseason heat tolerant Indian cauliflower (*Brassica oleracea* var. *botrytis* L.). SABRAO J Breed Genet 47(2):124–132

Saha P, Kalia P, Sharma M, Singh D (2016) New source of black rot disease resistance in *Brassica oleracea* and genetic analysis of resistance. Euphytica 207:35–48

Sarikamis G, Marquez J, MacCormack R, Bennett RN, Roberts J, Mithen R (2006) High glucosinolate broccoli: a delivery system for sulforaphane. Mol Breed 18:219–228

Sharma SR, Kapoor KS, Gill HS (1995) Screening against Sclerotinia rot (*Sclerotinia sclerotiarum*), downy mildew (*Peronospora parasitica*) and black rot (*Xanthomonas compestris*) in caulifl ower (*Brassica oleracea* var. *botrytis* subvar. *cauliflora* DC). Indian J Agric Sci 65:916–918

Sharma SR, Singh PK, Chable V, Tripathi SK (2005) A review of hybrid cauliflower development. J New Seeds 6(2):151–193

Sharma BB, Kalia P, Yadava DK, Singh D, Sharma TR (2016) Genetics and molecular mapping of black rot resistance locus *Xca1bc* on chromosome B-7 in Ethiopian mustard (*Brassica carinata* a. Braun). PLoS One 11(3):e0152290. https://doi.org/10.1371/0152290

Sharma BB, Kalia P, Singh D, Sharma TR (2017) Introgression of black rot resistance from *Brassica carinata* to cauliflower (*Brassica oleracea botrytis* group) through embryo rescue. Front Plant Sci 8:1255

Shu J, Liu Y, Li Z, Zhang L, Fang Z et al (2016) Detection of the diversity of cytoplasmic male sterility sources in broccoli (*Brassica Oleracea* var. *Italica*) using mitochondrial markers. Front Plant Sci 7:927. https://doi.org/10.3389/fpls.2016.00927

Sigareva MA, Earle ED (1997) Direct transfer of a cold tolerant Ogura male sterile cytoplasm into cabbage (*Brassica oleracea* ssp. *capitata*) via protoplast fusion. Theor Appl Genet 94:213–220

Singh R, Sharma SR (2003) Cauliflower. In: Textbook of vegetables, tuber crops and spices. ICAR, New Delhi, pp 76–97

Singh B, Singh A, Pal AK, Banerjee MK (2002) Evaluation of self-incompatibility in Indian cauliflower. Veg Sci 29(2):142–145

Singh BK, Sharma SR, Singh B (2010) Heterosis for superoxide dismutase, peroxidase and catalase enzymes in the head of single cross-hybrids of cabbage (*Brassica oleracea* var. *capitata*). J Genet 89:217–221

Singh S, Sharma SR, Kalia P, Deshmukh R, Vinod SP, Sharma TR (2012) Molecular mapping of the downy mildew resistance gene *Ppa3* in cauliflower (*Brassica oleracea* var. *botrytis* L.). J Hortic Sci Biotechnol 87(2):137–143

Singh S, Sharma SR, Kalia P, Sharma P, Kumar V, Kumar R et al (2013) Screening of cauliflower (*Brassica oleracea* L. var. *botrytis* L.) germplasm for resistance to downy mildew [*Hyaloperonospora parasitica* constant (Pers.:Fr) Fr.] and designing appropriate multiple resistance breeding strategies. J Hortic Sci Biotechnol 88(1):103–109

Smith LB, King G (2000) The distribution of *BoCAL-a* alleles in Brassica oleracea is consistent with a genetic model for curd development and domestication of the cauliflower. Mol Breed 6(6):603–613

Stansell Z, Hyma K, Fresnedo-Ramírez J, Sun Q, Mitchell S et al (2018) Genotyping-by-sequencing of *Brassica oleracea* vegetables reveals unique phylogenetic patterns, population structure and domestication footprints. Hortic Res 5(1):38

Stephenson P, Baker D, Girin T, Perez A, Amoah S, King GJ, Ostergaard L (2010) A rich TILLING resource for studying gene function in *Brassica rapa*. BMC Plant Biol 10(1):62

Sun D, Wang C, Zhang X, Zhang W, Jiang H, Yao X et al (2019) Draft genome sequence of cauliflower (*Brassica oleracea* L. var. *botrytis*) provides new insights into the C genome in Brassica species. Hortic Res 6(1):82

Swarup V, Brahmi P (2005) Cole crops. In: Dhillon BS, Tyagi RK, Saxena S, Randhawa GJ (eds) Plant genetic resources: horticultural crops. Narosa Publishing House, New Delhi, pp 75–88

Tester M, Langridge P (2010) Breeding technologies to increase crop production in a changing world. Science 327:818–822

Thakur H, Vidyasagar (2016) Comparing of self-incompatibility and cytoplasmic male sterility systems for the performance of F_1 hybrids in cabbage (*Brassica oleracea* var. *capitata* L.). Electron J Plant Breed 7(3):602–610

Thakur JC, Singh N, Kumar JS (2004) Genetic analysis of some economic traits in main season cauliflower. Agric Res J 41(1):68–73

Till BJ, Reynolds SH, Greene EA, Codomo CA, Enns LC et al (2003) Large-scale discovery of induced point mutations with high-throughput TILLING. Genome Res 13:524–530

Till BJ, Burtner C, Comai L, Henikoff S (2004) Mismatch cleavage by single-strand specific nucleases. Nucleic Acids Res 32:2632–2641

Tonguc M, Griffiths PD (2004) Transfer of powdery mildew resistance from *Brassica carinata* to *Brassica oleracea* through embryo rescue. Plant Breed 123:587–589

Triques K, Piednoir E, Dalmais M, Schmidt J, Le Signor C et al (2008) Mutation detection using ENDO1: application to disease diagnostics in humans and TILLING and EcoTILLING in plants. BMC Mol Biol 9:9

Tsunoda S, Hirata K, Gomez-Campo C (1980) *Brassica* crops and wild allies. Japan Scientific Societies Press, Tokyo

Vanlalneihi B, Saha P, Kalia P, Singh S, Saha ND, Kundu A, Singh N, Bhowmik A (2019a) Genetic and principal component analysis for agro-morphological traits, bioactive compounds, antioxidant activity variation in breeding lines of early Indian cauliflower and their suitability for breeding. J Hortic Sci Biotechnol 15:1–3

Vanlalneihi B, Saha P, Kalia P, Jaiswal S, Kundu A, Saha ND, Sirowa SS, Singh N (2019b) Chemometric approach based characterization and selection of mid-early cauliflower for bioactive compounds and antioxidant activity. J Food Sci Technol 57:293. https://doi.org/10.1007/s13197-019-04060-6

Varalakshmi V (2009) Heterosis and combining ability for yield and its components in early cauliflower. Indian J Hortic 66:198–203

Varshney RK, Tuberosa R (2007) Genomics-assisted crop improvement vol. 1: genomics approaches and platforms. Springer, New York

Vaughan DA, Balasz E, Heslop-Harrison JS (2007) From crop domestication to super-domestication. Ann Bot 100:893–901

Verkerk R, Schreiner M, Krumbein A, Ciska E, Holst B et al (2009) Glucosiolates in Brassica vegetables: the influence of the food supply chain on intake, bioavailability and human health. Mol Nutr Food Res 53:S219–S265

Verma VK, Kalia P (2016) Comparative analysis of genetic diversity and its relation to heterosis in early and mid-maturity Indian cauliflower (*Brassica oleracea* var. *botrytis* L.). Indian J Hortic 73(4):518–525

Verma VK, Kalia P (2017) Combining ability analysis and its relationship with gene action and heterosis in early maturity cauliflower. Proc Natl Acad Sci India Sect B Biol Sci 87(3):877–884

Verma VK, Kalia P, Prasanna BM (2017) Genetic characterization of self-incompatible lines and strategies for heterosis breeding in cauliflower. Int J Veg Sci 23:411–429

Walters TW, Earle ED (1993) Organellar segregation, rearrangement and recombination in protoplast fusion-derived *Brassica oleracea* calli. Theor Appl Genet 85:761–769

Wang N, Shi L, Tian F, Ning H, Wu X, Long Y, Meng J (2010) Assessment of FAE1 polymorphisms in three *Brassica* species using EcoTILLING and their association with differences in seed erucic acid contents. BMC Plant Biol 10(1):137

Wang W, Zhang D, Yu S, Liu J, Wang D, Zhang F, Yu Y et al (2014) Mapping the *BrPur* gene for purple leaf color on linkage group A03 of *Brassica rapa*. Euphytica 199(3):293–302

Wang G, Lv J, Zhang J, Han S, Zong M, Guo N et al (2016) Genetic and epigenetic alterations of *Brassica nigra* introgression lines from somatic hybridization: a resource for cauliflower improvement. Front Plant Sci 7:1258. https://doi.org/10.3389/fpls.2016.01258

Warwick SI, Black LD (1991) Molecular systematics of *Brassica* and allied genera (subtribe *Brassicinae, Brassiceae*) chloroplast genome and cytodeme congruence. Theor Appl Genet 82:81–92

Yamagishi H, Bhat SR (2014) Cytoplasmic male sterility in *Brassicaceae* crops. Breed Sci 64(1):38–47

Yamagishi H, Nakagawa S, Kinoshita D, Ishibashi A, Yamashita Y (2008) Somatic hybrids between arabidopsis thaliana and cabbage (*Brassica oleracea* L.) with all chromosomes derived from *A. thaliana* and low levels of fertile seed. J Jpn Soc Hortic Sci 77(3):277–282

Yang K, Nath UK, Biswas MK, Kayum MA, Yi GE, Lee J, Yang TJ, Nou IS (2018) Whole-genome sequencing of *Brassica oleracea* var. *capitata* reveals new diversity of the mitogenome. PloS one 13(3):e0194356

Yarrow SA, Burnett LA, Wildeman RP, Kemble RJ (1990) The transfer of 'Polima' cytoplasmic male sterility from oilseed rape (*Brassica napus*) to broccoli (*B. oleracea*) by protoplast fusion. Plant Cell Rep 9:185–188

Yousef EA, Mueller T, Boerner A, Schmid KJ (2018) Comparative analysis of genetic diversity and differentiation of cauliflower (*Brassica oleracea* var. *botrytis*) accessions from two *ex situ* genebanks. PloS One 13(2):e0192062

Yu H, Ito T, Zhao Y, Peng J, Kumar P, Meyerowitz EM (2004) Floral homeotic genes are targets of gibberellin signaling in flower development. Proc Natl Acad Sci USA 101:7827–7832

Yuan Y, Chiu LW, Li L (2009) Transcriptional regulation of anthocyanin biosynthesis in red cabbage. Planta 230:1141–1153

Yumei L, Xiaowu W, Yuan F, Peitian S, Limei Y, Anfu H (1998) Investigation and utilization of heterosis in kohlrabi (*Brassica oleracea* var. *gongylodes*). Acta Hortic 467:127–132

Zeng CL, Wang GY, Wang JB, Yan GX, Chen BY et al (2012) High-throughput discovery of chloroplast and mitochondrial DNA polymorphisms in Brassicaceae species by ORG-EcoTILLING. PloS one 7(11):e47284

Zhang W, Wang S, Yu F, Tang J, Shan X, Bao K, Yu L et al (2019) Genome-wide characterization and expression profiling of *SWEET* genes in cabbage (*Brassica oleracea* var. *capitata* L.) reveal their roles in chilling and clubroot disease responses. BMC Genomics 20(1):93

Chapter 6
Marker-Assisted Selection in Pea Breeding

**Akhilesh Sharma, Bhallan Singh Sekhon, Rohit Kumar,
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6.1 Introduction

Food insecurity, malnutrition and climate change are the key challenges for the scientific community of twenty-first century. National Research Council (NRC) (2006) defined food insecurity as 'limited access to safe and nutrition adequate food'. Insufficient intake of biologically essential nutrients was termed malnutrition by NRC (2006). Both overnutrition and undernutrition are the types of malnutrition, and agricultural scientists are mainly concerned with the latter type. Malnutrition/undernutrition is further classified into protein energy malnutrition (PEM) and micronutrient deficiency, ranked first and second, respectively, on the basis of their importance in the world. PEM could lead to growth failure in humans, while micronutrient deficiencies lead to various diseases. Climate change is an additional issue to be addressed in the list and has been designated as an issue of global importance nowadays. Focusing on the breeding and biotechnological aspects is a potential key to enable us to combat with these challenges of nutritional and food security that can arise from several abiotic and biotic stresses.

Garden pea (*Pisum sativum* L.), nutritionally rich (high in protein) vegetable crop, is gaining immense importance in the malnourished world. Fitness in crop rotations and the nitrogen-fixing ability of this crop make it an ideal crop in the present era of climate change (Sharma et al. 2019a, b). Thus, emphasis on improvement of this crop became an inevitable need. Pea is renowned for its role in facilitating

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pioneering discoveries in the field of genetics and plant breeding. Gregor Mendel published his preeminent research in 1866 using garden pea as a model organism for research leading to discovery of the principles of genetics (Wade 1937). In 1787, pea became the first plant species improved by controlled breeding crosses and subsequent selection through the work of Thomas Knight (Wade 1937; Davies 1995). Much effort has been devoted to pea improvement during the following two centuries.

Despite their limitations, conventional breeding approaches like pureline method and pedigree method followed by hybridization, single plant selection, backcrossing, single seed descent and recurrent selection were applied to develop new varieties with desirable characteristics and are important for conserving wild germplasm, sexual hybridization between contrasting parental lines, novel genetic variants and mutations (Werner et al. 2005). But these approaches have the limitations of time span and may take several years for varietal development. Furthermore, having high labour requirement and being prone to human errors, these methods were considered less effective (Nguyen et al. 2018). To overcome such limitations, marker-assisted selection (MAS) is an emerging approach for accelerating the improvement in the twenty-first century breeding programmes.

Garden pea is a highly self-pollinated crop due to cleistogamous nature and presence of keel in the flower. It has seven pairs of chromosomes ($2n = 2x = 14$) with an estimated haploid size of 4.5 Gb (Doležel et al. 1998) being tenfold larger than the model legume *Medicago truncatula* and is mainly dominated by the mobile elements. Macas et al. (2007) reported that even in crops like pea which have relatively large genomic size, their generated sequences were sufficient to reconstruct and analyse major repeat families corresponding to a total of 35–48% of the genome and will provide a starting point for investigations of large genomic size based on global comparative analysis and for the development of more sophisticated approaches for data mining. Recently, the first annotated chromosome-level draft genome using the inbred pea cultivar 'Cameor' (characterized by protein-rich seeds) has been published and will hasten the marker-assisted crop improvement programmes in pea (Kreplak et al. 2019). MAS is largely practiced for simple traits that have few major effects, and not for the complex polygenic quantitative traits like yield, for which MAS involving marker-assisted recurrent selection (MARS) and genome-wide selection (GS) seem to be more appropriate (Bernardo and Yu 2007; Heffner et al. 2009).

The new tools of biotechnology would enable researchers to effectively go for the transfer of genes for insulating varieties from different diseases and pests. For this purpose, a number of markers that are known to be associated with QTL/genes for some major economic traits are being deployed for MAS in pea breeding programmes. Current genomic knowledge and technologies can also facilitate the allele mining for novel traits and their incorporation from wild *Pisum* sp. into elite domestic backgrounds. More recently, functional maps, i.e. composed of genes of known function, were developed (Aubert et al. 2006; Bordat et al. 2011). Translational genomics is also beginning to assist in identifying candidate genes or saturating markers in a zone of interest of pea.

6.2 Molecular Markers

Specific molecules which show easily detectable differences among different strains of a species or among different species are called markers. Nucleotide sequences which can be investigated through the polymorphism present between the nucleotide sequences of different individuals are known as molecular markers (Nadeem et al. 2018). In general, molecular markers are classified on DNA hybridization-based, polymerase chain reaction (PCR)-based and sequence-based markers (Fig. 6.1 and Table 6.1) on the basis of their detection and, more particularly, analysis (Singh 2015). The hybridization-based, PCR-based and sequence-based markers are generally low-, medium- and high-throughput, respectively, often referred to as first-generation, second-generation and third-generation markers, respectively. Commonly used molecular markers in pea breeding programme are listed in Table 6.2. As variability in the germplasm is prerequisite for any genetic improvement programme, therefore, identification and morphological characterization of diverse germplasm is

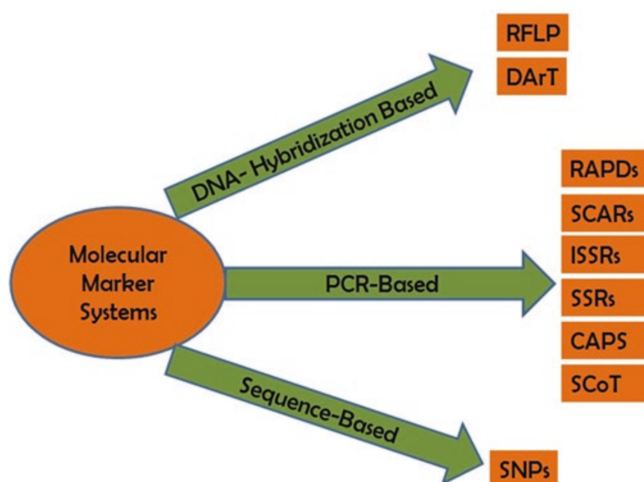

Fig. 6.1 Different molecular markers employed in crop improvement

Table 6.1 Commonly used molecular markers in crop improvement

Markers	Full form
RAPDs	Randomly amplified polymorphic DNAs
RFLP	Restriction fragment length polymorphism
DArT	Diversity array technology
SCARs	Sequence characterized amplified regions
ISSRs	Inter-simple sequence repeats
SSRs	Simple sequence repeats
CAPS	Cleaved amplified polymorphic sequence
SCoT	Start codon-targeted marker
AFLP	Amplified fragment length polymorphism
SNPs	Single nucleotide polymorphisms

a fundamental step towards plant improvement. Since morphological traits are environmental-dependent and time-consuming, molecular markers are implemented presently in germplasm profiling. Pea has been subjected to extensive characterization through morphological characters (Kalloo et al. 2005; Deepika et al. 2017) as well as different molecular marker systems such as RAPDs (Thakur et al. 2018), ISSRs (Kapila et al. 2011), SSRs (Ahmad et al. 2015) and EST-SSRs (Sharma et al. 2019a, b) to ascertain the extent of diversity present in given set of population. Molecular markers are becoming powerful tools to develop dense molecular genetic maps, understand genetic architecture of crop plant genomes, map agronomically important genes including quantitative trait loci (QTLs), to develop marker-assisted selection (MAS) and map-based cloning of useful genes, determine genetic diversity in crop germplasm and pathogen populations and precisely monitor alien introgression and analysis of evolutionary relationships.

Table 6.2 Commonly employed molecular markers in pea improvement

Markers	Nature of marker	Uses	References
1. *Powdery mildew*			
ScOPD 10_{650}	RAPD (OPD10650)-derived SCAR marker	Aids in efficient marker-assisted selection	Timmerman et al. (1994)
Sc-OPO-18_{1200} and Sc-OPE-16_{1600}	Two closely linked SCAR markers developed from RAPD markers: OPO18_{1200}, OPE16_{1600}	Sc-OPO18_{1200} identifying homozygous resistant individuals F_2 and subsequent segregating generations and Sc-OPE-16_{1600} will have greatest utility in selecting heterozygous BC\dn6 nF_1 individuals	Tiwari et al. (1998)
PSMPSAD51, PSMPSA5, PSMPSAD60, PSMPSAA374e, PSMPSAA369	SSR markers	Markers linked to er1. PSMPSAD60 was closely linked marker, at 10.4 cM distance from the er1 locus	Ek et al. (2005)
ScOPL61600 and PSMPSA5	SCAR marker and SSR marker	Both were linked to the er1mut2 locus, at a longer distance	Pereira et al. (2010)
ScOPX 04880	SCAR marker from RAPD marker OPX 04880	Differentiate homozygous resistant plants with more than 99% accuracy	Srivastava et al. (2012)
er1-2/MGB	STS dominant marker	Functional markers targeting the er1-2 allele	Pavan et al. (2013)
AD60 and c5DNAmet	SCAR marker and gene marker	Suggests that the resistance gene was an er1 allele	Sun et al. (2015)
PsMLO1-650	Coupling-phase marker	The functional marker for er1-6 could distinguish pea germplasm harbouring er1-6 from pea germplasm with non-er1-6 genotype	Wang et al. (2015), Sun et al. (2015a)
InDel111–120	Codominant functional marker specific for er1-7	This marker is of great importance for powdery mildew resistance in pea breeding programmes	Sun et al. (2016)

Table 6.3 Characteristics of different molecular markers

Characteristics	Types	Markers
Gene action	Dominant markers	RAPD, AFLP, DArT, ISSR, retrotransposons
	Codominant markers	RFLP, SSR, SNP
Reproducibility	High	RFLP, RAPD, SSR, SNP, DArT, retrotransposons
	Intermediate	AFLP
	Medium-high	ISSR
Polymorphism	Very high	RAPD
	High	AFLP, DArT, ISSR, retrotransposons, SSR, SNP
	Medium	RFLP
DNA quality required	High	RAPD, AFLP, DArT, retrotransposons, RFLP, SNP
	Low	ISSR, SSR
DNA quantity	High	RFLP
	Medium	RAPD
	Low	AFLP, DArT, retrotransposons, ISSR, SSR, SNP
PCR requirement	Yes	RAPD, AFLP, ISSR, retrotransposons, SSR, SNP
	No	DArT, RFLP
Cost	High	RFLP, AFLP, ISSR, SSR
	Less	RAPD
	Variable	SNP
	Cheapest	DArT, retrotransposons
Visualization	Agarose gel	RAPD, ISSR, retrotransposons, SSR, SNP
	Radioactive	RFLP
	SNP-vista	SNP
	Microarray	DArT
Sequencing	Yes	RFLP, SSR, SNP, DArT
	No	RAPD, AFLP, ISSR, retrotransposons
Genome abundance	Very high	RAPD, AFLP, SNP, DArT
	High	RFLP, retrotransposons
	Medium	ISSR, SSR

6.2.1 Ideal Molecular Marker

High degree of polymorphism, highly reproducible, evenly distributed throughout the genome, codominant nature, and rapid and easy detection along with low cost are the features of an ideal molecular marker (Mondini et al. 2009; Nadeem et al. 2018). Details regarding different molecular markers are presented in Table 6.3.

6.3 Molecular Mapping in Pea

Identification of a particular locus of a gene and the distance between the genes with the help of markers is termed as mapping. Mapping of a particular gene is classified into two types: genetic mapping and physical mapping. Genetic mapping helps in

identification of a particular gene, whereas physical mapping helps in measurement of true distance between the genes in base pairs for easy location of particular gene. Developing molecular maps is among the first steps of gene tagging and gene introgression strategies. In pea, genetic linkage was reported first time in 1911 by Vilmorin and Bateson (Vilmorin and Bateson 1911), the first genetic map was constructed by Wellensiek in 1925 (Wellensiek 1925), and Lamprecht (1948) published a full linkage map of pea with seven LGs. However, in 2000 a complete genetic map of seven chromosomes of pea was constructed with the help of RAPD and RFLP markers (Laucou et al. 1998; Rameau et al. 1998; Ellis and Poyser 2002). The first map based on genic markers like ESTs was constructed after the development of new sequencing techniques (Aubert et al. 2006; Bordat et al. 2011). Recently, first high-density pea SNP map defining all seven linkage groups was constructed and was found in syntenic relationship with the model legume *Medicago truncatula* and lentil (*Lens culinaris* Medik.) maps (Sindhu et al. 2014). Ma et al. (2017) have identified genome-wide SNPs and developed a linkage map for QTL identification for important seed mineral nutrients in pea which may further assist breeders in improvement of quality traits through MAS. Nowadays, SNPs are the markers of choice because of various merits because of their abundance, amenability to high throughput and easy scoring. Thus, these maps could be used in future molecular breeding efforts by enabling both the identification and tracking of introgression of genomic regions harbouring QTLs related to agronomic and seed quality traits.

6.4 New Molecular Breeding Strategies

Many important achievements in terms of improvement have been achieved in the last two decades with the help of conventional breeding. These include increase in grain yield, lodging resistance and resistance to various diseases and insects (powdery mildew resistance, *Ascochyta* blight resistance and resistance to pea weevil). However, conventional breeding strategies restrict the improvement programme as it is time-consuming and laborious and involves huge manpower. Thus to overcome these limitations, modern breeding strategies which include molecular approaches are being utilized for the development of new or improved variety with desire traits. Marker-assisted selection procedure is described in Fig. 6.2. These strategies include the following approaches:

6.4.1 Marker-Assisted Backcrossing (MAB)

Most commonly used method of incorporating one or more gene into elite variety in breeding is backcrossing. In this method of breeding, parent used has large number of desirable attributes but is deficient in only a few characteristics (Allard 1999). The method was first described in 1922 and was widely used between the 1930s and

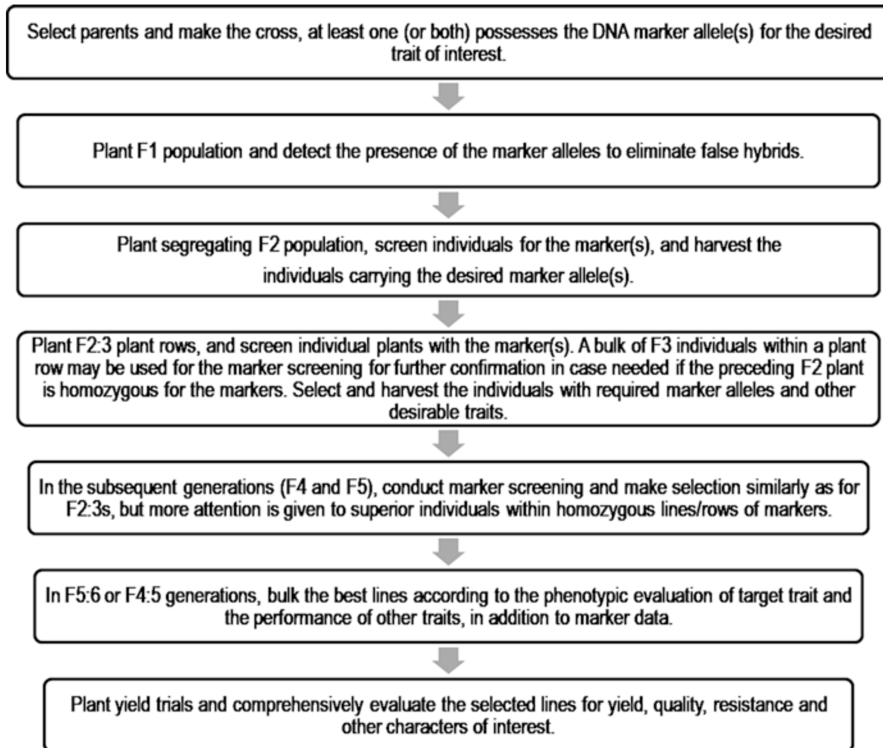

Fig. 6.2 Flow chart for MAS procedure with single cross example

1960s (Stoskopf et al. 1993). Introduction of markers has increased the efficiency of selection procedure. MAB procedure includes three steps of selection: foreground, recombinant and background selection (Holland 2004). The first step (foreground selection) involves use of molecular marker to identify/screen the desired gene or QTL (Hospital and Charcosset 1997). Second step (recombinant selection) involves the selection of backcross progeny with target gene and recombinants between the target locus and tightly linked flanking markers, and the last and final step (background selection) involves selection of recurrent parent (RP) by utilizing unlinked markers to the target locus. Foreground selection is useful for traits having time-consuming phenotypic screening. It is used to identify best plant for backcrossing as it can select reproductive traits in seedling stage. Foreground selection can also select recessive alleles which are difficult to select via conventional methods (Collard and Mackill 2008). Recombinant selection helps in reducing the size of the introgression which leads to linkage drag (Hospital 2005). The use of background selection during MAB to accelerate the development of a RP with an additional (or a few) genes has been referred to as 'complete line conversion' (Ribaut et al. 2002). Some examples of MAB in pea are being discussed in later section. We (unpublished data) have introgressed powdery mildew resistance genes into elite genotypes

of pea using molecular marker-assisted selection. We have developed powdery mildew-resistant versions of 'Pb89' and Bonneville introgressed with powdery mildew resistance genes *er1* and *er2*. Genetic stocks carrying *er1er1* as well as *er2er2* gene in the background of Pb89 and Bonneville were developed. The resultant lines had as much as 85% of background genome of recurrent parent, and all the introgressed lines were also subjected to phenotypic selection for horticultural traits of importance during the course of introgression. These superior genetic stocks can serve as pre-breeding material to be used in pea improvement programmes for powdery mildew resistance and can also be further crossed among themselves to pyramid both the genes into common genetic background. Some of the results related to foreground and background selection have been given in (Fig. 6.3a–c).

Besides, we are working on improvement of garden pea using conventional breeding approaches and developed varieties, namely, Palam Triloki (early maturing) and Palam Sumool (mid maturing). Further, research initiatives have been taken up to develop garden pea genotypes having long (>10 cm), well-filled (>10 seeds/pod), sweet and lush green pods along with high shelling. Keeping this in view, selections were carried out in the segregating generations of three intervarietal crosses to isolate progenies with desirable pod and quality traits as well as powdery mildew resistance by following pedigree method of selection (Sharma et al. 2019a). Before initiating this programme, genetic diversity of parents involved in the hybridization programme was tested using SSR markers. Finally, 36 (F_7) homozygous superior progenies were isolated. The best performing lines 'DPP-SP-6', 'DPP-SP-7', 'DPP-SP-17' and 'DPP-SP-22' for majority of economic traits also showed resistance to powdery mildew disease. The morphological characterization revealed that these genotypes had long to very long pods with slightly curved to curved characteristics and green to dark green colour. 'DPP-SP-6' (Fig. 6.4), being the most promising, could be recommended for cultivation as an alternative to existing varieties, in north western Himalayan region as it showed stable performance over varied environmental conditions (Rana 2019).

6.4.2 Marker-Assisted Gene Pyramiding (MAGP)

Combining various genes together in a single genotype is called as pyramiding. Pyramiding of genes is one of the important methods of conventional breeding. The application of DNA markers to plant breeding for gene pyramiding is termed as marker-assisted gene pyramiding (MAGP). Limitation of gene pyramiding in conventional breeding includes difficulties in identifying the plants with more than one gene. Moreover, one has to evaluate all traits tested which made assessment of plants from F_2 population with traits having destructive bioassays difficult. These limitations are overcome by DNA markers selections which are basically non-destructive. MAGP is most commonly used for combining multiple disease resistance genes to develop stable disease and insect resistance at the same time since pathogens frequently overcome single-gene host resistance over time due to the

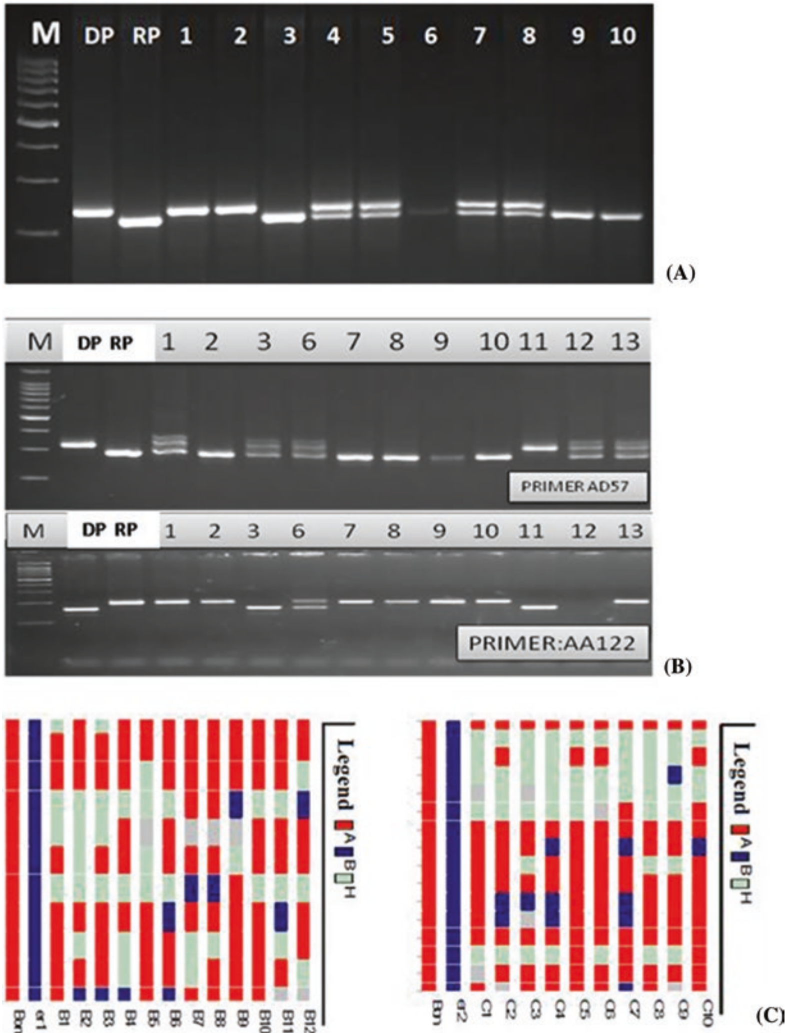

Fig. 6.3 (**a**) Genotyping of BC3F2 population of pea using AD60 (a foreground marker for er1**);** (**b**) background selection of BC3F2 population using different primers; (**c**) background of BC_3F_2 lines with introgressed *er1* gene, as depicted by the GGT software

emergence of new plant pathogen races (Kloppers and Pretorius 1997; Shanti et al. 2001; Singh et al. 2001). MAGP can be done by combining genes from multiple parents by using any of the mentioned approaches: multiple-parent crossing or complex crossing, backcrossing and recurrent selection. MAGP may also be used to facilitate the combination of QTLs for abiotic stress tolerances, especially QTLs effective at different growth stages. Another use could be to combine single QTLs that interact with other QTLs (i.e. epistatic QTLs). Three strategies, stepwise,

Fig. 6.4 (**a–d**) Garden pea genotype 'DPP-SP-6': profused pod bearing, long and bright green pods

simultaneous/synchronized and convergent backcrossing or transfer, are being followed for MAGP in breeding for crop improvement. In pea, the pyramiding of *er1*, *er2* and *Er3* genes into an elite background can be done for achieving broad and durable resistance against the powdery mildew.

6.4.3 Marker-Assisted Recurrent Selection (MARS)

MARS is one of the MAS approaches which is based on recurrent selection and mainly utilized for identifying and selecting multiple genomic regions with expression of complex traits and assembling best genotype within a single or across related populations (Eathington et al. 2007; Ribaut et al. 2010). By MARS approach genotypic selection and intercrossing among the selected individuals could be done in the same crop season for one cycle of selection (Jiang et al. 2007) which enhances the efficiency of recurrent selection and progress of the procedure particularly helping in integrating multiple favourable genes or QTLs (Gazal et al. 2015). General procedure for MARS is given in Fig. 6.2 (source: Jiang et al. 2007). For complex traits such as grain yield and biotic and abiotic resistance, MARS has been proposed for 'forward breeding' of native genes and pyramiding multiple QTLs (Ribaut et al. 2010).

6.4.4 Genome Selection

Genomic selection (GS) or genome-wide selection (GWS) is marker-based selection approach that uses available tens or hundreds of thousands of markers covering the whole genome for simultaneously selecting all genes which are in associated with at least some of the markers (Meuwissen et al. 2001; Meuwissen 2007). It is an upgrade form of MAS (Goddard 2009) and is rapidly gaining popularity among the breeders particularly for the traits which are difficult to measure. In GS approach, QTLs and gene identified are in linkage disequilibrium with at least one marker and help in reduction of the risk of missing small-effect QTLs (Guo et al. 2012). Genome-wide association (GWA) mapping has recently emerged as a valuable approach for refining the genetic basis of polygenic resistance to plant diseases, which are increasingly used in integrated strategies for durable crop protection (Desgroux et al. 2016). GS is much advance and powerful approach than MARS, but due to non-availability of surplus knowledge of GS, it could not become a popular MAS approach in breeding for crop improvement (Nakaya and Isobe 2012). However, Carpenter et al. (2018) have reported the efficacy of GS in predicting *Ascochyta* blight in pea and have suggested GS as potentially useful for pea breeding programmes pursuing aschohyta blight resistance. GS could not work well with hundreds and thousands of crosses/populations at the same time. Thus, there is need for evaluating accuracy and cost-effectiveness of GS in practical breeding programmes (Heffner et al. 2009). However, GS involving the prediction of the date of beginning of flowering and 1000 seed weight using a subset of 331 SNP markers genotyped in a reference collection of 372 pea accessions by Burstin et al. (2015) seems to be a potential strategy in pea. Tayeh et al. (2015b) have further improved the prediction accuracies using GenoPea 13.2 K SNP Array (Tayeh et al. 2015a) by increasing the marker coverage of the genome.

6.5 Examples of MAS in Pea Breeding

Utilization of marker-assisted selection approach in plant breeding helped a lot in improving selection efficiency and reduced the time and effort needed for development of new cultivars of various crops. There is not much involvement of MAS approach in legumes improvement due to certain reasons which are as follows:

(a) Dearth of knowledge, physical capacity for genetic and genomic analysis and infrastructure facilities for conducting MAS in conventional plant breeding of legumes.
(b) Lack of genomic data in most of the pulse crops is major limitation to establish close linkage between molecular marker and trait of interest.
(c) Less interaction between biotechnologists and plant breeders.
(d) Conventional plant breeders hesitate to use MAS in their routine breeding programmes.

(e) Difference in biological system of pulse crops which have high plasticity and G × E interactions (Mackay 2009).

However, considerable efforts have been made in development of genomic resources during the past decades (Sato et al. 2010). To ensure optimal cost-effectiveness, molecular markers used for MAS should both permit efficient screening of large populations and show a high degree of reproducibility across laboratories (Mohan et al. 1997). RFLPs are reliable and yield codominant data but are also time-consuming and expensive, requiring relatively large amount of highly purified DNA, and they do not lend themselves to automation (Gupta et al. 1999). RAPD markers are notoriously unreliable with poor replication success among laboratories (Penner et al. 1993). In order to facilitate breeding for disease resistance, research for molecular markers linked to resistant genes has been carried out (Dirlewanger et al. 1994). RLFP markers p252, p254, p248, p227, p105 and p236 and RAPD markers H19, Y14 and YI5 are found linked to resistance genes mo, Fw and er. Some QTLs were also mapped for resistance to *Ascochyta pisi* race c. In the same way, Timmerman et al. (1994) identified a RAPD marker (PD10650) tightly linked to powdery mildew-resistant gene, er, and the RFLP marker GS185 closely linked to sbm-1 (Timmerman et al. 1993). SCAR markers are more reliable but are often developed from RAPD markers (Paran and Michelmore 1993), which might limit their utility. SSR markers, on the other hand, combine reliability and genomic abundance with high levels of polymorphism (Mohan et al. 1997). They do not require sophisticated DNA extraction methods and are ideally suited for high-throughput automated scoring and multiplexing (Tang et al. 2003). With PCR multiplexing, the cost for SSR analysis may be less than 0.5 USD per marker and individual (Frey et al. 2004). The main drawback of SSRs is the initial identification of primer sites to amplify SSR loci, a procedure which is time- and resource-demanding. In the present case, a large number of SSR markers are already available for pea (Burstin et al. 2001; Loridon et al. 2005), thereby obviating the need for initial SSR development. Thus, MAS using SSR markers will most likely become a valuable tool in pea breeding. Microsatellite (SSR) markers can be successfully used in marker-assisted selection for powdery mildew resistance breeding in pea. Nevertheless, by using flanking SSR markers, a high degree of reliability in differentiating resistant and susceptible genotypes can be reached. Given the superior performance and cost-effectiveness of SSR markers over other marker systems, SSR markers provide an attractive option for MAS of powdery mildew resistance in pea (Ek et al. 2005).

In pea breeding MAS has been mostly used in development of disease-resistant varieties (Table 6.4). *Erysiphe pisi* is the causative agent of powdery mildew disease in pea. Two single recessive genes (*er1* and *er2*) and one dominant gene (*Er3*) have been identified for powdery mildew resistance in pea germplasms to date. Majority of pea powdery mildew breeding programmes rely on the presence of the recessive gene *er1* as it presents complete resistance from powdery mildew by constraining pathogen penetration, while the *er2* interceded resistance is primarily in view of decrease in penetration success supplemented by post-penetration cell death (Fondevilla et al. 2006). Now, a dominantly inherited gene for resistance to powdery

Table 6.4 Molecular markers linked to desirable genes/QTL for marker-assisted selection in pea

Trait(s)	QTL/ genes	Type of markers	Genetic distance (cM)	References
Resistance to powdery mildew	er1	RFLP	11.0	Dirlewanger et al. (1994)
Resistance to powdery mildew	er-1	RAPD	2.0	Tiwari et al. (1998)
Resistance to powdery mildew	er1	PSMPAD60	10.4	Ek et al. (2005)
Resistance to powdery mildew	er-2	ScX17_1400	2.6	Katoch et al. (2010)
Resistance to powdery mildew	Er3	SSR	0.39	Cobos et al. (2018)
Resistance to *Fusarium* wilt	Fw	RFLP	6.0	Dirlewanger et al. (1994)
Resistance to pea common mosaic virus	mo	RFLP	15.0	Dirlewanger et al. (1994)
Resistance to *Fusarium* wilt	Fw	SCAR	1.2	Kwon et al. (2013)
Resistance to pea seed-borne mosaic virus	Sbm-1	STS	4.0	Frew et al. (2002)

mildew, named *Er3*, has been identified in *Pisum fulvum* (Fondevilla et al. 2007). *Er3*, unlike the gene *er2*, is not temperature-dependent. Difficulties related to the treatment of obligate pathogens like *E. pisi* along with recessive nature of *er1* and *er2* entangle determination of resistant progenies in breeding programmes. Therefore, molecular mapping of such genes is a prerequisite for any molecular-assisted breeding programme. The *er1* locus has been mapped on linkage group VI of the pea consensus map (Timmerman et al. 1994). Several workers have reported various DNA markers linked to er1, er2 and Er3, and marker-assisted selection for powdery mildew resistance in pea has been reviewed by Ghafoor and McPhee (2012).

Humphry et al. (2011) and Pavan et al. (2011) reported that genetic and phyto-pathological features of *er1* resistance are similar to those of barley, Arabidopsis, and tomato *MLO* powdery mildew resistance, which is caused by the loss of function of specific members of the *MLO* gene family. To date, seven *er1* alleles (*er1-1* to *er1-7*) have been identified conferring the er1-resistant phenotype in pea germplasms (Humphry et al. 2011; Pavan et al. 2011, 2013; Sun et al. 2015a, b). All alleles except for *er1-2* and *er1-7* are the result of point mutations, including single base substitutions and deletions, in the *PsMLO1* coding sequence. Therefore, many functional markers have been developed by many workers for the er1. Cobos et al. (2018) localized Er3 gene in pea genetic map of susceptible pea cv. Messire (er3er3) and a resistant near isogenic line of Messire (cv. Eritreo, Er3Er3) with SSR markers and found that SSRs (AA349 and AD61) were linked to Er3 gene and RAPD and SCAR markers which were already linked to this gene (Cobos et al. 2018).

Pea seed-borne mosaic virus (PSbMV) is an economically important viral pathogen affecting pea crop across globe. PSbMV resistance is conferred by a single recessive gene (*eIF4E*), localized on LG VI (*sbm-1* locus). SSR and SNPs were developed for identifying resistance to PSbMV (Smýkal et al. 2010). Marker-assisted backcross selection for PSbMV resistance gene in locally adapted breeding lines from 'Lifter' has been carried successfully recently (Scegura 2017). *Fusarium*

wilt is also one of the most widespread diseases of pea. A single gene (*Fw)* located on chromosome 3 provides resistance to *Fusarium wilt* race 1. Earlier reported AFLP and RAPD markers have not succeeded in MAS because of map distance and linkage phase. Thus SCAR markers were used for MAS in pea. Three SCAR markers (Fw_Trap_480, Fw_Trap_340 and Fw_Trap_220) which are tightly linked to and only 1.2 cM away from the *Fw* locus were found ideal for *Fusarium wilt* race 1 resistance (Kwon et al. 2013).

6.6 Future Prospects

To make MAS approach successful in pea breeding programme, breeders have to develop mapping populations and map the important gene/QTL related to a particular trait which will help in identifying tightly linked markers for MAS. Advanced techniques like TILLING could be used for mining rare alleles of genes controlling important traits. The draft genome of pea published recently in September 2019 by an international consortium has further equipped the breeders with a valuable tool and is expected to further facilitate the prospects of hastening pea improvement and allow more efficient use of available genetic diversity in the pea crop. High-throughput molecular markers like SNP and SSR are widely utilized for improving understanding of complex traits, relations among traits, and between target trait and environments at genetic levels. These markers will certainly provide cheap and large-scale screening of segregating individuals for selecting desirable genotypes and will make MAS breeding of pea crop more broad, useful and efficient as well as cost-effective in future. MAS approach could be used to evaluate germplasm, map genes and characterize complex traits effortlessly. Breeding cycle could be reduced, and offspring can be selected at any time of the year by MAS as it allows selection at seedling stage. With the complete knowledge of genome sequence of pea crop, markers could be developed linked to different desired traits and can be used further in breeding for development of improved and new cultivars.

References

Ahmad S, Kaur S, Palmer NDL (2015) Genetic diversity and population structure of *Pisum sativum* accessions for marker trait association of lipid content. Crop J 3:238–245

Allard RW (1999) Principles of plant breeding, 2nd edn. Wiley, New York

Aubert G, Morin J, Jacquin F et al (2006) Functional mapping in pea, as an aid to the candidate gene selection and for investigating synteny with the model legume Medicago truncatula. Theor Appl Genet 112:1024–1041. https://doi.org/10.1007/s00122-005-0205-y

Bernardo R, Yu J (2007) Prospects for genomewide selection for quantitative traits in maize. Crop Sci 47(3):1082–1090

Bordat A, Savois V, Nicolas M et al (2011) Translational genomics in legumes allowed placing in silico 5460 Unigenes on the pea functional map and identified candidate genes in *Pisum sativum* L. Andrews BJ, editor. G3 58(1):93–103. https://doi.org/10.1534/g3.111.000349

Burstin J, Deniot G, Potier J et al (2001) Microsatellite polymorphism in Pisum sativum. Plant Breed 120:311–317

Burstin J, Salloignon P, Chabert-Martinello M, Magnin-Robert JB, Siol M, Jacquin F et al (2015) Genetic diversity and trait genomic prediction in a pea diversity panel. BMC Genomics 16:105. https://doi.org/10.1186/s12864-015-1266-1

Carpenter S, Mishra P, Ghoshal C, Dash P, Wang L, Midha S et al (2018) A strain of an emerging Indian pathotype of Xanthomonas oryzae pv. oryzae defeats the rice bacterial blight resistance gene xa13 without inducing a clade III SWEET gene and is nearly identical to a recent Thai isolate.. bioRxiv [preprint. https://doi.org/10.1101/384289

Cobos MJ, Satovic Z, Rubiales D, Fondevilla S (2018) Er3 gene, conferring resistance to powdery mildew in pea, is located in pea LGIV. Euphytica 214:11

Collard BC, Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philos Trans R Soc Lond Ser B Biol Sci 363(1491):557–572. https://doi.org/10.1098/rstb.2007.2170

Davies RD (1995) Peas Pisum sativum. In: Smartt J, Simmonds NW (eds) Evolution of crop plants. Longman scientific and technical, Essex England

Deepika, Mahajan R, Lay P et al (2017) Assessment of genetic variability, heritability and genetic advance among *Pisum* accessions. Vegetos 30(Special):482–488. https://doi.org/10.5958/2229-4473.2017.00125.2

Desgroux A, Anthoënel VL, Roux-Duparque M et al (2016) Genome-wide association mapping of partial resistance to *Aphanomyces euteiches* in pea. BMC Genomics 17:124. https://doi.org/10.1186/s12864-016-2429-4

Dirlewanger E, Isaac PG, Ranade S et al (1994) Restriction fragment length polymorphism analysis of loci associated with disease resistance genes and development traits in *Pisum sativum* L. Theor Appl Genet 88:17–27

Doležel J, Greilhuber J, Lucretti S, Meister A, Lysák MA, Nardi L, Obermayer R (1998) Plant genome size estimation by flow cytometry: inter-laboratory comparison. Ann Bot 82(suppl_1):17–26

Eathington SR, Crosbie TM, Edwards MD et al (2007) Molecular markers in commercial breeding. Crop Sci 47:154–163

Ek M, Eklund M, Von Post R et al (2005) Microsatellite markers for powdery mildew resistance in pea (*Pisum sativum* L.). Hereditas 142:86–91

Ellis THN, Poyser SJ (2002) An integrated and comparative view of pea genetic and cytogenetic maps. New Phytol 153:17–25. Blackwell Science Ltd. https://doi.org/10.1046/j.0028646X.2001.00302.x

Fondevilla S, Carver TLW, Moreno MT et al (2006) Macroscopic and histological characterisation of genes *er1* and *er2* for powdery mildew resistance in pea. Eur J Plant Pathol 115:309–321

Fondevilla S, Carver TLW, Moreno MT et al (2007) Identification and characterisation of sources of resistance to *Erysiphe pisi* Syd. In *Pisum* spp. Plant Breed J 126:113–119

Frew TJ, Russell AC, Timmerman-Vaughan GM (2002) Sequence tagged site markers linked to the *smb*1 gene for resistance to pea seed borne mosaic virus in pea. Plant Breed 121:512–516

Frey JE, Frey B, Sauer C et al (2004) Efficient low-cost DNA extraction and multiplex fluorescent PCR method for marker-assisted selection in breeding. Plant Breed 123:554–557

Gazal A, Dar ZA, Lone AA, Abidi I, Ali AG (2015) Molecular breeding for resilience in maize. IASET JANS 7(2):1057–1063

Ghafoor A, McPhee K (2012) Marker assisted selection for developing powdery mildew resistant pea cultivars. Euphytica 186:593–607

Goddard M (2009) Genomic selection: prediction of accuracy and maximisation of long term response. Genetica 136:245–257

Guo ZG, Tucker DM, Lu JW (2012) Evaluation of genome-wide selection efficiency in maize nested association mapping populations. Theor Appl Genet 124:261–275

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their applications in wheat breeding. Plant Breed 118:369–390. https://doi.org/10.1046/j.1439-0523.1999.00401.x

Heffner EL, Sorrells ME, Jannink JL (2009) Genomic selection for crop improvement. Crop Sci 49:1–12

Holland JB (2004) Implementation of molecular markers for quantitative traits in breeding programs-challenges and opportunities. In: Proceedings of 4th international crop science, Brisbane, 26 Sept–1 Oct
Hospital F (2005) Selection in backcross programmes. Philos Trans R Soc B 360:1503–1511. https://doi.org/10.1098/rstb.2005.1670
Hospital F, Charcosset A (1997) Marker-assisted introgression of quantitative trait loci. Genetics 147:1469–1485
Humphry M, Reinstädler A, Ivanov S et al (2011) Durable broad-spectrum powdery mildew resistance in pea er1 plants is conferred by natural loss-of-function mutations in *PsMLO1*. Mol Plant Pathol 12:866–878
Jiang GL, Dong Y, Shi J, Ward RW (2007) QTL analysis of resistance to Fusarium head blight in the novel wheat germplasm CJ 9306. II. Resistance to deoxynivalenol accumulation and grain yield loss. Theor and Appl Genet 115(8):1043–1052
Kalloo G, Rai M, Singh J et al (2005) Morphological and biochemical variability in vegetable pea (*Pisum sativum* L.). Veg Sci 32(1):19–23
Kapila K, Naryal S, Dhiman KC (2011) Analysis of genetic diversity among garden and field pea genotypes of higher Indian Himalayas. J Plant Biochem Biotechnol 21(2):286–291
Katoch V, Sharma S, Pathania S et al (2010) Molecular mapping of pea powdery mildew resistance gene er2 to pea linkage group III. Mol Breed 25(2):229–237
Kloppers FJ, Pretorius ZA (1997) Effects of combinations amongst genes Lr13, Lr34 and Lr37 on components of resistance in wheat to leaf rust. Plant Pathol 46:737–750. https://doi.org/10.1046/j.1365-3059.1997.d01-58.x
Kreplak J et al (2019) A reference genome for pea provides insight into legume genome evolution. Nat Genet 51:1411–1422
Kwon SJ, Smykal P, Hu J, Wang M, Kim SJ, McGee RJ, McPhee K, Coyne CJ (2013) User-friendly markers linked to *Fusarium* wilt race 1 resistance *Fw* gene for marker assisted selection in pea. Plant Breed 132: 642–648
Lamprecht H (1948) The variation of linkage and the course of crossing over. Agric Hort Genet 6:10–49
Laucou V, Haurogne ÂK, Ellis N et al (1998) Genetic mapping in pea. 1. RAPD-based genetic linkage map of Pisum sativum. Theor Appl Genet 97:905–915
Loridon K, McPhee K, Morin J et al (2005) Microsatellite marker polymorphism and mapping in pea (Pisum sativum L.). Theor Appl Genet. In Press
Ma Y, Coyne CJ, Grusak MA et al (2017) Genome-wide SNP identification, linkage map construction and QTL mapping for seed mineral concentrations and contents in pea (*Pisum sativum* L.). BMC Plant Biol 17:43. https://doi.org/10.1186/s12870-016-0956-4
Macas J, Neumann P, Navratilova A (2007) Repetitive DNA in the pea (Pisum sativum L.) genome: comprehensive characterization using 454 sequencing and comparison to soybean and Medicago truncatula. BMC Genomics 8: 427. https://doi.org/10.1186/1471-2164-8-427
Mackay TFC (2009) A-maize-ing diversity. Science 325:688–689
Meuwissen T (2007) Genomic selection: marker assisted selection on a genome wide scale. J Anim Breed Genet 124:321–322
Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome wide dense marker maps. Genetics 157:1819–1829
Mohan M, Nair S, Bhagwat A, Krishna TG, Yano M, Bhatia CR, Sasaki T (1997) Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol Breed 3:87–103. https://doi.org/10.1023/A:1009651919792
Mondini L, Noorani A, Pagnotta MA (2009) Assessing plant genetic diversity by molecular tools. Diversity 1(1):19–35
Nadeem MA, Nawaz MA, Shahid MQ et al (2018) DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnol Biotechnol Equip 32(2):261–285. https://doi.org/10.1080/13102818.2017.1400401
Nakaya A, Isobe SN (2012) Will genomic selection be a practical method for plant breeding? Ann Bot 110:1303. https://doi.org/10.1093/aob/mcs109

National Research Council (2006) Food insecurity and hunger in the United States: an assessment of the measure. The National Academies Press, Washington, DC. https://doi.org/10.17226/11578
Nguyen GN, Norton SL, Rosewarne GM et al (2018) Automated phenotyping for early vigour of field pea seedlings in controlled environment by colour imaging technology. PLoS One 13(11):e0207788. https://doi.org/10.1371/journal.pone.0207788. pmid: 30452470
Paran I, Michelmore R (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:985–993
Pavan S, Schiavulli A, Appiano M et al (2011) Pea powdery mildew *er1* resistance is associated to loss-of-function mutations at a MLO homologous locus. Theor Appl Genet 123:1425–1431
Pavan S, Schiavulli A, Appiano M et al (2013) Identification of a complete set of functional markers for the selection of *er1* powdery mildew resistance in *Pisum sativum* L. Mol Breed J 31:247–253
Penner GA, Bush A, Wise R, Kim W et al (1993) Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. Genome Res 2:341–345
Pereira G, Marques C, Ribeiro R, Formiga S, Dâmaso M, Sousa MT, Farinhó M, Leitão JM (2010) Identification of DNA markers linked to an induced mutated gene conferring resistance to powdery mildew in pea (Pisum sativum L.). Euphytica 171(3):327
Rameau C, DeÂnoue D, Fraval F et al (1998) Genetic mapping in pea 2 identification of RAPD and SCAR markers linked to genes affecting plant architecture. Theor Appl Genet 97:916–928. https://doi.org/10.1007/s001220050972
Rana C (2019) Stability analysis for pod yield and component traits and validation of powdery mildew resistance in garden pea. PhD Dissertation submitted in CSK Himachal Pradesh Agricultural University
Ribaut JM, Jiang C, Hoisington D (2002) Simulation experiments on efficiencies of gene introgression by backcrossing. Crop Sci 42:557–565
Ribaut JM, Vicente MC, Delannay X (2010) Molecular breeding in developing countries: challenges and perspectives. Curr Opin Plant Biol 13:1–6
Sato S, Isobe S, Tabata S (2010) Structural analyses of the genomes in legumes. Curr Opin Plant Biol 13:1–17
Scegura A (2017) Marker assisted backcross selection for virus resistance in pea (*Pisum sativum* L.). North Dakota State University of agriculture and applied science, Fargo
Shanti ML, George MLC, Cruz CMV et al (2001) Identification of resistance genes effective against rice bacterial blight pathogen in eastern India. Plant Dis 85:506–512. https://doi.org/10.1094/PDIS.2001.85.5.506
Sharma A, Sekhon B, Sharma S, Kumar R (2019a) Newly isolated intervarietal garden pea (Pisum sativum L.) progenies (F_7) under north western Himalayan conditions of India. Exp Agric 1–12. https://doi.org/10.1017/S0014479719000115. Published online Apr 2019
Sharma R, Dar AA, Mahajan R et al (2019b) Molecular and biochemical characterisation of Indian germplasm of *Pisum sativum* L. Proc Natl Acad Sci India Sect B Biol Sci 90:103. https://doi.org/10.1007/s40011-018-01069-3
Sindhu A, Ramsay L, Sanderson LA et al (2014) Gene-based SNP discovery and genetic mapping in pea. Theor Appl Genet 127(10):2225–2241. https://doi.org/10.1007/s00122-014-2375-y
Singh BD (2015) Plant breeding: principles and methods. Kalyani Publishers, New Delhi, p 697
Singh S, Sidhu JS, Huang N et al (2001) Pyramiding three bacterial blight resistance genes (*xa*5, *xa*13 and *Xa*21) using marker-assisted selection into indica rice cultivar PR106. Theor Appl Genet 102:1011–1015. https://doi.org/10.1007/s001220000495
Smýkal P, Šafářová D, Navrátil M et al (2010) Marker assisted pea breeding: eIF4E allele specific markers to pea seed-borne mosaic virus (PSbMV) resistance. Mol Breed 26:425
Srivastava RK, Mishra SK, Singh AK, Mohapatra T (2012) Development of a coupling-phase SCAR marker linked to the powdery mildew resistance gene 'er1'in pea (Pisum sativum L.). Euphytica 186(3):855–866
Stoskopf NC, Tomes DT, Christie BR (1993) Plant breeding: theory and practice. Westview Press Inc, San Francisco/Oxford

Sun S, Fu H, Wang Z et al (2015a) Discovery of a novel *er1* allele conferring powdery mildew resistance in Chinese pea (*Pisum sativum* L.) landraces. PLoS One. https://doi.org/10.1371/journal.pone.0147624

Sun S, Fu H, Wang Z et al (2015b) Resistance to powdery mildew in the pea cultivar Xucai 1 is conferred by the gene *er1*. Crop J 3:489–499

Sun S, Deng D, Wang Z et al (2016) A novel *er1* allele and the development and validation of its functional marker for breeding pea (*Pisum sativum* L*.)* resistance to powdery mildew. Theor Appl Genet 129:909–919

Sun S, Wang Z, Fu H, Duan C, Wang X, Zhu Z (2015) Resistance to powdery mildew in the pea cultivar Xucai 1 is conferred by the gene er1. The Crop Journal 3(6):489–499

Tang SX, Kishore VK, Knapp SJ (2003) PCR-multiplexes for a genome-wide framework of simple sequence repeat marker loci in cultivated sunflower. Theor Appl Genet 107:6–19

Tayeh N, Aluome C, Falque M, Jacquin F, Klein A, Chauveau A et al (2015a) Development of two major resources for pea genomics: the GenoPea 13.2K SNP Array and a high density, high resolution consensus genetic map. Plant J 84(6):1257–1273. https://doi.org/10.1111/tpj.13070. [Epub ahead of print]

Tayeh N, Klein A, Le Paslier MC, Jacquin F, Houtin H, Rond C et al (2015b) Genomic prediction in pea: effect of marker density and training population size and composition on prediction accuracy. Front Plant Sci 6:941. https://doi.org/10.3389/fpls.2015.00941

Thakur B, Sharma S, Sharma I et al (2018) Diversity analysis of pea genotypes using RAPD markers. Legum Res 41(2):196–201

Timmerman GM, Frew TJ, Allison AL et al (1993) Linkage mapping of *sbm*-1, a gene conferring resistance to pea seed-borne mosaic virus, using molecular markers in *Pisum sativum*. Theor Appl Genet 85:609–615

Timmerman GM, Frew TJ, Weeden NF et al (1994) Linkage analysis of er-1, a recessive *Pisum sativum* gene for resistance to powdery mildew fungus *(Erysiphe pisi* D.C.). Theor Appl Genet 88:1050–1055

Tiwari KR, Penner GA, Warkentin TD (1998) Identification of coupling and repulsion phase RAPD markers for powdery mildew resistance gene er-1 in pea. Genome 41(3):440–444. https://doi.org/10.1139/g98-014

Vilmorin PD, Bateson WA (1911) Case of Gametic coupling in *Pisum*. Proc R Soc B Biol Sci R Soc 84:9–11. https://doi.org/10.1098/rspb.1911.0040

Wade BL (1937) Breeding and improvement of peas and beans. Year book of agriculture

Wang Z, Fu H, Sun S, Duan C, Wu X, Yang X, Zhu Z (2015) Identification of powdery mildew resistance gene in pea line X9002. Acta Agron Sin 41:515–523

Wellensiek SJ (1925) Genetic monograph on *Pisum* [Internet] Martinus Nijhoff. Available: https://books.google.ru/books?id=hJjYOwAACAAJ

Werner K, Friedt W, Ordon F (2005) Strategies for pyramiding resistance genes against the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2). Mol Breed 16:45–55. https://doi.org/10.1007/s11032-005-3445-2

Chapter 7
Efficient Methods for the Improvement of Temperate Root Vegetables

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7.1 Beetroot (*Beta vulgaris* L.)

7.1.1 Introduction and Importance

Beetroot (*Beta vulgaris* L.) is also called garden beet, red beet, and table beet. Beetroot and sugar beet along with the leafy vegetable, Swiss chard, are all members of the same species, *Beta vulgaris*. Despite being distinguished by various subspecies designations by taxonomists, these crops are all fully sexually compatible and will readily cross when flowering in proximity to one another. Root and leaves also have medicinal value against infection and tumor (Navazio et al. 2010a). Betanins, obtained from the roots, are used industrially as red food colorants. The color of red/purple beetroot is due to a variety of betalain pigments, unlike most other red plants, such as red cabbage, which contain anthocyanin pigments. The composition of different betalain pigments can vary, resulting in strains of beetroot which are yellow or other colors in addition to the familiar deep red. Cultivars of this group are used as storage vegetable, for canning, for juice production, and for the production of natural dyes for food industry. The crop is of great importance in Central and East Europe because it ensures a supply of consumers with fresh vegetable during the winter. Furthermore, it is assumed that the consumption of garden beet reduces the cancer risk (Hoppe 1981). In view of the nutritive and dietary properties as well as the potential value for beet breeding in general, this crop deserves more attention than it received in the past (Baranski et al. 2001).

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7.1.2 Crop History

The garden beet is thought to have originated in the western reaches of the Mediterranean region in southwestern Asia and was introduced into Europe during the middle Ages. According to Burkill (1935), beetroot was introduced in India in remote time, and then via sea route, it was taken by the Arabs to China in 1850 AD. Beet thought to be originated in Europe and North America and spread eastward from Mediterranean area. Its wild forms occur around the Mediterranean, Asia Minor, and the Near East. The present-day cultivated garden beet has probably originated from *Beta vulgaris* L. ssp. *maritime*, a variable species of the Mediterranean region, possibly by means of hybridization with *Beta patula*, a closely related species of Portugal and the canary island. *Beta vulgaris* L. ssp. *maritime* has been found to grow wild on seashores in Britain and through Europe and Asia to the Indies (Kalila 2004). It has been speculated that the original form of the crop was an annual, probably a winter annual. It is thought that through selection as a food plant that could be stored through the winter that as the crop spread northward the beet developed an enlarged storage root and become a biennial.

7.1.3 Improvement of Beetroot

Beet has nine pairs of chromosomes and is a diploid, with $2n = 2x = 18$. Tetraploid and triploid sugar beet have been developed and are often used in commercial cultivars, but table beet cultivars are solely diploid. Garden beet group is one of the four cultivar groups of *Beta vulgaris* L. ssp. *vulgaris* (Lange et al. 1999). Cultivated beets are propagated from seed. Botanically, a table beet seed is a fruit, termed a 'seed ball' (Hayward 1938). When the seed ball forms by the aggregation of multiple flowers, as is typical in cultivated beets, a multigermed fruit is produced from which one or more true seeds can germinate. The multigerm (also known as polygerm) property of beetroot seed has long been recognized as a significant issue in producing roots of an even size, shape, and harvest maturity. It affects factors such as germination rates and planting density, which in turn affects growth and subsequent quality characteristics of the roots. Development of monogerm beet seeds began in the early 1900s in an attempt to overcome these problems; however it was not until the 1960s that commercial monogerm cultivars became available (Meikle 1981; Nottingham 2004) with most of this work focusing on sugar beet rather than beetroot. Although some monogerm varieties are available for garden beet, Australian growers utilize polygerm seed for economic reasons. This means that the plant density issues are still relevant in the low-cost, low-input processing beetroot industry today (Donald 2012).

7.1.4 Genetics of Beetroot

In beetroot two genes govern root color, and red coloration is dominant to yellow or white, but the intensity of red coloration is influenced by minor polygenes (North 1979). Further five alleles have been identified at R locus and three alleles at Y locus. Male sterility has been reported to be governed by gene 'X' in 'S' cytoplasm, with fertility dominant to sterility. A second gene 'Z' causing partial male fertility has also been reported. Quantitative differences in the color of red beetroot are determined by the ratio of violet betacyanin to yellow betaxanthin pigments. Root with high, medium, and low betacyanin to betaxanthin ratios have violet, red, and orange color, respectively (Watson and Gabelman 1984). The ratio of betacyanin to betaxanthine was found to be controlled by triallelic system at the R locus with incomplete dominance (Wolyn and Gabelman 1989).

7.1.5 Major Breeding Objectives

High root yield, dark red uniformly colored, uniform root shape, absence of internal white rings in roots, slow bolting habit, monogerm seed, and resistance to downy mildew (*Peronospora farinosa* f. sp. *betae*) and powdery mildew (*Erysiphe communis* f. sp. *betae*).

7.1.6 Genetic Resources

Beetroot is cross compatible with all of the forms of *Beta vulgaris* including the noncultivated weedy types. This provides a vast array of natural variation for plant breeders which as yet have remained largely untapped. The monogerm character and CMS have been transferred from sugar beet, but there is little work published on utilization of genetic resources for introduction of pest and diseases resistance. Hammer et al. (1990) considered the genic base of modern cultivar was narrow. Previously, however, Holland (1957) had found great variation among beetroot cultivars. He classified over 200 cultivars into 40 groups on the basis of their root and foliage characteristics.

7.1.7 Strategies Methods of Improvement

A clear-cut objective of breeding is a useful prerequisite for greater chances of success in the improvement of a crop. The breeding method depends upon breeding system, life cycle, mode of reproduction, sex forms, and genetic architecture. The commonly used breeding methods for crop improvement are selection, hybridization, backcrossing, and heterosis breeding through utilization of hybrid vigor.

7.1.7.1 Mass Selection

Mass-pollinated seed will be collected separately from each group or possibly from single plants for progeny testing and sown separately. It is most likely that some group progenies will perform better than others and further selections could be made only from these (Watts 1980). While selecting the roots, quality of each root can be assessed by cutting a vertical wedge and replacing it afterward. Selected sampled roots can be grown on to produce seeds. Growing in relatively infertile soils accentuates color differences and makes it easier to choose the darkest colored roots. Premature bolting occurs most readily in the long days and relatively at low temperatures. Early sowing and growing at northerly latitudes encourages bolting and can aid selection for resistance to bolting. Selection for resistance can also be achieved very easily by subjecting a large number of seedlings to long days (16 h of day light supplemented by artificial light from tungsten bulbs), selecting those which did not produce flowering heads from 8 to 10 weeks of treatment, and mass pollinating the plants which grew on to produce high quality roots. Beetroot when subjected to self-pollination shows considerable inbreeding depression. Therefore, as a rule, this crop is improved by mass selection or by more sophisticated techniques. In mass selection, desirable plants in a population are selected on the basis of their phenotypic characteristics. The selected plants are then bulked and intermated. The degree of heritability is by far the most important of the factors that determine the efficiency of mass selection.

7.1.7.2 Heterosis and Male Sterility

Gabelman (1974) reported that monogerm character and cytoplasmic male sterility (CMS) were transferred from sugar beet. With the introduction of cytoplasmic male sterility (CMS) system, the production of hybrids has been facilitated. Male sterility was governed by the segregation of gene ‘X’ in ‘S’ cytoplasm, with fertility dominant to sterility (Bliss 1965). Another gene ‘Z’ with partial male fertility and complete dominance over male sterility was also reported, but it was independent of and hypostatic to ‘X’. For hybridization, if dominant marker is available, it can be used to avoid emasculation of female parent, and the selfed plants can be rogued either at seedling stage or at root stage. If the pollen parent does not possess any marker gene, it would be necessary to emasculate the flowers before crossing since these are hermaphrodite. During pollination care should be taken to avoid contamination from foreign pollen by wind. The bags covering the plants of the male and female parents should not be opened on a windy day, and pollination must be done when the air is still, preferably in a glasshouse or plastic cage (Swarup 1991).

7.1.7.3 Mutation Breeding

Chemical mutagen (dimethyl sulfate, DMS, N-methyl-N-nitrosourea, MNU; N-ethyl-N nitrosourea, ENU) and physical mutagen (gamma rays and ultraviolet irradiation) have been used to induce variation in beetroot (Pink 1993). Cultivar Avon Early has been bred by selecting from among a population derived from a large number of cultivars of the Detroit type. The plants were raised early on the year and subjected to continuous light followed by cold treatment to induce bolting. The level of resistance has then to be maintained by selection during seed multiplication. This variety has good resistance to downy mildew. Crimson Globe and Detroit Dark Red, two introductions are becoming acceptable and popular cultivar of beetroot in India.

7.1.7.4 Hybrid Seed Production

Sowing seed parent and pollen parent in 1:1 ratio can produce the commercial F_1 hybrid seed in fields isolated from other compatible crops or varieties by at least 1000 m, since beetroot is anemophilous. Zoning scheme can also be employed to confine seed production of different hybrids in separate geographical areas. Hybrid seed production is feasible only if monogerm character and cytoplasmic male sterility system are available. Dominant marker, if available, can also be made use of for the purpose, but then self's needs to be thinned.

7.1.7.5 Polyploidy Breeding

In beetroot tetraploid plants have been reported among the triploid progeny of crosses between diploid beetroot and tetraploid sugar beet. These were produced for the cytological and biochemical studies (Akhramenka 1988), and although backcrossing to the diploid beetroot parent produced tetraploid which more closely resembled beetroot (Akhramenka and Kazyrevich 1989), it seems unlikely that tetraploid cultivars will be produced.

7.1.7.6 Use of Biotechnology in Beetroot

Development of transgenic sugar beet having herbicide resistance in the USA is now a reality. Little, if any, work has been done on transgenics in beetroot. However, it is quite likely that any genetic transformation accomplished in sugar beet likewise can be translated in beetroot. There are reports on significant genetic variability for regeneration ability from leaf disk callus in sugar beet. SNP markers have been developed for each of nine linkage groups in sugar beet, and similar developments are expected in beetroot. Sabir and Ford-Lloyd (1991) in their study included four beetroot cultivar for mass production in vitro regenerants and found that shoot

culture of beetroot was not successful. They obtained more success with regenerants from adventitious buds. Girod and Zryd (1987) used callus culture derived from the cotyledon explants to investigate clonal variability and light induction of betalain synthesis.

7.1.8 Commercial Seed Production

The following point must be taken into consideration while producing quality seed of beetroot:

1. Beetroot is a cool season crop and thrives best at moderate temperatures between 15.5 and 25 °C, which encourages steady, vigorous growth. At the end of the first season of vegetative growth, the beet seed crop requires a vernalization period where roots are exposed to temperatures at or below 10 °C for at least 8 weeks. In the second year of growth, the beet seed crop can be more sensitive to heat, especially during the early stages of seed development, from anthesis (the period of flower opening to fruit set) until the embryo and endosperm have fully formed. The best beet seed production locales have cool, wet spring weather to establish a sturdy frame of vegetative growth, followed by cool, relatively dry summer weather to support optimum pollination, fertilization, and seed development.
2. Root being economic part, it suited to a fairly deep, well-drained sandy loam. The optimum soil pH for beets is a narrow range, between 5.8 and 6.2. In seed crops, the amount of available nitrogen should not be too high in order to avoid excessive vegetative growth before bolting, as this promotes lodging of the plants during seed set.
3. For seed production, the planting and sowing of seed should be adjusted in such a way that quality roots must get ready by the onset of winter to meet vernalization. Beet seed is produced using one of two basic methods, seed-to-seed or root-to-seed depending on your goals. The seed-to-seed method is the easier and more efficient of the two. Caution should be used in heavy or frequently water logged soils as excessive moisture can cause rot at the growing point. As warm spring weather returns, the beets put on a flush of new vegetative growth. Because of this, it is very important to start with seed that is known to have a high degree of genetic purity and therefore guaranteed to produce a high percentage of roots that are true to type for the particular variety being grown.
4. Root-to-seed method of planting requires that the beetroots are lifted and selected, and then they are either replanted within hours or stored in a cooler, root cellar, or in a pit in the ground and are replanted the following spring. The advantage of this method is that it affords to evaluate each root and decide if it is worthy of contributing its traits to the next generation. Additionally, it also produces beet seed in climates that are too cold for the seed-to-seed method to be used. When using the root-to-seed method, the roots should be (1) free of

any physical damage, disease, and insect infestation, (2) true to type for all major traits inherent in the particular variety, and (3) prepared for storage by trimming off most of the tops while retaining the apical growing point of the shoot.

5. For long-term storage, preparation of roots for storage in a cooler or root cellar the beetroots needs to be properly prepared for storage. Roots that are stored for replanting to produce a seed crop are called stecklings. Therefore, there are several methods used in preparing the stecklings for storage in a controlled environment. Stecklings are prepared by giving 1/3 cut to top portion (foliage) and 1/4 to lower (root). Roots/stecklings must also be stored at a relative humidity of at least 95%.
6. Flowering and pollination in garden beet is predominantly wind pollinated and has extremely light pollen that is easily carried long distances by the wind. Perfect flowers usually occur in clusters of two to five, with one bearing an extended bract that encircles the cluster. Monogerm varieties have single flowers borne in bract axils, therefore no fusing of multigerm seed balls.
7. Each individual plant must have pollen from a genetically different individual in the population (or another population) to produce viable seed. This encourages almost complete cross-pollination between individuals in a population.
8. Beetroot requires an isolation distance of 4–4.5 km. Isolation requirements can vary depending on location and size of production. It is also important to remember that the table beet is only one of several crops that belong to the species *Beta vulgaris*, and therefore it is fully sexually compatible and will readily cross with sugar beet, fodder beet (mangel), and Swiss chard.
9. For genetic maintenance and improvement, the population size is very important. Being cross-pollinated species and in order to maintain adequate genetic diversity and elasticity in any open-pollinated condition, it is important to harvest seed from a minimum of 120–200 plants to avoid inbreeding depression.
10. Selection should be done at several points in the life cycle when possible, including at the seedling stage, at the market or eating stage, and during flowering to maximize reproductive health and seed yield. Beets are commonly selected on the basis of traits, viz., seedling vigor, leaf size, shape and color of petioles, root shape, root color, root crown, and smoothness for genetic maintenance.
11. Seed maturation depends on variety, climate, and planting date. Beet seed formation usually starts anywhere from 6 to 7 weeks after flower stalk initiation. A standard method used to judge maturity of the beet seed crop is a visual assessment of the color of the seed ball. Harvest should occur when between 60% and 80% of the seed balls on at least 90% of the plants in the field have turned a tannish-brown shade, typical of mature beet seed.
12. Finally, for seed harvest, the commercial beet seed crop is rarely staked, so there is a tendency for the crop to lean in all directions, thereby making mechanical cutting or swathing without causing excessive amounts of seed shattering nearly impossible. The seed plants should be placed in the windrows in such a way that they receive sufficient airflow to allow even drying even with heavy

dew or light rains that may occur during this after ripening. After this drying period, threshing can be done either by a belt thresher, stationary rotating thresher, or a self-propelled combine that is manually fed. Threshing should be done early in the day, mid-morning in most climates, as light dew on the plants will prevent much of the potential shattering that can occur when the plants are picked up for threshing. Further drying of the seed crop should occur at this point in a well-ventilated warm space with additional heat added as necessary. After cleaning and proper drying, the seeds are stored in moisture proof container.

7.2 Carrot (*Daucus carota* L.)

7.2.1 Introduction and Importance

Carrot (*Daucus carota* L.) is a widely grown root vegetable of the Apiaceae family. The first certain recorded use of carrot roots as a vegetable is from the tenth century in Afghanistan. From the Middle East, carrots gradually spread north to Europe and also travelled east to China and Japan, presumably via the Silk Road, over the next several centuries. From the earliest documentation in the Middle East till the sixteenth century in both Europe and Asia, cultivated carrots were less refined than our modern crop and were either purple or yellow in color (Navazio et al. 2010b). Orange carrots were first appeared as a genetic variant in Europe in the sixteenth century and soon after became very popular. From then until present, much of the breeding in this crop has concentrated on eating qualities and the intensity of orange color. Carrots can be placed into two basic types. The western type includes the orange, white, purple, and yellow forms originating and developed in the Mediterranean Basin and Europe. Alternately, the subtropical carrot type is adapted to the hotter, subtropical conditions of China, Japan, and Southeast Asia. These may be yellow, purple to reddish-purple, or red in color. It is an important source of *alpha* and *beta*-carotene, precursors of vitamin A in human nutrition, in many cultures worldwide. Temperate cultivar of carrot is grown as an annual for its fleshy root but is a true biennial requiring 2 years for flowering and seed production.

7.2.2 Germplasm Resources and Management

The availability of germplasm is an essential resource for carrying out a successful breeding program for any crop. The extent of carrot and other *Daucus* species held in germplasm collection is relatively small. Approximately 5600 accession are held worldwide with over 1000 of these at Vavilov Institute in Russia (VIR) (Frison and

Serwinski 1995). An important *Daucus* germplasm collection is also maintained by the Genetic Resource Unit of Horticultural Research International, Wellesbourne, UK, and the Crop Genetic Resources, Wageningen, the Netherlands.

7.2.3 Crop Improvement

Carrot is a diploid with nine chromosome pairs. The chromosome is short and fairly uniform in length. The intensive selection in the eighteenth and nineteenth century led to a great diversity of types in open-pollinated varieties as regards leaves and roots. The shape of root can be circular, obovate, triangular, obtriangular, or narrowly oblong. A great variation also exists for harvesting time (Stein and Nothangel 1995). The gene pool in the Europe has been divided into seven groups named Amsterdamer, Berlicumer, Chantenay, Danvers, de Colmar, Nantes, and Pariser Market indicating the regions where breeding of each particular variety in Europe began. The typical carrot in the USA and Canada, Imperator, is not included. For the classification of the cultivated and the examination of their homogeneity, 27 characters are used (UPOV 1976). In contrast to the large number of varieties, the gene pool of the cultivated carrot is limited due to a unique mutation in the orange color. In addition the use of inbred lines for hybrid seed production is still increasing. The genus *Daucus* contains 22 species, while the *Daucus* carrots complex, which includes the cultivated carrot, more than 15 subspecies have already been described. The main distribution is in Europe and Mediterranean region, although some species are found in southwest and central Asia and tropical Africa. Carrot is an outcrossing biennial species but can be handled as an annual in breeding programs. Carrot has no self-incompatibility system, but inbreeding depression is severe in many wild and domesticated germplasm stocks. Nevertheless, it has been possible to select lines which are able to withstand severe inbreeding and in few cases beyond ten generations of self-pollination.

7.2.4 Major Breeding Objectives

High root yield; scarlet/orange color roots; high carotene content in roots; uniformity in root shape, size, and color; thick fleshed roots; thin and self-colored core in roots; broad shouldered; cylindrical; uniformly tapering or stump root carrot with non-branching (Fig. 7.1) habit early rooting free from cracking and splitting, high sugar and dry matter in roots, slow bolting habit, smooth root surface for fresh and processing market, root with texture succulent, brittle and juicy, multipigment colored varieties, long field staying capacity, free from harsh flavor (monoterpenoides and sesquiterpenoides), resistant to nematodes (*Meloidogyne incognita*), Alternaria blight (*Alternaria dauci*) and Cersospora leaf blight (*Cercospora carotae*).

Fig. 7.1 (**a**) Forking of carrot roots/forked roots should be avoided for breeding and seed production purposes. (**b**) Splitting of temperate carrot roots takes place due to adverse climatic conditions should be avoided for breeding and seed production purposes. (**c**) Quality roots of temperate carrots should be used for breeding and seed production purposes

7.2.5 Development of Cultivar

The inflorescence of carrot is a compound umbel and protandrous. A primary umbel can have over 1000 flowers at maturity, whereas secondary, tertiary, and quaternary umbels bear fewer flowers (Peterson and Simon 1986). Flower development is centripetal, i.e., the flowers to dehisce first are on outer edges of the outer umbellets. All open-pollinated varieties suffer from inbreeding depression and a limited degree of uniformity, and hybrid breeding of carrot has now been started by the carrot breeders intensively to improve uniformity. In case where extreme uniformity seems to be unnecessary, e.g., for juice or pulp production or for the regions with weakly developed agriculture, breeding of synthetics would also be worthwhile. Carrot breeding in the past 150 years has resulted in varieties with high yield, a short growing period, and excellent root color (Stein and Nothangel 1995).

The improvement of uniformity and vigor that many carrot hybrids provide is primarily responsible for the gradual replacement of open-pollinated cultivars. Many open-pollinated cultivars are grown by the growers which do not require the advantage of hybrids or the inability to justify the higher cost of hybrid seed. The discovery of two distinct genic-cytoplasmic types of male sterility (CMS) has provided a system for commercial production of hybrid carrot cultivar. Hybrid carrot seed production in the USA relies almost entirely upon petaloid CMS since all sources of brown anther CMS tend to develop some male fertile flowers in tertiary or even secondary umbels. However, carrot hybrid produced in the Europe and Asia more often uses brown anther CMS since male flower is not reported to occur in their seed production areas (Bonnet 1985). Hand emasculation is very difficult to produce hybrid seeds, and lack of practicably useful chemical agents for emasculation or inducing male sterility in carrot signifies the utmost importance of CMS for

the development of highly adapted and uniform hybrid varieties of carrot. Breeding of carrot *(Daucus carota* L.) has become increasingly dominated by hybrid varieties based on seed production from inbreds, with male sterility in the female line. As a means of improving the potential of carrot hybrid seed yields, the three-way hybrid is being given considerable thought, and some combinations are being made. The male sterile (female) seed parent line, for example, would be a hybrid of two lines, say A × B, which preferably should be uniform in their major horticultural characteristics, yet when hybridized would show hybrid vigor for good seed yield. This A × B male sterile hybrid, with good seed yielding potential, is planted alongside male fertile (pollen-producing) line C; seed harvested from the A × B plants is, then, a three-way hybrid, (A × B) × C. A great deal of work is required to develop and test such combinations, as well as to maintain them for seed production purposes (Prasanth et al. 2014). Moreover, hybrid carrot seeds cost more to produce the open-pollinated seed because a portion (20–40%) of the seed produced in a field is derived from the pollen parent and is not saleable. Additionally, the male sterile plants used in the hybrid seed production often produce less seed per plant than the male fertile. This is especially true for the petaloid form of CMS. Carrot hybrids are usually three-way crosses (A × B) × C, since the hybrid vigor in a single cross F_1 female seed parent usually results in much greater seed production than that of an inbred male sterile parent. Single cross hybrid, A × B, is on average more uniform than three-way crosses and does not require an extra year to produce F_1 seed parent stock. Thus, if seed productivity of single cross is adequate, they are used.

7.2.6 Strategies and Methods of Improvement

7.2.6.1 Mass Selection

The most promising roots of a given variety or breeding progeny are selected and then planted together in the open to cross-pollinate at random. Superb uniformity for given characteristics, such as color, shape, and taste, can hardly be expected from this method. Yet, as exemplified by such varietal types as Red-Cored Chantenay and Nantes, important gross characteristics can be maintained with reasonable attention to selection; and, certainly, some improvement is possible. Most of the breeding programs for carotene use the traditional in exact selection method that is done by visually rating the carrot roots. Roots with the best exterior color are selected, giving preference to those that exhibit orange color spreading well down to the taproot ('red tails'). Major selection criteria are color intensity, indistinct cambium zone, and perfect color match between phloem and xylem. Limits of visual selection are around 120 ppm of total carotenoids. Difficulties with visual selection differentiating dark-orange from red-orange roots are also commonly reported. Therefore, alternative analytical procedures are necessary to provide more accurate evaluation that will ensure genetic gain by selection. Carotenoids in root have been determined using spectrophotometric analysis and thin-layer chromatography.

7.2.6.2 Hybrid Breeding

It has led to considerable improvements in the percentages of marketable roots, as well as a marked increase in quality. An F_1 hybrid, in reality, can also be considered as any first generation cross involving two established varieties (or strains) or two breeding stocks of any type. In carrot, and other cross-pollinated vegetable crops, the potential value of F_1 hybrids arising from inbred combinations lies in vastly improved uniformity of various characteristics. There are distinct problems involved in development of the hybrid carrot. It is a biennial, and, in cold climates, short cuts are necessary via greenhouses or shipment to warm production areas after induction of potential flowering by cold storage. Loss of high quality roots by various organisms causing decay is serious; and inbreeding generally results in weak vigor and low seed yields (some inbreds are better than others). Maintenance of inbred lines is costly; two lines must be maintained for the male sterile (female) parent, from which seed is obtained; a third line, the pollen-producing inbred must also be maintained as one of the F_1 parents (Nothnagel et al. 2000).

Three potentially new cytoplasmic plasmic male sterile systems based on the cytoplasm *of Daucus carota*, *Daucus carota gummifer*, *Daucus carota maritimus*, and *Daucus carota gadecaei* have been identified in carrot for utilization in breeding programs. Genetic analyses suggested that a nuclear-cytoplasmic interaction determined the male sterility of the three sources.

(a) *Brown anther (Sa) type*: Formation of brownish unfertile anthers, commonly known as the brown anther sterility phenotype, is one such trait being utilized in F_1 hybrid breeding programs as stated by Sota and Yoko (2019). The anthers are formed but degenerate and shrivel before anthesis. Brown anther (Sa) cytoplasmic male sterility (CMS) cytoplasms have been isolated from several open-pollinated varieties as well as from wild populations. Genetic analysis of the trait has revealed that two duplicate genes (one recessive and one dominant) and two complimentary dominant fertility restorer genes have been reported in the brown anther type. The brown anther (ba) male sterility was first discovered in the cultivar 'Tender Sweet' and reported by Welch and Grimball (1947). Expression of the brown anther sterility was due to a homozygous recessive locus Ms5 or a dominant allele for Ms4, but dominant allele of either of the two complimentary loci would restore the fertility. Kozik et al. (2012) reported that brown anther sterility is characterized by formed but unrolled, shriveled filaments and brownish anthers which are a result of tapetum degeneration. It was the first type used for developing hybrid carrot varieties, but recently petaloid, the second type of sterility, is the more widely employed in carrot breeding programs, as CMS lines of the petaloid type have been found to maintain their male sterility better than those of brown-anther type (Davey 1999; Bach et al. 2002).

(b) *Petaloid type*: Petaloid sterility is manifested as the replacement of stamens with a second additional whorl of petals or petal-like, bract-like, or carpelloid structures (Erickson et al. 1982; Kitagawa et al. 1994). The stamens are replaced

by five petals or bract-like structures and found in Cornell wild carrot. The most widely used type of CMS in carrot is petaloid type. It also has occurred spontaneously in feral populations of *D. carota* ssp. *carota* from North America and Sweden and in a *D. carota* ssp. *maritimus* accession from Portugal. It is also indicated that at least two dominant duplicate genes control the expression of petaloid stamen. Additional CMS phenotypes have been observed in carrot lines containing cytoplasms from *Daucus carota* subspecies but not yet incorporated in to cultivated carrots for hybrid breeding. Petaloid sterility is commercially used for hybrid seed production in the world. This is manifested as the replacement of stamens with petals (white petaloidy) or both stamens and petals with green bract like structures (green petaloid) (Kitagawa et al. 1994). It is stable across a wide range of environments through flowering and seed production. In carrots, the Sp-cytoplasm causes transformation of stamens into petal-like organs, while plants carrying normal N-cytoplasm exhibit normal flower morphology. In carrots (*Daucus carota* L.) carrying the Sp-cytoplasm, stamens are replaced with petal-like organs, and thereby male reproductive function is eliminated. Such carrots represent a more general phenomenon of cytoplasmic male sterility (CMS), which refers to as maternally inherited impairment of pollen production.

7.2.6.2.1 Use of Male Sterility in Hybrid Development and Its Maintenance

Thompson (1961) and Hansche and Gabelman (1963) were the first scientist to detect and analyze male sterility. The first carrot hybrids were sold in the early 1960s in the USA. Today more than 100 hybrid varieties exist worldwide. The percentage of hybrids is 60–90% in Europe for early and late varieties, and in the USA, the value has reached to almost 100%. The development of the male sterile and maintainer line is very difficult and laborious process due to the dominant state of the male sterility. Crossing, backcrossing, selfing, and testing of the progenies in the following two generations, including the isolation of the positive progenies, are characteristic steps in the breeding process. The breeder is forced to eliminate the male fertile plants or phenotypes with the partial male fertility within the ms line developed and to develop new lines with low inbreeding depression. Such lines can be found but they rarely have good combining ability.

The petaloid type of CMS (Cornell type) with its maintainer line and a restorer line (for fertility) having a good combining ability is used for commercial hybrid seed production. A new CMS line from sterile cytoplasm of 'Wisconsin Wild' has been released for hybrid seed production. Extensive genetic studies on carrot male sterility demonstrated a nuclear-cytoplasmic interaction for both CMS types. Thompson (1961) first described the cytoplasmic inheritance of petaloidy in carrot lines suggesting that the dominant alleles of each of the three duplicate nuclear genes, Ms1, Ms2, and Ms3, were necessary to maintain sterility for both cytoplasms, and dominant alleles at one or more epistatic loci could restore fertility (Timin and Vasilevsky 1997). Contrary hypotheses were postulated for the brown anther CMS

system. The results of Hansche and Gabelman (1963) and Banga et al. (1964) suggested that expression of the brown anther sterility was due to a homozygous recessive locus Ms5 or a dominant allele for Ms4, but dominant allele of either of the two complementary loci would restore the fertility. Several authors reported that both CMS systems can be influenced by specific environmental conditions, in particular high temperatures, which promote occurring fertile plants (Michalik 1979; Mehring-Lemper 1987; Wolyn and Chahal 1998; Borner et al. 1995). The effects of restorers can also provide valuable information on the level of sterility, even if the genes involved are not formally identified (Budar and Pelletier 2001). In this study, evaluation of the phenotypic uniformity within the carrot backcross populations in relation to several morphological traits of petaloidy expression was assessed (Sota and Yoko 2019). Genetics of male sterility expression in *Daucus carota* (carrot) is known to be complicated (Kozik et al. 2012). In this species, two different types of cytoplasmic male sterility (CMS) are known. Conversion of stamens into petal-like or carpel-like structures, known as the petaloid or carpeloid, is another type of male sterility known in this species (Linke et al. 2003). This phenotype is expressed as a result of nuclear-cytoplasmic genotype interaction. The cytoplasmic genotypes of the two types are known to be distinct. Different genetic models with the involvement of at least two genetic loci in the expression of petaloid in carrots have been proposed by past studies (Banga et al. 1964; Kozik et al. 2012). Furthermore, expression of the CMS phenotype is reported to be influenced by environmental backgrounds such as high temperatures (Wolyn and Chahal 1998). Therefore, molecular markers that enable genetic diagnosis of the male sterility phenotype are invaluable in carrot breeding. Recently, gene identification is facilitated by high-throughput sequencing technology. Notably, identification of the quantitative trait loci using bulked segregant analysis, or QTL-seq, is becoming a powerful strategy for the discovery of causal genetic loci of a phenotype (Takagi et al. 2013). Here we employed the QTL-seq approach to identify the causal gene locus of the petaloid phenotype in carrots.

A third CMS has been detected in an alloplasmic form of orange-colored roots of carrot originating from a cross between the wild carrot *D. carota gummifer* Hook. Fil. and the cultivated carrot *Daucus carota sativus* Hoffm (Nothnagel 1992). This type of male sterility, called 'gum' type, is characterized by a total reduction of anthers and petals. Recent results on the genetic mechanism suggest that an interaction of the 'gummifer' cytoplasm with a recessive allele (gugu) in the nucleus is responsible for the expression of such type of male sterility. The gum type can be potentially be used a source of CMS for carrot breeding.

Almost all of the hybrid seed production in carrot relies on a form of cytoplasmic male sterility (cms) which occurs naturally and was originally derived from wild carrot populations. To produce hybrid seed using this system, the female parent is a male sterile inbred carrot line that doesn't produce viable pollen but has fully functional female parts and will accept pollen from the male parental inbred line. The male parental carrot is fully fertile and will supply plenty of pollen to pollinate the female parent. The sterile female line is usually monitored and rouged for the occasional presence of male, pollen-producing plants. It is important to note that many of the inbred lines are poor seed yielders. For this reason many carrot F_1varieties are three-way crosses that use a single cross for the female parent to take advantage of

hybrid vigor for increased seed yield. The parent populations are commonly planted in a ratio of eight female rows to two male rows to maximize the amount of hybrid seed that is harvested. Once the effective pollination period has passed, the male rows are then mowed and incorporated to avoid mixing with seed from the female rows at harvest. The female rows, once mature, are harvested in the same manner as in standard seed production.

7.2.6.3 Synthetics

So-called synthetic varieties represent a third method for improving cross-pollinated crops. They arise from carefully selected breeding lines, somewhat similar in desired characteristics; when crossed together, these lines result in a superior open-pollinated stock. Some undesirable characters which might be present if random mass selection was practiced can be eliminated by this method. Once selected lines are combined, the synthetic is then maintained by open pollination. It may be that such types will play an interim role in carrot production while hybrids are evolving.

7.2.6.4 Recurrent Selection

This simply represents an extension of the method described in development of the synthetic. For example, after combining several good lines for a synthetic in open pollination, if we select and develop another round of selfed lines from the synthetic superior (after testing) to those originally used, we practice recurrent (repeated) rounds of selection. Time must be taken to determine whether the new round of lines, when again intercrossed in all directions, are in fact superior to the prior rounds. This process may be repeated indefinitely, as long as progress can be demonstrated.

7.2.6.5 Backcross Method

In this method a desirable parent (for many characters), such as Nantes, is chosen for the recurring parent (continued crossing back to Nantes) with the object of growing the F_2 progeny of each cross, selecting for desired Nantes characters, plus a desired character or characters introduced from the other (nonrecurring) parent. For example, the other parent may have resistance to cracking. The idea is to select crack-resistant roots near Nantes in type and then to hybridize them again to Nantes so that we continue to add genes from Nantes, selecting for resistance to cracking in each backcross generation. In general, at least three or four rounds of backcrosses are necessary for mass transfer of the desired parent (Nantes) genes. More may be required, depending upon complications of the interties of crack resistance with desirable or undesirable inheritance units (genes). This is a very valuable approach in plant breeding, providing a highly desirable recurrent parent is available, and the 'intertie' complications are not serious.

7.2.7 Breeding for Quality Traits

Consumers today demand a uniformly orange-colored root without differences between phloem and xylem. The origin of the orange color is the carotenoid, carotene. More or less red-colored genotypes from Asian sources contain carotenoids, but in the form of lycopene, the main carotenoid in tomato. Nevertheless, only beta-carotene is important as provitamin A for quality. Orange-colored carrot and tomato are the main vegetables containing vitamin A. In Germany, Schuphan (1942) was the first to recommend breeding carrots with a high level of beta-carotene. A great variation in color intensity and level of carotene exists within the OP varieties. Therefore, uniformly colored OP varieties were initially selected that possessed a reduced yield. A problem is the variable color expression in the phloem and the xylem (heart). Two genes seem to be important for the different carotene content in both parts of roots (Simon 1984). A systematic breeding program for carotene content has been started in some regions only. In developed countries, the main attention has been directed toward uniform root color because the minimum level of vitamin A for humans is already provided by fruits and vegetables throughout the year. Recently this situation has changed, since beta-carotene is important for its anticarcinogenic and other health-promoting effects. Breeding of carrots with high a content of carotene is always necessary in regions where the food supply or the import of fruit is strongly limited. For a long time, the genetics of root color were available only without specific information concerning the chemical background. Recently, many genetic investigations have been performed to study the genes involved in the biosynthesis of carotenoids (especially beta-carotene), as well as in root color (Kust 1970; Buishand and Gabelman 1979; Iman and Gabelman 1968; Laferrier and Gabelman 1968; Krivsky and Sverepova 1985).

Peterson et al. (1988) and Simon et al. (1989) reported that genotypes had been selected with an extremely high level of carotene (>500 ppm) in a crossing program using Asian genotypes, a fivefold higher level than in common varieties. The high carotene carrots 'HCM' and 'Beta III' are present in the 'Imperator' type, which is only important in the USA. Simon (1990) started experiments growing carrots in the developing world and under tropical conditions. By backcrossing, the high level of carotene can be transferred into other genetic backgrounds which are adapted to conditions in regions where vitamin A deficiency is prevalent. In hybridization experiments, F_1, progenies could be found which contain the same level of beta-carotene as the best parent but more detailed analysis is necessary. Since hybrid vigor has not been observed, at least one parent must have high beta-carotene content (Stein 1979). In general, beta-carotene is the main component of carotene in European varieties. Other components such as α, γ, δ, and ζ carotene are only present in small amounts. For breeding of high-carotene carrots, attention must be given to the composition of carotene when foreign material is introduced. So far, the genetics of the different components is unknown. Generally, only a weak correlation is observed between the orange color and the carotene content (Laferrier and Gabelman 1968). The application of HPLC analysis for selection of high-carotene

varieties was reported by Simon and Wolff (1987). Biophysical methods for color selection, based on the measurement of spectral transmission, have also been applied. In Europe, an increasing number of carrots are used as fresh or prepared dressing carrots (bunching carrots). Therefore, special requirements for taste and consistency exist. A typical sweet carrot taste based on two major components of content-free sugars and volatile terpenoids is demanded for all varieties for fresh market. Glucose, fructose, and sucrose form up to 95% of the free sugars. A great variation between the genotypes can be observed. Extensive investigations by Simon et al. (1980a, 1982) and Freeman and Simon (1983) have demonstrated a monogenic dominant inheritance for the high sugar content. The level of total dissolved solids and reducing sugar can be increased by some cycles of recurrent selection (Stommel and Simon 1989). A pleasant carrot taste is the effect of many components, especially of terpenoids (Simon et al. 1980b, 1982), which can mask the sweetening effects. In breeding, the taste has generally been analyzed organoleptically. The introduction of HPLC analysis (Freeman and Simon 1983) or HPTLC analysis (Hofer and Gennari 1994) enables an exact analysis of the related sugars and other taste-influencing components. To prevent an unsatisfying carrots taste in hybrid varieties, an analysis of basic lines with regard to specific components is necessary.

The 'cracking' of roots before or after harvest, especially in varieties of the early and midseason group, can decrease yield. The reasons for cracking are both genetic and environmental, particularly with regard to irrigation (Bienz 1968). Nevertheless, the influence of growth technology has been described. Investigations into inheritance have revealed that cracking susceptibility may be governed by a single dominant major gene but with a low heritability (Dickson 1966). Dowker and Jackson (1977) have also reported the effects of the drilling date, plant density, and growing season on cracking.

7.2.8 Use of Biotechnology

Interesting and exciting possibilities exist for the application of biotechnological tools for the carrot improvement. As molecular marker systems become extensive, their use in selecting for difficult or expensive to evaluate traits will become useful. Carrot is readily manipulated in tissue culture and genetically transformed, so that opportunities for biotechnologically engineering carrots are also excellent. Approximately 1000 RFLPs, 15 RAPDs, 250 AFLP, and 30 other molecular or biochemical loci have been mapped in several carrot populations to date.

According to Stein and Nothangel (1995) in their study remarks on carrot breeding, today, carrot breeding exists in all developed countries and has changed from mass selection to hybrid breeding. Only 20 genes have been described (Simon 1984), as well as about 50 isozyme, RFLP, and RAPD loci (Westphal and Wricke 1991; Schulz et al. 1994). Only the patchiest genetic knowledge exists concerning quality traits, e.g., root color (Kust 1970; Buishand and Gabelman 1979), sugar and carotene content (Banga et al. 1958) cytoplasmic male sterility

(Banga et al. 1964; Mehring Lemper 1987), and root shape (Frimmel 1938). Carrot serves as a model plant for biotechnological techniques and is used in experiments analyzing the physiology of plants; in vitro culture has been well developed and has been introduced into breeding programs. The possibility of producing artificial seeds by somatic embryogenesis offers new ways of maintaining special lines (Kitto and Janick 1985; Kamada et al. 1989; Liu et al. 1992). In recent years, experiments have aimed to find molecular markers for different characters, especially genes responsible for male sterility (ms) (Scheike et al. 1992; Steinborn et al. 1992; Schulz et al. 1994).

Carrot has been extensively studied as a model species for tissue culture, plant somatic embryogenesis, and protoplast fusion (Ammirato 1986) and was therefore predestined for transformation approaches. The first transgenic carrots were reported after *A. rhizogenes* infection by Tepfer (1984). Shortly after, Langridge et al. (1985) obtained transgenic plants by electroporation of suspension protoplasts with naked DNA. Later, transgenic plants were obtained by *A. tumefaciens* infection of various carrot plant explants and cells (Scott and Draper 1987; Thomas et al. 1989; Wurtele and Bulka 1989). Herbicide resistance was first introduced into carrot via direct gene transfer of the pat gene (Droge et al. 1992; Droge-Laser et al. 1994). Chen and Punja (2002) introduced the bar gene and Aviv et al. (2002) a mutant acetolactate gene (ALS) from *Arabidopsis thaliana* causing resistance to herbicide Imazapyr. A number of genes have been introduced to enhance resistance to fungal pathogens, such as chitinases, glucanases, thaumatin-like protein, osmotin, and lysozyme. Resistance has been engineered by using chitinases cloned from petunia and tobacco (Linthorst et al. 1990), from beans (Broglie et al. 1991) or from *Trichoderma harzianum* (Baranski et al. 2008). A thaumatin-like protein from rice was expressed in carrot and showed enhanced tolerance to six fungal pathogens (Chen and Punja 2002; Punja 2005). Transgenic carrots with the tobacco osmotin (AP24) in combination with a chitinase and a glucanase gene also expressed broad-spectrum tolerance (Tigelaar et al. 1996; Melchers and Stuiver 2000). Carrot lines which constitutively expressed a human lysozyme showed enhanced resistance to *E. heraclei* and *A. dauci* (Takaichi and Oeda 2000). The microbial factor (MF3) from *Pseudomonas fluorescens* enhanced the resistance to *Alternaria* sp. and *Botrytis cinerea* (Baranski et al. 2007). An interesting field is the production of biopharmaceuticals. A number of transgenic carrots have been engineered to produce proteins or potential human vaccines, such as enterotoxin (LTB) against cholera and diarrhea (Rosales-Mendoza et al. 2008), the MPT64 gene of *Mycobacterium tuberculosis* (Wang et al. 2001), the major hepatitis B virus surface protein (Imani et al. 2002), an immuno-dominant antigen of the measles virus (Bouche et al. 2003, 2005; Marquet-Blouin et al. 2003), and glutamic acid decarboxylase (GAD65) as an autoantigen in autoimmune type 1 diabetes mellitus (Porceddu et al. 1999; Avesani et al. 2003). Currently two approaches focus on functional foods or nutraceuticals. It was demonstrated that transgenic carrots expressing the *Arabidopsis* H+/Ca2+ transporter CAX1 increase their calcium content up to 50% compared with the control. Enhancing the concentration of bioavailable calcium in vegetables could prevent calcium malnutrition and reduces the incidence of osteoporosis (Park et al. 2004). Furthermore, carrots have been engineered into the ketocarotenoid biosynthetic526

E. Transgenic carrots converted up to 70% of total carotenoids to novel ketocarotenoids, showing that carrots are suitable for applications to the functional food, nutraceutical and aquaculture industries (Jayaraj et al. 2008; Jayaraj and Punja 2008). At the present time, there are no transgenic carrot cultivars or other Apiaceae commercially available on the market.

7.2.9 Commercial Seed Production

1. The best climate for temperate-type carrot seed production includes a fairly mild spring and a dry summer, but generally doesn't reach above 35 °C until late in the summer, after the seed is set and near maturity. For seed maturation and harvest, a dry climate with low precipitation is beneficial.
2. For proper growth and development loose, well-drained, deep loamy or sandy loam soils with good fertility are best.
3. Carrot seed is produced using two different methods, the 'seed-to-seed' or 'root-to-seed' method depending on your goals. The seed-to-seed method is the most efficient if seed stock is selected from genetically uniform lot. The root-to-seed method on the other hand affords selection based on the root characteristics.
4. Planting seed for the seed-to-seed or root-to-seed method should be sown into well-prepared soil with good moisture holding capacity. Seed is planted in summer, usually in July–August, depending on carrot type and location. After seed germination and seedling enlargement, thinning is necessary to achieve the desired growth within row spacing. With the root-to-seed method, carrots are sown much as a grower would for producing the vegetable. As the crop is planted in midsummer to plant at a proper spacing which will insure that roots achieve their optimum characteristic size and shape for selection upon lifting in the fall. Upon replanting stecklings in the spring at recommended spacings as per the types of cultivars being used for seed production. The density of the planting also influences which class of umbels will yield the highest proportion of seed. The carrot seed produced by the primary and second order umbels is universally regarded as superior to later forming seed of the third and fourth order umbels, due to its size and degree of maturity. Under higher-density plantings, the development of the later forming third and fourth order umbels are restricted, thereby benefiting the development and quality of seed from primary and secondary order umbels.
5. By root-to-seed method of planting, the carrot roots are 'lifted,' selected, and either replanted soon after the selection process or stored in a cooler or root cellar and planted the following spring. The advantage of this method is the ability to evaluate each root and decide if it is worthy of contributing to the next generation. The grower using this method must make sure that all the roots that are retained (1) receive adequate vernalization to bolt the following season, (2) confirm to the standards set by the selection criteria, and (3) are free of growth cracks, splits, disease, or any insect tunnels or damage. The main disadvantage of this method is that some types of carrots, notably Nantes, do not vernalized unless storage conditions are for an adequate duration. Proper storage of carrot

roots for the root-to-seed method is very important as some growers may need to store the roots for upward of 5 months before replanting in the spring. Roots of biennial crops like carrots that are stored for seed are known as 'stecklings.' Carrot roots must also be stored at 90–95% relative humidity to remain in good condition for subsequent growth when replanted. Cutting the roots when preparing carrot stecklings serves two primary purposes; (1) it allows the grower to evaluate for quality characteristics, and (2) it shortens the length of longer carrot types for easier transplanting. When cutting, the goal is to cut no more than 1/3 to 1/2 of the root off at the bottom or taproot end of the carrot. This gives you a chance to see both the intensity of the color of the carrot and the core size and a piece that can be used for taste and textural analysis.

6. Pollination in carrot is caused by insects, and like most crops in the Apiaceae, they are very attractive to a wide diversity of pollinator species. If diverse insect habitat exists in the area, then wild pollinators can supply a substantial amount of pollination to the crop. Depending on location, size of crop, and availability of pollinator species, the grower may need to manage pollination by placing honeybee hives in the field.
7. For quality seed production, carrot requires that there is no wild carrot or Queen Anne's lace (*Daucus carota* var. *carota*) within a 1- to 2-mile area of the potential production field. This common weed will readily cross with cultivated carrots resulting in off types of gnarled, white roots. The standard recommended minimum isolation distance of 1.6 km between carrot seed crops of the same 'crop type' should be observed.
8. Proper genetic maintenance and improvement, maintaining the genetic integrity of open-pollinated carrot varieties that are good performers, require attention to details, knowledge of carrot traits, and a higher level of commitment to selection than for many other vegetables. Carrots are more prone to inbreeding depression from 'over selection' (selecting too narrowly) than most cross-pollinated crops. A minimum of 150 root sand preferably over 200 should be retained in the final intermating population to maintain adequate genetic variability.
9. For harvesting at proper stage, the king or primary umbel is the first to ripen. The seed will turn from a dark green to brown and will actually begin to detach from the umbel, but because of the racemes, or little hooks that cover the seed, they often latch together and remain on the umbel surface. However, waiting until all of the late forming umbels ripen is seldom economically viable as this seed tends to be of lower quality and strong winds will begin to dislodge the seed from the king umbels which is generally the best seed. This crop is very vulnerable to shattering due to high winds and rain at this stage, so timing and being mindful of the weather are important in timing this step. For proper threshing it takes an experienced operator. Further seed cleaning with screens and forced air is necessary to get a clean seed crop. The stems will still have fair bit of moisture so make sure to manually turn the crop to get it uniformly dry. Don't pile the crop any higher than 2–3 ft. deep, and try to keep the pile loose and fluffy to allow air flow through the crop. Supplementary air from fans may be necessary to keep mold from forming on the mature seed until threshing (Navazio et al. 2010b).

7.3 Radish (*Raphanus sativus* L.)

7.3.1 Crop History and Importance

Radish (*Raphanus sativus* L.), one of the oldest vegetable domesticates, was originally cultivated in China. The ancestral form of radish was probably closely related to the modern green and red fleshed Chinese radishes that are still in favor across Asia today. Radish probably originated in Europe and Asia. It has been under extensive cultivation in Egypt since long. It was introduced in England and France in the beginning of the sixteenth century. In 1806, it was introduced in America. Radish does not exist in wild state; it is believed to have originated from *R. raphanistrum* which is widely distributed as weed in Europe. These older types are true biennials requiring a period of cold treatment or vernalization to initiate flowering (bolting) and produce seed. It is a major root vegetable crop in the family Brassicaceae that is cultivated worldwide, serving as a source of carbohydrates, nutrients, phytochemicals, and dietary fiber for human nutrition. Nevertheless, radish is not only a vegetable crop but also an important source of medicinal compounds. Radish is a close relative of other *Brassica* crops, such as Chinese cabbage, cabbage, mustard, and rape seed, as well as the model plant *Arabidopsis thaliana*. The most commercially important part of the radish is its elongated, fleshy taproot. Other parts of the radish, including leaves, young siliques, seeds, and seedling sprouts, are also consumed. Its different preparations are useful in curing liver and gallbladder problems. Roots are also used for treating urinary complaints and piles. In Eastern Asia, radish has occupied an important position in the seed industry, and diverse cultivars have been developed in both public and commercial breeding programs (Yu Hee et al. 2019). European varieties are small in size, mild in pungency, early in maturity, and rich in quality parameters.

7.3.2 Major Breeding Objectives

Very early rooting (20–30 days), high yield, white to multicolored, round to long stump roots with thin tap root and non-branching habit, non-pithy roots, mild in pungency as per consumer's preference (mild pungency) and free from bitterness, photo-insensitive varieties with slow and late bolting habit, high root to shoot ratio, and resistant to tolerant cultivars for various biotic and abiotic stresses.

7.3.3 Crop Improvement and Taxonomy

Taxonomy classifies radish within the family Brassicaceae into the section *Raphanus* DC (Kaneko and Matsuzawa 1993). Radish is easily crosses with the related species, such as Chinese small radish (*Raphanus sativus* var. *sativus* con var. *sinensis*

Saz.) and *R raphanistrum* L. Radish is open-pollinated, self-incompatible, diploid species with a chromosome number $2n = 18$ and small genome size. Radish breeding was practiced for centuries, by means of mass or pedigree selection. In the past two decades, the production of F_1 hybrids using cytoplasmic male sterility has widely replaced simple breeding methods based on morphological traits (Banga 1976). Uniformity of varieties is becoming a high priority goal in radish breeding. Over a third (35%) of currently grown radish varieties in Germany is F_1 hybrids, which indicates a significant increase during the period of the last 15 years (Bundessortenamt 1986, 2001). Most breeding work is aimed at further adaptation to different growing conditions, improved resistance to *Peronospora parasitica* Tul. and *Albugo candida* (Pers.) Kuntzel, and improved marketing conditions (Vogel 1996). Specific market preferences strongly influence the selection of morphological traits of roots considered in the breeding process of radish. Thus, garden radish is bred for round, light-red-cored roots; French breakfast radish type has a unique oblong red root shading to white at the tip, whereas giant radish type possesses a stronger, red fleshed root, wider in diameter and not prone to sponginess. For the optimum exploitation, the parental line should be derived from genetically unrelated germplasm pools, commonly referred to as heterotic groups.

Radish is cross-pollinated due to sporophytic system of self-incompatibility. It shows considerable inbreeding depression on selfing. It is entomophilous. It is pollinated mainly by wild honeybees and wild flower flies. Stigma receptivity is maintained up to 4 days after anthesis. Selfing can be accomplished by bud pollination. The flower buds are pollinated 2 days prior to opening by their own pollen be applying fresh pollen from previously bagged flowers of the same plant. Emasculation is not necessary in bud pollination. After pollination, the buds are to be protected from foreign pollen by enclosing the particular branch bearing those buds in a muslin cloth bag.

7.3.4 Hybrid Development

In crossing the same technique is used as in bud pollination except that in the crossing, the buds of the female parent are emasculated a day prior to opening and are pollinated by pollen collected from the flowers of the male parent which were also bagged before opening. The artificial pollination is done by hand by shaking the pollen over the stigma directly from the freshly opened but previously bagged buds of male parent. When a large quantity of crossed seed is required, the roots of radish of female and male parents are planted in alternate rows, spaced 60 cm apart. Later about 3–4 days before opening of buds, the plants are covered under an insect-proof wire net or plastic cage of 22–24 mesh. Usually two plants, one female and one male, are covered under small cage, or sometimes a cage is used to cover four plants, two female and two male plants. A small honeybee colony is placed inside the cage, 3–4 days before opening of buds. This method is followed when it is possible to rogue out the selfed or sibmated plants in the seedling or root stage with the

help of a dominant marker gene or when the male and female lines are homozygous for self-incompatibility alleles but are cross compatible. This procedure can also be used to produce sibmated seeds to maintain a variety under insect-proof cages. However, in this case, it will be necessary to place about 20–30 plants under a cage to avoid inbreeding depression. A wire net or plastic net cage of 3 × 3 × 2.5 m (height) with a small door on one side is convenient for this purpose.

7.3.5 Strategies and Methods of Improvement

7.3.5.1 Genes Involved in Bolting and Flowering Network in Radish

To know networks of genes responsible for bolting and flowering regulation, transcripts in leaves at proper growing stages in late-bolting radish have been comprehensively surveyed by a high-throughput RNA sequencing technology (Nie et al. 2016). Among 53,642 unigenes of radish, 24 candidate genes associated with the plant circadian clock mechanism, such as CO and GI, were identified. In addition, 142 potential genes, including flowering pathway integrators such as FT, SOC1, and LFY, and their regulators such as FLC were also identified as genes associated with bolting and flowering. A similar experiment conducted by Jung et al. (2016), using two inbred lines with different flowering times, demonstrated that the expression patterns of genes related to the vernalization pathway showed expected correlations with the phenotypes of the two lines, suggesting that the vernalization pathway is conserved between radish and *Arabidopsis*. In addition to analysis of expressed unigenes, microRNAs (miRNAs) related to bolting and flowering times have been collected (Nie et al. 2015). Forty-two known miRNAs and 17 novel miRNAs were identified as bolting-related miRNAs. Among the candidate genes described above, several genes have been investigated to elucidate their function in radish. In the GI gene of radish, transgenic radish plants with low expression of GI caused by the introduction of an antisense GI sequence driven by CaMV35S promoter were produced, and a significant delay in both bolting time and flowering time (about 18 days) was observed in the transgenic plants compared with wild-type radish plants. This result suggests that GI is obviously involved in both traits and that the bolting time and flowering time could be controlled by regulation of the GI expression level. As described in the above paragraphs, FLC is the most significant gene for bolting and flowering times in *Arabidopsis* and *Brassica* species. In radish, three FLC paralogous genes, i.e., RsFLC1, RsFLC2, and RsFLC3, which are orthologous to BrFLC1, BrFLC2, and BrFLC3, respectively, have been identified in radish by de novo assembled transcriptome analysis (Yi et al. 2014; Nie et al. 2015). According to our search based on the information of radish genome sequences published by Kitashiba et al. (2014) and Jeong et al. (2016), RsFLC1, RsFLC2, and RsFLC3 are distributed on three chromosomes, i.e., Rs7, Rs2, and Rs3, respectively. Each gene has a genome structure similar to that in *A. thaliana* and *Brassica* species, i.e., seven exons and six introns (Yi et al. 2014) with high similarity. Although all the RsFLC

genes were expressed in radish under nonvernalized conditions, the respective expression levels were decreased in proportion to the duration of cold exposure (Yi et al. 2014). In particular, the expression patterns of RsFLC1 and RsFLC3 exhibited strong correlations with bolting and flowering times. Overexpression of all the RsFLC genes caused late-flowering phenotypes in *Arabidopsis*, indicating that every single RsFLC gene functions as a regulator of bolting and flowering times.

7.3.5.2 Male Sterility and Fertility Restorer Genes

The use of male sterility to breed F_1 cultivars has increased because of high F1 seed purity. In the USA, excessive dependence on the T-type male sterile cytoplasm in corn caused extensive losses due to southern leaf blight. The current situation in radish, which relies on just a few male sterile cytoplasms, seems to be similar to that in corn. We need to develop new male sterile cytoplasm by using interspecific crosses among Brassicaceae. In addition, a study of how Ogura-type cytoplasm has little pleiotropic effect on floral characters other than the stamen without limiting the attraction for pollinator insects is needed.

Various methods, viz., introduction, mass selection, pedigree, polyploidy, mutation, interspecific, and intergeneric, have played major role in the development of new cultivars and in improvement of various attributes. Mass selection is effective in improving qualitative or highly heritable characters, color and shape roots in radish. The selection intensity in each generation depends on the population size from which selection is to be made. The decision on selection intensity also takes into account character under selection, genetic makeup of variety, heritability, and linkage relationship of the character. Modified mass selection procedures based on the progeny testing recommended by Rajan (1996) are useful in radish. This is practiced in landraces/cultivars collected from the farmer's field. Roots are allowed to reach an overmature stage. They are dug up and leaves (but not growing points) removed. Bare roots after discarding the undesirable types are immersed in a container of water. Roots which float being pithy and full of air spaces are discarded, and only the large sinking roots are retained for seed production in isolation en masse. Small sinkers are also rejected. Cultivar Pusa Desi, Punjab Safed, Arka Nishant, and CO-1 were developed through this method. Variety Pusa Himani has been developed as the selection from cross between Black radish and Japanese white. Cultivar Pusa Reshmi is also such a selection from a cross Green Top × Desi which has been identified by pedigree method. A polyploid radish with $2n = 36$ was produced through colchiploidy, e.g., Sofia Delicious ($2n = 36$) and Semilong Red Giant ($2n = 36$) have yielded more than diploids developed through polyploidy methods. Three types of radishes *R. indicus*, *R sativus* Sen., and *R. raphanistroides* are cross compatible. This hybridization technique is being done to develop heat-tolerant lines and resistant to diseases and pests by interspecific hybridization. Varying degrees of sterility were observed when radish was crossed with Abyssinian mustard (*Brassica carinata*), Chinese cabbage, and turnip (*B. rapa*). Daniels et al. (2005) reported flowering of *R. sativus* plants could coincide with either winter or

spring *Brassica napus*. In *R. sativus* plants growing in or near a field of glufosinate-resistant *B. napus* in the UK, Daniels et al. (2005) found no *R. sativus* × *B. napus* hybrids. Further, progeny from the sampled *R. sativus* plants was all susceptible to glufosinate. Hybrids between *B. napus* and *R. sativus* have been obtained in several studies with the aid of ovule culture or embryo rescue and also by hand pollination (Gupta 1997). All artificially produced hybrids were male sterile. However, in natural crosses, Ammitzboll and Jorgensen (2006) obtained an average of 0.6 seeds per pod when male sterile *B. napus* plants were used as the female and a radish cultivar as the pollen parent. Huang et al. (2002) in hand crosses also produced many hybrids on Ogura male sterile plants. All seeds produced proved to be F_1 triploid hybrids with low pollen fertility (0–15%). It is highly probable that the presence of radish cytoplasm in the male sterile *B. napus* parent greatly facilitated *R. sativa* pollen penetration of the stigma. Further studies need to be carried out with this cross since *R. sativa* crosses easily with *R. raphanistrum* (Snow et al. 2001).

7.3.5.3 Hybrid Breeding

Heterosis has been observed for yield, root weight, and vitamin C content. This has been a common breeding approach in Japan. Self-incompatibility (SI) plays an important role in seed production of commercial hybrid cultivars in *Cruciferae* vegetables for preventing self-pollination, but this trait also makes it difficult to proliferate both parental lines, and hybrid cultivars of radish inbred lines which are self-incompatible are produced by 5–6 generations of selfing through bud pollination, while selfing, only the plants with desirable root shape, size, color, and other quality considerations are advanced to the next generation. During the recent years, a number of F_1 hybrids have been developed in radish by public and private agencies across the world, due to the increasing craze for F_1 hybrids. India is not far behind in the race. F_1 hybrids have been found advantageous for earliness, high early and total yield, root quality, uniform root development, delayed pithiness, resistance to insect pests, disease, and wider adaptability. Before the release of these hybrids, all the radish hybrids marketed in India were imported seeds, mainly from Japan and Korea. However, the trend of using F_1 seed is increasing every year. The indigenously developed and produced F_1 hybrid seed will be available to the farmers on a very competitive rate too. Hybrid seeds in radish can be produced by (1) emasculation and hand pollination, (2) free insect pollination, (3) using self-incompatible lines, and (4) using male sterile lines, but for commercial seed production of F_1 hybrid seed, we exploit self-incompatible and male sterile lines. Before going for hybrid and hybrid seed production, the steps necessary for the production of hybrid seed in radish are (1) inbreeding and production of inbred lines, (2) testing of combining ability, (3) improvement of inbred lines/varieties, and (4) finally production of hybrid seeds. In general, the system of breeding remains the same as followed in other root/brassica vegetables.

7.3.5.4 Hybrid Seed Production

Sporophytic type (oppositional factor hypothesis) also exists in radish. Incompatible reaction of pollen grains is controlled by the genotype of the plant on which they are borne while that of style is governed by its own genotype. The parental lines may be developed by the same procedure as adopted in other Brassica vegetables; however, there is as a whole not much progress in developing self-incompatible lines in radish. In a practical breeding program, the recommended isolation distance is being followed to avoid outcrossing. Single, double, and triple cross hybrids can be made. For the production of hybrid seed in different ratios (1:1, 2:1, 3:1) may be adopted depending upon the demand and quality. The market demand is mostly for single cross hybrids because of higher uniformity. An excess of nitrogen causes poor pod set in radish. Spraying of 30 ppm boric acid or 0.2% multiplex at flowering stage is useful for seed setting. Radish is less preferred by bees in comparison to cauliflower, cabbage, turnip, etc., necessitates keeping a beehive in the seed crop. For maintenance purposes, the self-incompatibility can be overcome by bud pollination or by carbon dioxide at relatively high concentration (3–5%) for 2 h in the evening on other day and continued for 3–4 weeks. In bud pollination, the flower buds are pollinated 2 days prior to their opening by their own pollen. It has been observed that the seed is better when the buds are pollinated 2 days before opening when there is at least 30% flowering on the plant rather than bud pollinating only a day prior to opening just at the commencement of flowering. The 'seed-to-seed' and 'root-to-root' methods are followed. From the seed production point of view, the winter and summer radish cultivars of temperate regions are similarly dealt, and the seed is produced in the hills (European types).

Nowadays, male sterility is commercially being used in the development of many hybrids. Ogura (1968) reported gene-cytoplasmic male sterility (CMS) in radish, which is due to an interaction between a recessive nuclear gene ms and sterile (s) cytoplasm. Later, it was reported that there are two pairs of ms genes in the nucleus (Kalloo and Bergh 1993); hence the genotype of male sterile line (A) could be $Sms^1ms^2ms^2$, maintainer line (B) $Nms^1ms^2ms^2$, and pollen parent line (C) $Nms^1ms^1ms^2ms^2$. Genic male sterility is also found in radish, carrying sterility in heterozygous forms, but the genic male sterility is not of much importance for commercial production, when other systems are available. The F_1 hybrid seed production is done by using A and C lines in different ratios as stated above, as the yield of F_1 seed varies ratio to ratio of A and C lines.

7.3.5.5 Mutation Breeding

Mutation breeding of radish has not been successfully performed because it is difficult to distinguish induced mutations and variations present in radish cultivars. Furthermore, most mutated traits are controlled by recessive alleles and are not suitable for hybrid breeding, which combines dominant alleles of parents. However, all the landraces having characteristic traits have been developed by spontaneous mutations.

Wang et al. (2015) induced compound-leaf mutant of radish by treatment of ethyl methanesulfonate (EMS) and analyzed the photosynthetic, agronomic, microstructural, and quality traits of the mutant and compared them with those of wild-type. Net photosynthetic rate was approximately 30% higher, and total chlorophyll content was approximately 36% higher in mutant than in wild-type. However, the root weight of the mutant was only half of the wild-type. Compared with wild-type, the mutant showed 75% higher vitamin C content, 39% higher total soluble solids content, and 12% lower soluble sugar content. The stomatal density was higher in compound leaves than in simple leaves. Compound leaves contained six chloroplasts per guard cell while only five in simple leaves. The degree of stomatal opening was greater in compound leaves. Compared with simple leaves, compound leaves showed thinner and looser vascular bundles and phloem cells, smaller petiole diameter, and higher density of parenchyma cells. A sequence-related amplified polymorphism analysis showed that ethyl methanesulfonate induced DNA mutations at several loci.

7.3.5.6 Use of Biotechnology

Modern market has high requirements, so it is important to enhance the genetic diversity and accelerate the breeding radish process. Actually it takes about 8–10 years to create new varieties and F_1 hybrids using conventional breeding methods, which is too long for the modern market. Modern biotechnological methods cut the time for production of fully homozygous lines to 1 year, thus shortening the breeding process. Biotechnological methods are already included into the selection process of many vegetable crops of Brassicaceae Burnet family; however, no efficient protocols developed for the species *Raphanus sativus* L. Only sporadic articles on obtaining of Daikon double haploids (DH-plants) can be found in the literature. Major genetic improvement of radish has been achieved by conventional plant breeding methods, such as crossing. However, these methods are time and labor consuming. In recent years, advances in plant genetic engineering have opened a new avenue for crop improvement, and various transgenic plants with novel agronomic characteristics have been produced. The success in plant genetic engineering is dependent upon several factors, from which an efficient tissue culture system, with high plant regeneration potential, is a crucial option. However, to the best of our knowledge, there is limited information on the plant regeneration from cell and tissue cultures of radish. Previous attempts to regenerate plants from leaf protoplasts of radish were not successful. However, the use of radish as one of the donors in the protoplast fusion studies has produced several novel fertile intergeneric somatic hybrids. The aim of this research was to study for the first time the effects of explant types and culture media on the potential of calli production from four cultivars of radish (Aazami and Hassanpouraghdam 2009).

Curtis (2003) has revealed that radish can be genetically modified by a technique called ‘floral dipping’. This system has been used successfully to delay both bolting and flowering in radish by the cosuppression of the photoperiodic gene, GIGANTEA. Future research could use this system to improve the pharmaceutical

value of the crop for global usage. The first reported production of transgenic radish by a method known as 'floral dip' used the commercially important Korean cultivar 'Jin Ju Dae Pyong'. This procedure of gene transfer is currently the only system available for producing transgenic radish. It appears, therefore, that the transformation of radish by floral dipping results in a high frequency of sibling transformants. GIGANTEA (GI), a gene involved in regulating photoperiodic flowering and controlling circadian rhythms, was cloned by two independent research groups. Previous studies have shown that GI mutants of *Arabidopsis* exhibit delayed flowering. Because of the taxonomic closeness between *Arabidopsis* and radish, attempts to delay bolting and flowering in radish were investigated by transferring an *Arabidopsis* antisense GI gene fragment into the crop to downregulate the expression of native GI.

Park et al. (2006) demonstrated 'FB plate' and the PCR-based selection on *S*-locus as effective method of DNA extraction which was found to be a feasible tool for developing new breeding methods for enhancing purity and increasing production of hybrid seeds. A new breeding method was developed to increase the uniformity of F_1 hybrid seeds and cut the cost of seed production using near-isogenic lines on double cross.

Kohzuma et al. (2017) reported that radish possesses a large sink organ (the root), implying that photosynthetic activity in radish can be enhanced by altering both the source and sink capacity of the plant. However, since radish is a self-incompatible plant, improved mutation breeding strategies are needed for this crop. TILLING (Targeting Induced Local Lesions in Genomes) is a powerful method used for reverse genetics. Further, they have also developed a new TILLING strategy involving a two-step mutant selection process for mutagenized radish plants: the first selection is performed to identify a BC^1M^1 line, that is, progenies of M^1 plants crossed with wild-type, and the second step is performed to identify BC^1M^1 individuals with mutations. They focused on Rubisco as a target, since Rubisco is the most abundant plant protein and a key photosynthetic enzyme, and found that the radish genome contains six RBCS genes and one pseudogene encoding small Rubisco subunits. They screened 955 EMS-induced BC^1M^1 lines using our newly developed TILLING strategy and obtained six mutant lines for the six RsRBCS genes, encoding proteins with four different types of amino acid substitutions. Finally, they selected a homozygous mutant and subjected it to physiological measurements.

Cheng et al. (2013) developed novel TRAP markers based on expressed sequence tag (EST) and resistance gene analog (RGAs) and applied to the genetic diversity analysis of radish genotypes. TRAP is a novel molecular marker technique which has been effectively used in genetic diversity analysis of germplasm and genetic mapping. Dendrograms constructed by UPGMA method showed that these genotypes could be clustered into four groups. Interestingly, these groups were in highly accordance with the results of resistance evaluation to turnip mosaic virus (TuMV). A cultivar identification diagram (CID) was made manually to discriminate the 30 radish genotypes using four polymorphic TRAP primer combinations. The results indicated that TRAP is an efficient genetic marker system, which could provide an effective tool for genetic mapping and for marker-assisted selection in radish breeding programs.

7.3.6 Commercial Seed Production

For commercial seed production of temperate radish, the following key points must be taken into consideration (Fig. 7.2):

1. Like carrot, radish is also a cool season vegetable crop. For seed production, superior roots are best formed at temperatures that rarely 26 °C. During seed set and maturation, however, radish seed crops may tolerate slightly higher temperatures.
2. Radishes require higher soil tilth and fertility than most other vegetable crops. To insure the development of well-shaped roots that are a true reflection of a variety's potential, it is best to grow the roots on a lighter soil, preferably a sandy loam, silt loam, or high organic matter soil.
3. As with other root crops, radish varieties can only be properly maintained for trueness to type if they are grown using the 'root-to-seed method' which allows for selection of the roots before seed production begins. The radish roots, or 'stecklings', are first produced by planting the seed in much the same way that a commercial radish vegetable crop is grown. With this method, roots are then pulled from the ground at edible maturity, selected based on root morphological characteristics (appearance), and then replanted in a field at a wider inter- and

Fig. 7.2 (**a**) True to type selected healthy root of radish for further breeding and seed production purpose. (**b**) Full bloomed healthy crop stand of radish. (**c**) Flowering and pollination stages with proper provision of pollinators (Honeybees boxes) for quality pod and seed development in radish. (**d**) Healthy pod development and maturation stage in radish

intra-row spacing to provide adequate space for the subsequent reproductive stages that lead to a seed crop.

4. Radish is a cross-pollinated species with perfect flowers. It is pollinated by a variety of pollinating insects attracted to the abundance of flowers over an extended blooming period. As with most other cultivated members of the Brassicaceae family, radish is self-incompatible. Therefore, each pollination event comes from an outcross between two different radish plants of the same population. Initiation of flowering (bolting) in radish is not day-length sensitive, but most radishes do require some degree of vernalization to induce flowering.
5. As a cross-pollinated species, radish can cross easily from one radish type to another. Isolation distances of at least one mile should be maintained from another radish population if grown in an open landscape with few natural barriers.
6. Proper genetic maintenance and size of population are very important. The extreme outcrossing (cross-pollination) of radish is ideal for maintaining adequate genetic mixing in a diverse varietal population. It is imperative to keep the level of inherent diversity and in order to avoid inbreeding depression. Seed should be collected from a minimum of 120–200 plants in any commercial radish seed crop.
7. The criteria of selection are similar to that of carrot. Beyond selecting for the quickest or earliest germinating plants, early selection should include recognition of shape, size, color, and ability of the seedlings and roots to grow under less than optimum conditions.
8. Radish seed crops mature in approximately 150 days depending on variety, climate, and planting date. Radish seed is ready to harvest when about 60–70% of the seed pods turn from green yellow to brown and lose their fleshy appearance, becoming papery thin and light. The plants are then placed in a windrow to cure in the field for 10–14 days. Threshing can be done without machinery on a small scale by stomping on or driving over the pods. Once threshed, radish seeds may be separated from the pods, chaff, and debris by screening and winnowing the seed. If the chaff is adequately dried, much of it should be lighter than the seed and may also be removed by winnowing (separating by weight), by either dropping the material in front of a fan by hand or using a gravity table.

7.4 Turnip (*Brassica rapa* L.)

7.4.1 Crop History and Importance

Turnip is a cool season root vegetable grown mainly in temperate regions and subtropics. It is a frost hardy biennial that is grown as annual for their enlarged fleshy taproot. It has been cultivated in Europe for over 4000 years. It was introduced to Canada in 1540 by Cartier during the voyage of exploration and then taken to Mexico in 1586 and to Virginia in 1610 (Peter 1998). The Mediterranean area is thought to be the primary center of origin of European types (Bailey 1965), while Eastern Afghanistan with adjoining area of Pakistan is considered to be another primary

center with Asia minor, Transcaucasus, and Iran, as secondary center (Sinskaia 1928). The parents of cultivated turnip are found wild in Russia, Siberia, and Scandinavia (Thompson and Kelly 19,657). It is widely distributed in Europe mainly in Germany, the UK, Russia, and Asian countries, i.e., Afghanistan, Pakistan, Iran, China, and India (Purewal 1957). It is now cultivated throughout the world. Turnip leaves and roots are used as vegetable and salad. The roots are also cooked along with the tender top. European types of turnip are relatively sweet and more palatable and may be eaten sometimes raw. The Asiatic turnips use to prepare good pickles and to a limited extent in salads (Choudhary 1967; Nath et al. 1987). In some places it is used in several delicious dishes including with meat (Gopalan et al. 1971).

7.4.2 *Major Breeding Objectives*

Early in maturity, uniformity in size and shape, root color as per consumer's preferences (white, purple and creamish types), stump rooted varieties with thin tap root and non-branching habit, slow bolting habit, high dry matter (8–9%) in roots, and resistance to club root, powdery mildew, turnip mosaic virus, white rust, phyllody, cabbage root fly and turnip root fly (Fig. 7.3).

Fig. 7.3 (**a**, **b**) Selection of healthy and true roots of turnip and preparation of stecklings for further breeding and seed production purpose. (**c**) Turnip at flowering stages in temperate regions. (**d**) Healthy crops stand and seed setting turnip in temperate regions of Himachal Pradesh

7.4.3 Strategies and Methods of Crop Improvement

Turnip (*Brassica rapa* L., $2n = 2x = 20$, formerly *B. campestris* subsp. *rapifera*) belongs to the large *Brassica* family and bears similar yellow (and occasionally white) flowers. It does not cross readily with the oleracea (cabbage types) and belongs to a separate species, *B. campestris*. Another crop swede (*B. napus* L. var. *napobrassica*) is directly related to turnip. The swede is an amphidiploid with 38 chromosomes and is known to be a natural hybrid between *B. campestris* ($2n = 20$) and *B. oleracea* ($2n = 18$). Neither of the ancestral types is known accurately, but it is thought that the cross occurred numerous times where the two species overlap in their natural habitat (from Western Europe to Eastern Asia). The interspecific cross has been synthesized by several workers, but the result is not noticeably swede-like. Cross-incompatibility between the parental species is quite marked, and pollen tubes tend to become distorted in the style. Any successful fertilization seems to result in the production of a sterile hybrid. Both are cultivated as annual root crops for both animal and human consumption. The storage organ is a swollen hypocotyl.

This crop is highly cross-pollinated due to self-incompatibility and male sterility. It has a sporophytic system of self-incompatibility. Davey (1931) investigated the genetics of flesh and skin color in turnip and swede as well. White flesh is dominant to yellow in both the species and is governed by a single locus in turnip, whereas skin color is determined by two independent loci conditioning the presence or absence of green or red pigmentation respectively. Mero and Honma (1985) found that bolting in the progeny of a cross between Chinese cabbage and turnip was controlled by two major additive genes. However, there was also an association between strong bolting resistance and the turnip phenotype.

Major germplasm collections are present in the gene banks in Japan, the UK, and the USA. Breeding work in turnip is almost at halt expecting for the maintenance of existing cultivars as marketed by the major International Japanese and European seed companies. Frandsen (1941) reported that *Brassica rapa* hybridizes readily with *B. napus* to produce triploid hybrids ($3n = 29$) which are almost sterile. Spontaneous and artificial amphidiploids ($2n = 58$) (Frandsen and Winge 1932; Olsson 1963; McNaughton 1973) might be used to introgress useful characteristics from one species to another. The *Brassica oleracea* can be crossed with difficulty with *B. rapa*. Hybridization can be facilitated by embryo culture (Harberd 1969). Artificial form of *Brassica napus* can be synthesized from the diploid parent species and have been used to introgress disease resistance from *B. oleracea* to *B. rapa* (McNaughton 1976).

Various breeding methods have been employed for the improvement of this crop. The mass selection method of breeding has been commonly employed to breed several open-pollinated cultivars. Plants from selected roots allowed mass-pollination in isolation. An increase over 25% over the base population was observed in mass selected progeny. Variety Improved Golden Ball has been selected through mass selection from a population of Golden Ball. Mass selection is the simplest and

oldest breeding method practiced to improve turnip species (Hoen 1968). Selfing with selection increases the effectiveness of the practice and the quantity of genetic enhancement attained in a breeding program through increasing the frequency of desirable alleles (Solieman et al. 2012).

Recurrent selection is the predominant method plant breeder's use for developing improved seed. This method is very effective in improving yield and other character by accumulation of additive genetic variance. This is highly efficient in cross-pollinating crops for creating superior germplasm. In the simple recurrent selection, the best individual is selected, and their selfed are intercrossed, and further selection is made in intercross progenies. When a population plateau is off its potential, recurrent selection may be useful. Involvement of large number of parents may be helpful for accelerating desirable gene frequency in the population. The last intermated population is used as base population for further improvement through different breeding methods. It is a cyclic process for advancing one or more traits. With each cycle, superior parents are selected and mated to produce progeny for the next round of selection. The expectation is that favorable alleles at loci will increase in frequency and assemble through recombination with subsequent generations (Briggs and Goldman 2006). A non-waxy mutant has been developed using gamma rays on Pusa Sweti. The mutant has showed resistance to aphids developed by mutation. Polyploid could be developed through colchicine treatment. It was found that first-generation polyploidy had low fertility, which could be rectified by crossing and selection. Tetraploids had high root yield but reduced dry matter content. A trisomic line with $2n = 21$ was isolated in turnip that showed reduced fertility compared with diploid in open, cross-, and self-pollination.

7.4.3.1 Heterosis Breeding

Different methods followed in hybrid seed production of turnip are (a) finding out combinations having higher specific combining ability effects and (b) growing selected two lines together in a mixed crop where female lines have recessive marker gene so that selfed progeny may be discarded in hybrids. Pollen sterile lines and self-incompatible lines are also used in hybrid seed production.

There has been greater interest among breeders to produce hybrid cultivars of fodder swedes utilizing self-incompatibility. However, the same has not been achieved in case of horticultural types. Heterosis has been reported for several characters including the yield of root and leaf. Japanese breeders have been successful in developing several F_1 hybrid cultivars of white turnip. There has been some progress in breeding of F_1 hybrid turnip cultivars in the USA, but the acceptance of these cultivars has been poor. However, considering the higher level of heterosis for root yield and availability of sporophytic system of self-incompatibility, it is suggested that hybrid cultivars of turnip should be developed following the procedures as outlined in case of other root vegetables.

7.4.3.2 Intergeneric Hybridization

A triploid ($2n = 28$) was identified from a cross between a tetraploid fodder radish and a tetraploid stable turnip; triploid had nine bivalent and ten univalents. *B. rapa*, a widespread weed of cultivated and disturbed lands, is also grown as a vegetable and oilseed crop. The weedy type differs from the cultivated oilseed form only in the primary seed dormancy trait. Plant breeders of *B. rapa* and *B. napus* have known for many years that these two species readily cross in nature and they were not surprised that natural interspecies gene flow was demonstrated in several countries. Normally the highest hybrid frequencies occur when individual, self-incompatible plants of *B. rapa* are present in *B. napus* fields. In the field, more hybrids are produced on *B. rapa* plants than on *B. napus* plants (Jorgensen and Andersen 1994; Hauser et al. 1997), primarily due to their respective self-incompatible and self-compatible breeding systems. However, in reciprocal hand crosses, more hybrids per cross are found when *B. napus* is the female (Downey et al. 1980). Natural interspecific hybridization between *B. rapa* and *B. napus* varies widely, depending on the environment under which the plants develop and the design of the experiment, particularly the ratio of *B. rapa* to *B. napus* plants. Compared to the parent species, natural interspecific hybrids have reduced fertility and poor seed set, averaging two to five seeds per pod (Jorgensen and Andersen 1994). The survival rate of hybrid seedlings is also low, with <2% survival (Scott and Wilkinson 1998), reducing the rate of introgression. Interspecific vegetative and reproductive competition strongly impacts the relative and absolute fitness of the hybrids. The rate of introgression of a *B. napus* trait into the *B. rapa* genome will greatly depend on the selection pressure exerted on the gene (Scott and Wilkinson 1998; Snow and Jorgensen 1999).

7.4.3.3 Synthetic Varieties

Allard (1960) defines a synthetic variety as one ‘that is maintained from open pollinated seed following its synthesis by hybridization in all combinations among a number of selected genotypes’. This method, which is widely used in breeding forage crops, is also effective for the breeding of oil seed *B. rapa*. Equal amounts of seed from varieties or recurrent lines that arise from widely different gene pools are mixed and sown in Syn.-0 isolation plots. Seed harvested from the Syn.-0 plot constitutes the Syn.-1 generation. Syn.-1 seed from a two component synthetic will consist of 25% from each parental genotype and 50% hybrid seed. Thus, if the parental lines are good combiners, a significant amount of heterosis can be captured. Normally, despite the high multiplication rate (1000: 1), there is insufficient Syn.-1 seed for commercialization so that Syn.-1 seed is sown to provide commercial Syn.-2 seed. This procedure has been used in Canada to produce the first commercial *B. rapa* synthetic varieties, Hysyn 100 and Hysyn 110. Because of the large number of genotypes within the parental lines, there is very little loss in heterosis between the Syn.-1 and Syn.-2 generations (Falk and Woods 2003). If the market is very large, a Syn.-3 generation could be added. Synthetics are formed by mechanically mixing two or three parental lines (Buzza 1995) to constitute the Syn-1

generation, which is generally the commercial seed. All the synthetic generations yielded significantly more than the parents including the Syn-0, that is, the mixing of the parents. Reported yield increase ranged between 2% and 8% (Syn-0), 10 and 14% (Syn-1), and 5% and 10% (Syn-2).

7.4.3.4 Composites

The composites are formed by mixing seeds of male sterile hybrid plants (75–80%) with seeds of fertile plants (20–25%) as a pollen source. But their performance has varied depending on weather conditions during flowering. Composites are more sensitive, when compared with open-pollinated cultivars, to cold, wet conditions during pollination resulting in decreased seed set (Kightley 1999). Renard et al. (1995) suggested that some of the yield increase in composite hybrids could be the result of the lower-energy requirements of male sterile plants or of the hybridity of the cytoplasms (cybrids).

7.4.3.5 Use of Biotechnology

Comparative mapping, using more than 20 linkage maps for *B. oleracea*, *B. rapa*, *B. nigra*, *B. napus*, and *B. juncea*, has contributed greatly to the understanding of chromosome homology and colinearity. Marker-assisted selection and chromosome mapping came into general use in the 1980s with the development of restriction fragment length polymorphisms (RFLP) techniques that resulted in the first linkage maps for *B. rapa* (Song et al. 1995) and *B. napus* (Landry Benoit et al. 1991). This technique was important in identifying genomes and their chromosomes and locating genes and qualitative trait loci (QTLs), which are DNA regions containing a gene or genes that regulate traits of agronomic or quality interest. The technique of TILLING (Targeting Induced Local Lesions in Genomes) can be used to identify a series of mutations (alleles) in a target gene by hetero duplex analysis (McCallum Claire et al. 2000). This method combines a standard technique of mutagenesis with a chemical mutagen such as ethyl methanesulfonate (EMS), six with a sensitive DNA screening technique that identifies single-base mutations (also called point mutations) in a target gene. *B. rapa* TILLING services are available from Rev. Gen UK in the UK (John Innes Centre).

Root protoplast of turnip cultivar Snowball regenerated roots via a callus culture stage but failed to produce any shoot (Ziemborska and Harney 1986). Whole turnip plants and protoplast have been successfully transformed utilizing a modified cauliflower mosaic virus genome (Paszkowski et al. 1986). Techniques of DNA hybridization have been used to assess sequence homologies of turnip mtDNA with other plant species and between the nuclear genome of turnip and *Arabidopsis* and their somatic hybrid produced by protoplast fusion (Miroshnichenko and Volkov 1984; Miroshnichenko et al. 1986). RFLP was used by Ohkawa (1985) to demonstrate that male sterile cytoplasm of *B. napus* was the same as that of *B. rapa* but different from that of *B. oleracea*.

References

Aazami MA, Hassanpouraghdam MB (2009) The effects of different culture media on the callus production of radish (*Raphanus sativus* L.). Rom Biotechnol Lett 14(4):4519–4523

Akhramenka GD (1988) Cytological and biochemical characteristics of polyploid forms of beetroot. Vestsi Akademii Navuk BSSR, Biyalagichnykh Navuk 5:33

Akhramenka GD, Kazyrevich TP (1989) Effect of backcrosses on alteration of characters in recombinant forms of tetraploid beetroot. Vestsi Akademii Navuk BSSR, Biyalagichnykh Navuk 1:20

Allard RW (1960) Principles of plant breeding. Wiley, New York

Ammirato PV (1986) Carrot. In: Evans DA, Sharp WR, Ammirato PV (eds) Handbook of plant cell culture, vol 4. Macmillan, New York, pp 457–499

Ammitzboll H, Jorgensen RB (2006) Hybridization between oilseed rape (*Brassica napus*) and different populations and species of *Raphanus*. Environ Biosaf Res 5(1):3–13

Avesani L, Falorni A, Tornielli GB, Marusic C, Porceddu A, Polverari A, Faleri C, Calcinaro F, Pezzotti M (2003) Improved in planta expression of the human islet autoantigen glutamic acid decarboxylase (GAD65). Transgenic Res 12:203–212

Aviv D, Amsellem Z, Gressel J (2002) Transformation of carrots with mutant acetolactate synthase for Orobanche (broomrape) control. Pest Manag Sci 58:1187–1193

Bach IC, Olesen A, Simon PW (2002) PCR-based markers to differentiate the mitochondrial genomes of petaloid and male fertile carrot (*Daucus carota* L.). Euphytica 127:353–365. https://doi.org/10.1023/A:1020314802236

Bailey LH (1965) The Standard encyclopedia of horticulture Bailey Volume (1,2 &3):3403–3404

Banga O (1976) Radish (*Raphanus sativus* L.). In: Simmonds NW (ed) Evolution of crop plants. Longman, London, pp 60–62

Banga O, de Bruyn JW, van Bennekom JL, van Keulen HA (1958) Selection of carrots for carotene content. Euphytica 7:247–256

Banga O, Petiet J, van Bennekom JL (1964) Genetical analysis of male-sterility in carrots (L.). Euphytica 13:75–93. https://doi.org/10.1007/BF00037521

Baranski R, Grzebelus D, Frese L (2001) Estimation of genetic diversity in a collection of the garden. Euphytica 122:19–29

Baranski R, Klocke E, Nothnagel T (2007) Enhancing resistance of transgenic carrot to fungal pathogens by the expression of Pseudomonas fluorescence microbial factor (MF3) gene. Physiol Mol Plant Pathol 71:88–95

Baranski R, Klocke E, Nothnagel T (2008) Chitinase CHIT36 from Trichoderma harzianum enhances resistance of transgenic carrot to fungal pathogens. J Phytopathol 156:513–521

Bienz DR (1968) Evidence for carrot splitting as an inherited tendency. Proc Am Soc Hortic Sci 93:429–433

Bliss FA (1965) Inheritance of male sterility in table beets (*Beta vulgaris* L.). Diss Abstr 25:6901

Bonnet A (1985) Breeding of carrot male sterile lines and F_1 and three-way hybrids creation. In: Eucarpia – Breeding of root crops, pp 30–37

Börner T, Linke B, Nothnagel T, Scheike R, Schulz B, Steinborn R, Brennicke A, Stein M, Wricke G (1995) Inheritance of nuclear and cytoplasmic factors affecting male sterility in *Daucus carota* L. In: Kück U, Wricke G (eds) Genetic mechanisms for hybrid breeding. Blackwell Wissenschafts- Verlag, Berlin, pp 111–122

Bouche FB, Marquet-Blouin E, Yanagi Y, Steinmetz A, Muller CP (2003) Neutralising immunogenicity of a polyepitope antigen expressed in a transgenic food plant: a novel antigen to protect against measles. Vaccine 21:2065–2072

Bouche FB, Steinmetz A, Yanagi Y, Muller CP (2005) Induction of broadly neutralizing antibodies against measles virus mutants using a polyepitope vaccine strategy. Vaccine 23:2074–2077

Briggs WH, Goldman IL (2006) Genetic variation and selection response in model breeding populations of *Brassica rapa* following a diversity bottleneck. Genetics 172(1):457–465

Broglie K, Chet I, Holliday M (1991) Transgenic plants with enhanced resistance to the fungal pathogen *Rhizoctonia solani*. Science 254:1194–1197

Budar F, Pelletier G (2001) Male sterility in plants: occurrence, determinism, significance and use. C R Acad Sci III 324(6):543–550. https://doi.org/10.1016/S0764-4469(01)01324-5

Buishand JG, Gabelman WH (1979) Investigations on the inheritance of color and carotenoid content in phloem and xylem of carrot roots (L.). Euphytica 28:611–632

Bundessortenamt (1986) Beschreibende Sortenliste: wurzelgemuse, Zweibelgemuse, Kohlgemuse, Hulsenfruchte

Bundessortenamt (2001) Beschreibende Sortenliste: wurzelgemuse, Zweibelgemuse, Kohlgemuse, Hulsenfruchte

Burkill IH (1935) A dictionary of the economic products of the Malay Peninsula. Crown Agents, London

Buzza GC (1995) Plant breeding. In: Kimber DS, DI MG (eds) Brassica oil seeds, production and utilization. CAB International, Wallingford, pp 153–175

Chen WP, Punja ZK (2002) Transgenic herbicide- and disease-tolerant carrot (*Daucus carota* L.) plants obtained through *Agrobacterium*-mediated transformation. Plant Cell Rep 20:929–935

Cheng D, Fenjiao Z, Liwang L, Xu L, Yinglong C, Wang X, Cecilia L, Yu R, Yiqin G (2013) TRAP markers generated with resistant gene analog sequences and their application to genetic diversity analysis of radish germplasm. Sci Hortic 161:153–159

Choudhary B (1967) Vegetables. National Book Trust India, New Delhi

Curtis Ian S (2003) The noble radish: past, present and future. Trends Plant Sci 8(7):305–307

Davey VMM (1931) Colour inheritance in swedes and turnips and its bearing on the identification of commercial stocks. Scott J Agric 14:303

Davey JC (1999) Deploying male-sterility as a grouping character in carrot distinctness, uniformity and stability trials. In: Proceedings of the third international symposium on the taxonomy of cultivated plants. Edinburgh, 20–26 July 1998, pp 431–433

Dickson MH (1966) The inheritance of longitudinal cracking in carrots. Euphytica 15:99–101

Donald I (2012) Beetroot stand management. Final Report HAL Project VG 06117

Dowker BD, Jackson JC (1977) Variation studies in carrots as an aid to breeding. The effects of environments within a site on the performance of carrot cultivars. J Hortic Sci 52:299–307

Downey RK, Klaasen AJ, Stringham GR (1980) Rapeseed and mustard. In: Fehr WR, Hadley HH (eds) Hybridization of crop plants. American Society of Agronomy, Crop Science Society of America, Madison, pp 495–509

Droge W, Broer I, Puhler A (1992) Transgenic plants containing the phosphinothricin-N-acetyltransferase gene metabolize the herbicide L-phosphinothricin (glufosinate) differently from untransformed plants. Planta 187:142–151

Droge-Laser W, Siemeling U, Puhler A, Broer I (1994) The metabolites of the herbicide L-phosphinothricin (glufosinate)-identification, stability, and mobility in transgenic, herbicide-resistant, and untransformed plants. Plant Physiol 105:159–166

Erickson EH, Garment MB, Peterson CE (1982) Structure of cytoplasmic male-sterile and fertile carrot flowers. J Am Soc Hortic Sci 107(4):698–706

Falk KC, Woods DL (2003) Seed yield of successive synthetic generations in summer turnip rape. Can J Plant Sci 83(2):271–274. https://doi.org/10.4141/P01-182

Frandsen KJ (1941) Contribution to the cytogenetics of *Brassica napus* L., *Brassica campestris* L., and their hybrid the amphidiploid *Brassica napocampestris*. Arsskr K Vet Landobhojsk 1941:59

Frandsen KJ, Winge O (1932) *Brassica napocampestris*, a new constant amphidiploid species hybrid. Hereditas 16:212

Freeman RE, Simon PW (1983) Evidence for simple genetic control of sugar type in carrot (*Daucus carota* L.). J Am Soc Hortic Sci 108:50–54

Frimmel F (1938) Karottenzuchtung. Zuchter 10:181–185

Frison EA, Serwinski J (1995) Directory of European institutions holding crop genetic resource collection. IPGRI, Rome

Gabelman WH (1974) F1 hybrids in vegetable production. In: Antoszwski R, Harrison L, Nowosielski J (eds) Proceedings of XIX international horticultural congress, vol III, Warsaw, 11–18 Sept 1974, 419 ISHS, Wageningen

Girod PA, Zryd JP (1987) Clonal variability and light induction of betalain synthesis in red beet cell cultures. Plant Cell Rep 6:27

Gopalan C, Ram Sastri BV, Balasubramanium SC (1971) Nutritative value of Indian food. National Institute of Nutrition, Hyderabad

Gupta SK (1997) Production of interspecific and intergeneric hybrids in Brassica and Raphanus. Cruciferae Newsletter Eucarpia 19:21–22

Hammer K, Stanarius A, Kuhne T (1990) Differential occurrence of beet cryptic viruses-a new tool for germplasm characterization and evolutionary studies in beet. Euphtytica 45:23

Hansche PE, Gabelman WH (1963) Digenic control of male sterility in carrots (Daucus carota L.). Crop Sci 3:383–386

Harberd DJ (1969) A simple effective embryo culture technique for Brassica. Euphytica 18:425

Hauser TP, Jorgensen R, Ostergard H (1997) Preferential exclusion of hybrids in mixed pollination between oil seed rape (Brassica napus) and weedy Brassica campestris (Brassicaceae). Am J Bot 84:756–762

Hayward H (1938) The structure of economic plants. The MacMillan Company, New York

Hoen K (1968) Heritability and genetic correlations in turnips (Brassica campestris L. var. rapa). Euphytica 17(3):352–356

Hofer R, Gennari D (1994) HPTLC -Methode zur quatitativen Zuckerbestimmung in Mohren (Dcnicus carota L.). Vortr Pflanzenzuchtg 28:318–320

Holland H (1957) Classification and performance of varieties of red beet. Rep Natl Veg Res Stn for 1956, 16

Hoppe HA (1981) Taschenbuch der Drogenkunde. De Gruyter, Berlin/New York

Huang B et al (2002) Production and cytogenetics of intergeneric hybrids between Ogura CMS Brassica napus and Raphanus raphanistrum. Cruciferae Newsletter Eucarpia 24:25–27

Iman MK, Gabelman WH (1968) Inheritance of carotenoids in carrots (Daucus carota L.). Proc Am Soc Hortic Sci 93:419–428

Imani J, Berting A, Nitsche S, Schaefer S, Gerlich WH, Neumann KH (2002) The integration of a major hepatitis B virus gene into cell-cycle synchronized carrot cell suspension cultures and its expression in regenerated carrot plants. Plant Cell Tissue Org Cult 71:157–164

Jayaraj J, Punja Z (2008) Transgenic carrot plants accumulating ketocarotenoids show tolerance to UV and oxidative stresses. Plant Physiol Biochem 46:875–883

Jayaraj J, Devlin R, Punja Z (2008) Metabolic engineering of novel ketocarotenoid production in carrot plants. Transgenic Res 17:489–501

Jeong YM, Kim N, Ahn BO, Oh M, Chung WH, Chung H, Jeong S, Lim KB, Hwang YJ, Kim GB, Baek S, Choi SB, Hyung DJ, Lee SW, Sohn SH, Kwon SJ, Jin M, Seol YJ, Chae WB, Choi KJ, Park BS, Yu HJ, Mun JH (2016) Elucidating the triplicated ancestral genome structure of radish based on chromosome-level comparison with the Brassica genomes. Theor Appl Genet 129:1357–1372

Jorgensen RB, Andersen B (1994) Spontaneous hybridization between oilseed rape (Brassica napus) and weedy B. campestris (Brassicaceae): a risk of growing genetically modified oilseed rape. J Bot 81:1620–1626

Jung WY, Park HJ, Lee A, Lee SS, Kim YS, Cho HS (2016) Identification of flowering-related genes responsible for differences in bolting time between two radish inbred lines. Front Plant Sci 7:1844

Kalloo G, Bergh BO (1993) Genetic improvement of vegetable crops. Pergamon Press, Oxford

Kamada H, Kobayashi K, Kiyosue T, Harada H (1989) Stress induced somatic embryogenesis in carrot and its application to synthetic seed production. In Vitro Cell Dev Biol 25:1163–1168

Kaneko Y, Matsuzawa Y (1993) Radish (Raphanus sativus L.). In: Kalloo G, Bergh BO (eds) Genetic improvement of vegetable crops. Pergamon Press, Oxford, pp 487–505

Kightley SPJ (1999) The introduction of oilseed rape hybrids in the United Kingdom. GCIRC Bull 16:74–79

Kitagawa J, Gerrath JM, Posluszny U, Wolyn DJ (1994) Developmental and morphological analyses of homeotic cytoplasmic male sterile and fertile carrot flowers. Sex Plant Reprod 7:41–50. https://doi.org/10.1007/BF00241886

Kitashiba H, Li F, Hirakawa H, Kawanabe T, Zou Z, Hasegawa Y, Tonosaki K, Shirasawa S, Fukushima A, Yokoi S, Takahata Y, Kakizaki T, Ishida M, Okamoto S, Sakamoto K, Shirasawa K, Tabata S, Nishio T (2014) Draft sequences of the radish (Raphanus sativus L.) genome. DNA Res 21:481–490

Kitto SL, Janick J (1985) Production of synthetic seeds by encapsulating asexual embryos of carrot. J Am Soc Hortic Sci 110:277–282

Kohzuma K, Chiba M, Nagano S, Anai T, Ueda MU, Oguchi R, Shirai K, Hanada K, Hikosaka K, Fujii N (2017) Mutant selection in the self-incompatible plant radish (Raphanus sativus L. var. sativus) using two-step TILLING. Breed Sci 67:268–276

Kozik Elżbieta U, Renata N, Marzena N, Barbara D (2012) Level of sterility and morphological flowers differentiation of petaloid male-sterile plants of carrot. J Agric Sci 4(2):187–194

Krivsky K, Sverepova AG (1985) Hybridization of cultural and wild carrots (Daucus carota L.) Eucarpia-a meeting on breeding of root vegetables. 6–9 Sept, Olomouc, pp 51–62

Kust AF (1970) Inheritance and differential formation of colour and associated pigments in xylem and phloem of carrot Daucus carota L. PhD Thesis, University of Wisconsin

Laferrier L, Gabelman WH (1968) Inheritance of color, total carotenoids, alpha-carotene and beta-carotene in carrots (Daucus carota L.). Proc Am Soc Hortic Sci 93:408–418

Landry Benoit S, Nathalie H, Takeomi E, Harada John J, Lincoln SE (1991) A genetic map for Brassica napus based on restriction fragment length polymorphisms detected with expressed DNA sequences. Genome 34(4):543–552. https://doi.org/10.1139/g91-084

Lange W, Brandenburg WA, De Bock Th SM (1999) Taxonomy and cultonomy of beet (Beta vulgaris L.). Bot J Linn Soc 130:81–96

Langridge WHR, Li BJ, Szalay AA (1985) Electric-field mediated stable transformation of carrot protoplasts with naked DNA. Plant Cell Rep 4:355–359

Linke B, Nothangel T, Borner T (2003) Flower development in carrot CMS plants: mitochondria affect the expression of MADS box genes homologous to GLOBOSA and DEFICIENS. Plant J 34:27–37

Linthorst HJM, van Loon LC, van Rossum CMA, Mayer A, Bol JF, van Roekel SC, Melenhoff EJS, Cornelissen BJC (1990) Analysis of acidic and basic chitinase from tobacco and petunia and their constitutive expression in transgenic tobacco. Mol Plant Micro Interact 3:252–258

Liu JR, Jeon JH, Yang SG, Lee HS, Song NM, Jeong WJ (1992) Dry type of carrot (Daucus carota L.) artificial seeds. Sci Hortic 51:1–11

Marquet-Blouin E, Bouche FB, Steinmetz A, Muller CP (2003) Neutralizing immunogenicity of transgenic carrot (Daucus carota L.)- derived measles virus hemagglutinin. Plant Mol Biol 51:459–469

McCallum Claire M, Luca C, Greene Elizabeth A, Steven H (2000) Targeting induced local lesions in genomes (TILLING) for plant functional genomics. Plant Physiol 123(2):439–442. http://www.plantphysiol.org/content/123/2/439.full

McNaughton IH (1973) Brassica napocampestris (2n = 58), I. Synthesis, cytology, fertility and general considerations. Euphytica 22:301

McNaughton IH (1976) Swedes and rapes. In: Simmonds NW (ed) Evolution of crop plants. Longman, London

Mehring Lemper M (1987) Genetisch Züchterische Untersuchungenzur Schaffung von Hybrid sortenbei Möhren (Daucus carota L.). Dissertation, Universität Hannover

Meikle RAR (1981) Factors affecting the germination and establishment of monogerm sugar beet. BSc Hons Thesis, University of Edinburgh, Edinburgh

Melchers LS, Stuiver MH (2000) Novel genes for disease-resistance breeding. Curr Opin Plant Biol 3:147–152

Mero CE, Honma S (1985) Inheritance of bolting resistance in an intraspecific Chinese cabbage x turnip cross. Hort Sci 20:881

Michalik B (1979) Stability of male sterility in carrot under different growth conditions. De L'Academie Polonaise Des Sciences 26(12):827–832

Miroshnichenko GP, Volkov RA (1984) Formation of parasexual hybrids between Arabidopsis thaliana and Brassica campestris may be due to similarity of their DNA. Dokl Akad Nauk SSSR 276:489

Miroshnichenko GP, Volkov RA, Borisyuk NV (1986) Structure of the genomes of fodder turnip, Arabidopsis and their somatic hybrid. Biokhimiya 51:84

Nath P, Velayudhan S, Singh DP (1987) Vegetable for tropical regions. ICAR, New Delhi

Nie S, Xu L, Wang Y, Huang D, Muleke EM, Sun X, Wang R, Xie Y, Gong Y, Liu L (2015) Identification of bolting-related microRNAs and their targets reveals complex miRNA-mediated flowering-time regulatory networks in radish (Raphanus sativus L.). Sci Rep 5:14034

Nie S, Li C, Xu L, Wang Y, Huang D, Muleke EM, Sun X, Xie Y, Liu L (2016) De novo transcriptome analysis in radish (Raphanus sativus L.) and identification of critical genes involved in bolting and flowering. BMC Gen 17:389

North C (1979) Plant breeding and genetics in horticulture. The Macmillan Pres Ltd, London, p 150

Nothnagel T (1992) Results in the development of alloplasmic carrots (Daucus carota sativus Hoffm.). Plant Breed 109:67–74

Nothnagel T, Pand S, Linke B (2000) Male sterility in populations of Daucus and the development of alloplasmic male sterile lines of carrot. Plant Breed 145:112–119

Nottingham S (2004) Beetroot, vol 2012. http://www.stephennottingham.co.uk/beetroot.htm

Ogura H (1968) Studies on the new male sterility in Japanese radish with special reference to the utilization of this sterility towards practical raising of hybrid seeds. Mem Fac Agric Kagoshima Univ 6:39–78

Olsson G (1963) Induced polyploids in Brassica in recent plant breeding research. Svalof 1946–1961, Akerberg and Hagbg, eds pollinations between oilseed rape (Brassica napus) and weedy B. campestris (Brassicaceae), polymorphisms detected with expressed DNA sequences. Genome 34(4):543–552. https://doi.org/10.1139/g91-084

Park S, Kim CK, Pike LM, Smith RH, Hirschi KD (2004) Increased calcium in carrots by expression of an Arabidopsis H+/Ca2+ transporter. Mol Breed 14:275–282

Park S-H, Lee S-S, Choi J-K, Yoon M-K, Kim K-T, Lim S-H, Mok I-G (2006) Marker-assisted selection of self-Incompatibility allows an efficient double cross breeding in radish (Raphanus sativus L.). In: Proceeding of joint meeting: crucifer genetics workshop & 4th ISHS symposium on Brassicas

Paszkowski J, Pisan B, Shillito RD, Hohn T, Hohn B, Potrykus I (1986) Genetic transformation of Brassica campestris var. rapa protoplasts with engineered cauliflower mosaic virus genome. PI Mol Biol 6:303

Peter KV (1998) Genetics and breeding of vegetable crops. Indian Council of Agricultural Research, New Delhi

Peterson CE, Simon PW (1986) Carrot breeding. In: Bassette MJ (ed) Breeding vegetable crops. AVI Publ. Co., Westport, pp 321–356

Peterson CE, Simon PW, Rubatzky VE, Strandberg JO (1988) Beta 111 carrot. Hortic Sci 23:917

Pink DAC (1993) Beetroot. In: Kaloo G, Bergh BO (eds) Genetic improvement of vegetable crops. Pergamon Press, Oxford, pp 473–477

Porceddu A, Falorni A, Ferradini N, Cosentino A, Calcinaro F, Faleri C, Cresti M, Lorenzetti F, Brunetti P, Pezzotti M (1999) Transgenic plants expressing human glutamic acid decarboxylase (GAD65), a major auto antigen in insulin-dependent diabetes mellitus. Mol Breed 5:553–560

Prasanth K, Darshan S, Gangadhara K (2014) Utilization of male sterility for hybrid seed production in vegetables. Trends Biosci 7(17):2370–2375

Kalila P (2004) Root vegetable crops. J New Seeds Innov Prod Biotechnol Qual Market 6(2/3):266–267

Punja ZK (2005) Transgenic carrots expressing a thaumatin like protein display enhanced resistance to several fungal pathogens. Can J Plant Pathol 27:291–296

Purewal SS (1957) Vegetable cultivation in Northern India. Tech Bull 39, ICAR, New Delhi

Renard M, Delourme R, Vallee P, Morice J, Pierre J, Pelletier G, Budar F, Primard C, Bonhomme S, Grelon M, Hunzinger J, Darrozes G, Defossez H, Hunzinger J (1995) New concepts in rapeseed F1 hybrid breeding. In: Proceedings of 9th international rapeseed congress, Cambridge, A-7

Rosales-Mendoza S, Soria-Guerra RE, Lopez-Revilla R, Moreno-Fierros L, Alpuche-Solıs AG (2008) Ingestion of transgenic carrots expressing the Escherichia coli heat-labile enterotoxin B subunit protects mice against cholera toxin challenge. Plant Cell Rep 27:79–84

Sabir AA, Ford-Lloyd BV (1991) Processing crop plant germplasm in vitro for mass production of regenerants: a case study with beet. J Biotechnol 17:257
Scheike R, Gerold E, Brennicke A, Mehring-Lemper M, Wricke G (1992) Unique patterns of mitochondrial genes, transcripts and proteins in different male sterile cytoplasms of Daucus carota. Theor Appl Genet 83:419–427
Schulz B, Westphal L, Wricke G (1994) Linkage groups of isozyme, RFLP and RAPD markers in carrot (Daucus carota L. sativus). Euphytica 74:67–76
Schuphan W (1942) Biochcmische Sortenpriifungan Gartenmohrenalsneuzeitliche Grundlagefiirplanvollc Ziichtungsarbeiten. Ziichtcr 14:25–43
Scott RJ, Draper J (1987) Transformation of carrot tissue derived from proembyonic suspension cells: a useful model system for gene expression in plants. Plant Mol Biol 8:265–274
Scott SE, Wilkinson MJ (1998) Transgene risk is low. Nature 393(320):320
Simon PW (1984) Carrot genetics. Plant Mol Biol Report 2:54–63
Simon PW (1990) Carrot and other horticultural crops as a source of provitamin A carotenes. Hortic Sci 25:1495–1499
Simon PW, Wolff XY (1987) Carotenes in typical and dark orange carrots. J Agric Food Chem 35:1017–1022
Simon PW, Peterson CE, Lindsay RC (1980a) Genetic and environmental influences on carrot flavor. J Am Soc Hortic Sci 105:416–420
Simon PW, Peterson CE, Lindsay RC (1980b) Correlation between sensory and objective parameters of carrot flavor. J Agric Food Chem 28:559–562
Simon PW, Peterson CE, Lindsay RC (1982) Genotype, soil, and climate effects on sensory and objective components of carrot flavor. J Am Soc Hortic Sci 107:644–648
Simon PW, Rubatzky VE, Peterson CE, Kammerlohr DS (1989) High carotene mass carrot population. Hortic Sci 24:174–175
Sinskaia EN (1928) The oleiferous plants and root crops of the family cruciferae. Bull Appl Bot Genet Plant Breed 19(3):648
Snow AA, Jorgensen RB (1999) Fitness costs associated with transgenic glufosinate tolerance introgressed from Brassica napus subsp. Oleifera (oilseed rape) into weedy Brassica rapa. In: Lutmann PJW (ed) Gene flow and agriculture: relevance for transgenic crops, British crop protection council symposium proceedings, vol 72, pp 137–142
Snow AA, Uthus KL, Culley TM (2001) Fitness of hybrids between weedy and cultivated radish: implications for weed evolution. Ecol Appl 11(3):934–943
Solieman TH, Abdel-Razzak HS, Doss MM, El-Gazzar AR (2012) Efficiency of mass selection and selfing with selection breeding methods on improving some important characters of three eggplant cultivars. Am Eurasian J Agric Environ Sci 12(3):342–351
Song GK et al (1995) Rapid genome change in synthetic polyploids of Brassica and its implications for polyploid evolution. Can J Plant Sci 83(2):271–274. Proceedings of the National Academy of Science, turnip rape
Sota F, Yoko S (2019) Molecular identification of the causal locus for the petaloid phenotype in Daucus carota. Breed Sci 69:186–188
Stein M (1979) Untersuchungenzu Heterosiseffektenbeiwichtigen Inhaltsstoffen von Gemiise. Tag-Ber, Akad Landwirtsch Wiss. DDR, Berhn 168, pp 401–409
Stein M, Nothangel TH (1995) Some remarks on carrot breeding (Daucus carota sativus Hoffm.). Plant Breed 114:1–11. 179-9541/95/1401-0001
Steinborn R, Weihe A, Boerner T (1992) Mitochondrial genome diversity within a cultivar of Daucus carota (ssp. sativus) revealed by restriction fragment analysis of single plants. Plant Breed 109:75–77
Stommel JR, Simon PW (1989) Phenotypic recurrent selection and heritability estimates for total dissolved solids and sugar type in carrot. J Am Soc Hortic Sci 114:695–699
Swarup V (1991) Breeding procedure for cross pollinated vegetable crops. ICAR, New Delhi, p 118
Takagi H, Abe A, Yoshida K, Kosugi S, Natsume S, Mitsuoka C, Terauchi R (2013) QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. Plant J 74:174–183

Takaichi M, Oeda K (2000) Transgenic carrots with enhanced resistance against two major pathogens, Erysiphe heraclei and Alternaria dauci. Plant Sci 153:135–144
Tepfer D (1984) Transformation of several species of higher plants by Agrobacterium rhizogenes: sexual transmission of transformed genotypes and phenotypes. Cell 37:959–967
Thomas JC, Guiltinan MJ, Bustos S, Thomas T, Nessler C (1989) Carrot (Daucus carota) hypocotyl transformation using Agrobacterium tumefaciens. Plant Cell Rep 8:354–357
Thompson DJ (1961) Studies on the inheritance of male-sterility in the carrot (Daucus carota L. var. sativa). Proc Am Soc Hortic Sci 78:332–338
Tigelaar H, Stuiver MH, Molendijk L, Troost-van Deventer E, Sela-Buurlage MB, Storms J, Plooster J, Sijbolts F, Custers J, Apotheker de Groot M, Melchers LS (1996) Broad spectrum fungal resistance in transgenic carrot plants. Phytopathology 86:57
Timin NI, Vasilevsky VA (1997) Genetic peculiarities of carrot (Daucus carota L.). J Appl Genet 38A:232–236
UPOV (1976) Guidelines for the conduct of tests for distinctness, homogeneity and stability. International Union for Protection of new varieties of plant, carrot (Daucus carota L). TG/49/3
Vogel G (1996) Handbuch des speziellen Gemusebaus. Ulmer Verlag, Stuttgart
Wang LJ, Ni DA, Chen YN, Lee ZM (2001) The expression of Mycobacterium tuberculosis MPT64 protein in transgenic carrots. Acta Bot Sin 43:132–137
Wang F, Guangyuan L, Shuangchen C, Yan J, Wang S (2015) Characterization of a new leaf-compound radish mutant (Raphanus sativus L.). J Appl Hortic 17(2):115–120
Watson JF, Gabelman WH (1984) Genetic analysis of betacyanine, betaxanthine and sucrose concentrations in roots of table beet. J Am Soc Hortic Sci 109:386
Watts L (1980) Flower and vegetable plant breeding. Grower Books, London, p 179
Welch JE, Grimball EL (1947) Male sterility in the carrots. Science 106:594. https://doi.org/10.1126/science.106.2763.594
Westphal L, Wricke G (1991) Genetic and linkage analysis of isozyme loci in Daucus carota L. Euphytica 56:259–267
Wolyn DJ, Chahal A (1998) Nuclear and cytoplasmic interactions for petaloid male-sterile accessions of wild carrot (Daucus carota L.). J Am Soc Hortic Sci 123(5):849–835
Wolyn DJ, Gabelman WH (1989) Inheritance of root and petiole pigmentation in red table beet. J Hered 80:33
Wurtele ES, Bulka K (1989) A simple, efficient method for the Agrobacterium-mediated transformation of carrot callus cells. Plant Sci 61:253–262
Yi G, Park H, Kim JS, Chae WB, Park S, Huh JH (2014) Identification of three FLOWERING LOCUS C genes responsible for vernalization response in radish (Raphanus sativus L.). Hortic Environ Biotechnol 55:548–556
Yu Hee J, Seunghoon B, Joon LY, Ara C, Hwan MJ (2019) The radish genome database (Radish GD): an integrated information resource for radish genomics. Database 2019:1–10. https://doi.org/10.1093/database/baz009
Ziemborska JM, Harney PM (1986) Self-incompatibility in H2 generations derived from anther culture of hybrids between self-incompatible rape and self-incompatible rutabaga. In: Cruciferae genetics workshop III proceedings, 29–30 May, University of Guelph

Chapter 8
Rapid Methods of Improvement in Brinjal

A. S. Dhatt and M. K. Sidhu

8.1 Introduction

Brinjal (*Solanum melongena* L.), also known as eggplant, aubergine or guinea squash, is an important solanaceous crop of tropical and subtropical regions. It is cultivated in India, China, Japan, Indonesia, Bulgaria, Italy, France, the USA and several African countries. In Indian history, the first record of its occurrence published in *Origin of Cultivated Plants* indicated its origin in Asia (De Candolle 1886), which was further specified as Indo-Burma region (Vavilov 1951). Its origin in Africa and then domestication in Asia have also been narrated (Bhaduri 1951; Zeven and Zhukovsky 1975; Weese and Bohs 2010). Brinjal is cultivated on 18.67 lakh ha area with 497.82 lakh MT production and 26.7 MT ha^{-1} productivity in the world. India is the second largest producer of brinjal next to China and produces 135.58 lakh tonnes from 7.11 lakh ha area with 19.06 MT ha^{-1} productivity. Cultivation of brinjal is distributed in almost all states of the country, but maximum area is in West Bengal (1.61 lakh ha), followed by Odisha (1.25 lakh ha), Gujarat (0.76 lakh ha) and Bihar 0.58 lakh ha). The production share of brinjal is 8.3% of the total vegetable crops in the country. The extreme genetic variability in cultivated and wild forms made it declared as the 'National Vegetable' during the 'First Vegetable Science Congress' at Jodhpur during February 2019.

The genetic improvement of brinjal is carried out using conventional and biotechnological approaches. It is often cross-pollinated, where all principles and procedures applicable to the self-pollinated crops are followed for improvement. So far, introduction, selection and hybridization approaches are commonly followed with the objectives of high yield, but consumer preference for shape, size, colour and resistance to insect pests and diseases are also given importance. Purple and dark

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purple fruits are preferred in NorthWest India; green and variegated are popular in South India; and white are liked in the East India. Similarly, round and big fruits are used for *bharta*, small-round for stuffing and long in mix vegetables. Therefore, a large number of cultivars differing in size, shape and colour of fruits are grown in the country (Fig. 8.1). Wild species are source of resistance to insect pests and diseases as well as reserve of many quality compounds. Out of the 38 Asian species, 22 are available in India (Choudhury 1976). Species of primary gene pool like *S. melongena*, *S. incanum*, *S. xanthocarpum*, *S. indicum* and *S. maccani* are crossable and can be used for introgression of desirable alleles (San Jose et al. 2016; Kaushik et al.

Fig. 8.1 Genetic diversity in cultivated brinjal

2017). Traditionally, the principal method of crop improvement was selection from limited indigenous material, and with course of time, germplasm collection, evaluation, hybridization, mutation and biotechnological tools had been adopted (Isouard et al. 1979; Collonnier et al. 2001; Ansari et al. 2011; Kumchai et al. 2013). At the start of the twenty-first century, the genetic transformation against brinjal fruit and shoot borer gained a momentum (Kumar et al. 1998a). Brinjal is a widely adapted vegetable crop; therefore, various approaches followed for rapid improvement have been compiled in this chapter.

8.2 Germplasm Characterization and Utilization

Germplasm of *Solanum melongena* and allied species is conserved mainly ex situ in gene banks of AVRDC-the World Vegetable Centre, Taiwan; the Chinese Academy of Agricultural Sciences, China; National Bureau of Plant Genetic Resources (NBPGR), India; National Institute of Agrobiological Resources (NIAR), Japan; United States Department of Agriculture (USDA), USA; National Institute of Agricultural Research (INRA), France; Nijmegen Botanical Garden (NBG), the Netherlands and University of Birmingham, United Kingdom. NBPGR is conserving >2500, AVRDC >3,200, NIAR >400, USDA >920 and NBA >987 accessions of brinjal and its wild relatives (Mao et al. 2008; Taher et al. 2017). A more comprehensive database of eggplant-related germplasm holdings in Europe compiled by the EGGNET is about 6,000 accessions (Daunay et al. 2003).

Wild species are a good source of insect pest, disease and abiotic stress resistance (Stern and Bohs 2010; Chiarini and Mentz 2012; Vorontsova and Knapp 2012; Stern 2014; Sarkinen et al. 2015a, b, Sarkinen and Knapp 2016) and are given in Table 8.1.

Morphological and molecular characterization of brinjal germplasm has displayed wide genetic and regional dissimilarities for vegetative, flowering, fruiting and quality traits (Dhatt et al. 2017). Variation in chemical composition of species is useful for nutritional in brinjal. *Solanum aethiopicum* (scarlet) had lower level of carbohydrates (3.60 vs 6.48 g/100 g), starch (3.18 vs 6.15 g/100 g), vitamin C (11.6 vs 18.9 mg/100 g) and total phenolics (24.4 vs 144 mg/100 g), but higher values of TSS and ascorbic acid than *Solanum macrocarpon* (gboma). However, interspecific hybrids between scarlet and common eggplant displayed lower moisture content (79.0 g/100 g) and pH (5.15) values (San Jose et al. 2016). *S. insanum* is found to be a predominant source of chlorogenic acid (>65.0%). Hybrids between *S. melongena* × *S. insanum* are found intermediate in expression for phenolics and other traits (Kaushik et al. 2017). Anti-cancerous and antiparasitic glycoalkaloids, solamargine and solasonine are present in *S. melongena*, *S. aethiopicum* and *S. macrocarpon* (Kuo et al. 2000; Hall et al. 2006; Shiu et al. 2007; Mennella et al. 2010; Sanchez-Mata et al. 2010). Its high levels in *S. macrocarpon*, *S. sodomaeum*, *S. aethiopicum* and *S. integrifolium* may also cause toxicity.

Table 8.1 Characterization of wild species against insect pest and diseases of brinjal

Wild species	Resistant against insect pest/disease/nematode
Solanum integrifolium	Shoot and fruit borer, two-spotted spider mite, *Fusarium* wilt, *Phomopsis* blight, bacterial wilt
Solanum sisymbriifolium	Shoot and fruit borer, *Aphis gossypii*, *Fusarium* wilt, *Verticillium* wilt, *Meloidogyne incognita*, *Meloidogyne arenaria*, *Meloidogyne javanica*, *Meloidogyne hapla*
Solanum xanthocarpum	Shoot and fruit borer
Solanum khasianum	Shoot and fruit borer, *Meloidogyne incognita*
Solanum hispidum	Shoot and fruit borer, phytoplasma-little leaf, eggplant mosaic virus
Solanum macrocarpon	White fly, two-spotted spider mite, *Cercospora solani*
Solanum mammosum	*Aphis gossypii*
Solanum pinnatisectum	Colorado potato beetle
Solanum polyadenium	Colorado potato beetle
Solanum jamesii	Colorado potato beetle
Solanum berthaultii	Colorado potato beetle
Solanum chompatophilum	Colorado potato beetle
Solanum tffidum	Colorado potato beetle
Solanum capsici-baccatum	Colorado potato beetle
Solanum tarijense	Colorado potato beetle
Solanum chacoense	Colorado potato beetle
Solanum indicum	*Fusarium* wilt
Solanum incanum	*Fusarium* wilt, drought tolerance
Solanum torvum	*Verticillium* wilt, bacterial wilt, *Meloidogyne incognita*, *Meloidogyne arenaria, Meloidogyne javanica*
Solanum caripense	*Verticillium* wilt
Solanum persicum	*Verticillium* wilt
Solanum scabrum	*Verticillium* wilt
Solanum sodomaeum	*Verticillium* wilt, drought and salt resistance
Solanum gilo	*Phomopsis* blight
Solanum aethiopicum	*Meloidogyne incognita*, *Fusarium* wilt

8.3 Exploitation of Wild Species

Introgression of desirable traits from wild relatives has great potential, but crossability barriers and undesirable genetic drag are the limitations with the most of the species. Cultivated *S. melongena* found crossable with *S. insanum*, *S. incanum*, *S. integrifolium*, *S. gilo and S. macrocarpon*, but not with *S. indicum*, *S. zuccagnianum* and *S. sisymbriifolium* (Rao and Kumar 1980; Schaff et al. 1982). Nishio et al. (1984) crossed 11 wild species and made three groups based upon compatibility. The first group comprised of *S. melongena*, *S. incanum* and *S. macrocarpon*; the second of *S. integrifolium*, *S. gilo* and *S. nodiflorum*; and the third of *S. indicum*,

S. mammosum, *S. torvum*, *S. sisymbriifolium*, and *S. toxicarium*. Crosses were compatible within and between the first and second groups, but otherwise were incompatible. First backcross generations of interspecific hybrids of one primary gene pool species *S. insanum* and secondary gene pool species *S. lichtensteinii*, *S. dasyphyllum*, *S. anguivi*, *S. incanum* and *S. tomentosum* with cultivated *S. melongena* showed varying degree of success (Kouassi et al. 2016). Three new species, *S. lidii*, *S. vespertilio* and *S. campylacanthum* from secondary gene pool were also crossable with cultivated species. However, crosses of *S. elaeagnifolium* and *S. sisymbriifolium* produced parthenocarpic fruits with aborted seeds. The success of crosses between primary and secondary gene pool to get fertile and viable seed relied on direction of crosses and type of accessions used in the hybridization (Plazas et al. 2016; Afful et al. 2018). *Solanum torvum* is highly resistant to nematodes and wilt, but have the problem of cross-incompatibility and infertility with *S. melongena*. Use of *S. melongena* X *S. torvum* hybrid as male parent in backcrossing with cultivated species produced the viable seed (Sidhu and Dhatt 2019), and progenies with varying degrees of resistance to nematodes have been developed (Fig. 8.2; Dhatt and Sidhu, unpublished data). Undertaking backcross approach, resistance for *Fusarium oxysporum* from *S. aethiopicum* (Toppino et al. 2008); *Verticillium dahliae* from

Fig. 8.2 (1) *S. aethiopicum*, (2) *S. dasyphyllum*, (3) *S. xanthocarpum*, (4) *S. lichtensteineii*, (5) *S. sisymbriifolium*, (6) *S. grandiflorum*, (7) *S. insanum*, (8) *S. torvum*, (9) F_1 *S. melongena x S. torvum*, (10) segregants from three-way cross of *S. melongena x S. torvum*, (11) segregant from three-way cross of *S. melongena x S. insanum* (12) F_1 *S. melongena x S. lichtenteineii*, (13) F_1 *S. melongena x S. sisymbriifolium*

S. linnaeanum, *S. aethiopicum* and *S. incanum* (Prohens et al. 2012, 2013; Liu et al. 2015); draught tolerance from *S. incanum*; *Verticillium* wilt and salt tolerance from *Solanum sodomaeum* were introgressed (Toppino et al. 2009; Gramazio et al. 2017). *Solanum torvum* is highly resistant to nematodes and wilt but have the problem of cross-incompatibility and sterility with *S. melongena (sentence repeated)*. Alloplasmic male-sterile and fertility restorer lines representing the cytoplasm of wild relatives *S. anguivi*, *S. kurzii*, *S. virginianum*, *S. violaceum*, *S. aethiopicum* and *S. grandiflorum* have been developed (Saito et al. 2009a; Khan and Isshiki 2010; Khan et al. 2015; Garcha and Dhatt 2017) for utilization in the heterosis breeding.

To overcome the crossability barriers, biotechnological approaches can play a significant role to accelerate the improvement programmes in brinjal. Using embryo rescue approach, F_1 hybrids were obtained with tertiary gene pool species *S. sisymbriifolium*, but these hybrids were sterile. However, chromosome doubling with colchicine developed fertile amphidiploids (Khan et al. 2013a). Unlike tomato and potato, most of the interspecific crosses between secondary and tertiary gene pool remained limited to F_1 generation in brinjal (Rotino et al. 2014; Plazas et al. 2016). Gene designated as *SacMi* providing resistance to *Meloidogyne incognita* has been cloned and characterized from *S. aculeatissimum* (Zhou et al. 2018).

8.4 Development of Molecular Markers and Their Use

For accelerating the crop improvement programmes gene-based markers were required in brinjal. Initially, the already published DNA sequences were used to develop markers through in silico analysis (Stagel et al. 2008; Nunome et al. 2009). Later the construction of genomic libraries on the basis of whole genome sequences was used for development of different types of markers and its detail has been given in Table 8.2. Furthermore, the data generated from different sequences was compiled as “Eggplant Microsatellite DataBase” (EgMiDB) that allowed the location-specific identification of SSR markers on brinjal genome and simultaneously provided detailed information about the markers generated (Portis et al. 2018).

The foremost linkage map was developed in 1998 and continuously improved by using different types of markers from different mapping populations as given in Table 8.3:

8.4.1 Use of Molecular Markers

Molecular markers revealed that solanaceous vegetables evolved from a common ancestor. Comparison of genes of four solanaceous plants including brinjal revealed 9,489 brinjal scaffolds similar to tomato and conservation of 56 synteny blocks between these two crops (Fukuoka et al. 2012; Hirakawa et al. 2014). It endorsed the identification of potential candidate genes (QTLs) responsible for different traits

Table 8.2 Genome-based studies for marker development in brinjal

Aim of the study	Type of species	Plant and genetic material	Raw reads (M)	Coverage	Final assembly	Identified markers or genes	NCBI accession	References
Generation of RAD tag sequences	*S. melongena*	DNA leaves	10.9 m (P1) 12.12 m (p2)	77,876	45,390 sequences	10,089 SNPs 1600 Indels 1800 SSRs	–	Barchi et al. (2011)
Identification of miRNA	*S. melongena*	RNA of seedlings	30	–	5,940 miRNA	–	SRR833801 SRR833802	Yang et al. (2013)
Genome assembly	*S. melongena*	DNA of leaves	1,323	144.3X	33,873 scaffolds	4,536 SNPs 83,401 SSRs	DRR014074 DRR014075	Hirakawa et al. (2014)
Genome assembly	*S. melongena*	RNA of leaves, roots, fruits, flowers	1.4	0.48X				
Comparative transcriptome assembly for identification of resistant genes	*S. melongena*	RNA of leaves, root, stem	30	–	34,174 unigenes	621 resistant genes	SRR1104129	Yang et al. (2014)
	S. torvum	RNA of leaves, root, stem	54	–	38,185 unigenes/	815 resistant genes	SRR1104128	
Genome assembly	*S. melongena*	DNA of leaves	–	155X 35X 1X	12 pseudo molecules	–	–	Barchi et al. (2016)
Genome assembly	*S. melongena*	RNA of leaves	–	–	–	–	–	
Identification of putative allergens	*S. melongena*	RNA of fruit	89	–	149,224 transcripts	–	SRR1291243	Ramesh et al. (2016)

(continued)

Table 8.2 (continued)

Aim of the study	Type of species	Plant and genetic material	Raw reads (M)	Coverage	Final assembly	Identified markers or genes	NCBI accession	References
Whole transcriptome assembly	*S. aethiopicum*	RNA of leaf, floral bud, fruit	114	–	87,084 unigenes	164,127 SNVs 976 SSRs	SRR2229192	Gramazio et al. (2016)
Transcriptome assembly for whole genome	*S. incanum*	RNA of leaf, floral bud, fruit	105	–	83,905 unigenes	12,396 SNVs 1,248 SSRs	SRR2289250	
Transcriptome assembly for whole genome	*S. aculeatissimum*	RNA of root	28	–	69,824 unigenes	–	SRS1383901 SRS1383902	Zhou et al. (2016)
Identification of miRNA	*S. melongena*	RNA pistil	–	–	686 miRNAs	–	SRR3479276 SRR3479277	Wang (2017)
Transcriptomics for identification of parthenocarpic genes	*S. melongena*	RNA of flower buds	–	–	38,925 unigenes	258 up-regulated and 248 down-regulated genes	–	Chen et al. (2017)
Whole genome resequencing	*S. melongena* and *S. incanum*	DNA individual plantlet	1448	16.5 × 24.0×	10,916,466 high-quality variants	9,228,065 SNPs, 705, 687 Indels, 275, 467 MNPs, 707, 247 complex variations	SRR5796636 to SRR5796643	Gramazio et al. (2019)
Chromosome-anchored genome assembly for study of Solanaceae evolution	*S. melongena along with potato, tomato and capsicum*	DNA of leaf	–	1.16 Gb	34,916 protein-coding genes	667 gene families exclusive to brinjal	SRP078398	Barchi et al. (2019)
Selection trends in brinjal evolution through comparative transcriptomic genes	*S. sisymbriifolium*	RNA of leaf tissue	82.1	36.6 Mb	44,073	2734 to 6473 DEGs in different crosses	SRP127743	Wei et al. (2019)
	S. aethiopicum		61.8	43.8 Mb	51,519			
	S. integrifolium		74.8	40.5 Mb	45,838			
	S. melongena		65.8 to 75.5	44.8 Mb	58,677			

Table 8.3 History of linkage map development in brinjal

Mapping population	Map size	Linkage groups	Markers used	Average marker distance	References
Intra-specific F_2	716.7 cM	13	94 RAPD markers	8.8 cM	Nunome et al. (1998)
Intra-specific F_2	779.2 cM	21	88 RAPD and 93 AFLP	4.9 cM	Nunome et al. (2001)
Inter-specific F_2	1480 cM	12	233 RFLP tomato markers	7.6 cM	Doganlar et al. (2002)
Inter-specific F_2	716 cM	17	101 RAPDs, 54 AFLPs 7 SSRs	4.9 cM	Nunome et al. (2003)
Inter-specific F_2	736 cM	12	117 RAPD and 156 AFLP	2.7 cM	Sunseri et al. (2003)
Inter-specific F_2	1535 cM	12	110 COSII + 179 tomato-derived RFLPs	6.1 cM	Wu et al. (2009)
Intra-specific F_2	959.1 cM	14	236 SSR	4.3 cM	Nunome et al. (2009)
Inter-specific F_2	718.7 cM	12	212 AFLPs + 22 SSRs + 1 RFLP + 3 CAPS	3.0 cM	Barchi et al. (2010)
Intra-specific F_2	1285.5 cM	12	313 genomic SSR + 623 SNP and Indels	1.4 cM	Fukuoka et al. (2012)
Intra-specific F_2	884 cM	18	91 AFLPs, 26 SSRs 2 SRAPs	8.8 cM	Lebeau et al. (2013)
Inter-specific F_2	1518 cM	12	400 AFLPs, 348 RFLPs, 116 COSII	1.8	Doganlar et al. (2014)
Inter-specific F_2	1085 cM	12	88 AFLPs, 99 SSRs, 42 COSII, 9 CAPs, 4 SNP	1.4	Gramazio et al. (2014)
Intra-specific F_2	1280 cM	12	574 SNPs, 221 SSR	0.7	Hirakawa et al. (2014)

in solanaceous crops (Frary et al. 2003; Barchi et al. 2012; Hirakawa et al. 2014). It is imperative to understand the genetic diversity in the available germplasm for introgression of desirable traits for improvement of brinjal. Extensive work on development of molecular markers using cultivated and wild species can be useful to determine the relationships and their further use (Behera et al. 2006; Demir et al. 2010; Sunseri et al. 2010; Adeniji et al. 2012; Mahmoud and El-Mansy 2012; Boyaci et al. 2015).

Availability of SCAR and SSR marker for dark purple pigmentation of fruit peel can be used in MAS for dark coloured genotypes at seedling stage of brinjal (Bhanushree et al. 2018). For genomic-assisted breeding of parthenocarpic trait, one AFLP (smpc77) and DEGs have been reported (Shimomura et al. 2010; Chen et al. 2017). MAS can be employed in resistant breeding programmes for *Verticillium* wilt (Sunseri et al. 2003), *Fusarium* wilt (Mutlu et al. 2008; Toppino et al. 2008), bacterial wilt (Cao et al. 2009; Lebeau et al. 2013) and *Phomopsis* blight (Banik 2017). Hybridity of parents can also be assessed using molecular markers in brinjal (Mangal et al. 2016; Plazas et al. 2016). Molecular markers also make easy to understand the genetics of quantitative traits through comprehensive linkage maps. Fruit shape of brinjal is polygenic and was detected on LG 12 of brinjal (Lincoln

et al. 1992; Nunome et al. 1998, 2001). QTLs for fruit, stem and calyx tissue pigmentation were found on four linkage groups, with 68% of phenotypic variation in the traits on LG 7 (Nunome et al. 2001). QTL hot spot for plant hairiness was found in Chr 3, for prickliness in Chr 6 and for pigmentation in Chr 10 (Frary et al. 2014). Fruit-related QTLs were found to be concentred within specific chromosomal areas, which reflected involvement of either linkage or pleiotropy or both (Portis et al. 2014). Candidate genes for polyphenol oxidase activity (*PPO1*, *PPO2*, *PPO3*, *PPO4*, *PPO5*) were found near to the genes responsible for chlorogenic acid synthesis (*PAL*, *C4H*, *C3′H*, *HQT*) and QTLs for two domestication traits, fruit shape (*OVATE*, *SISUN1*) and prickliness (Gramazio et al. 2014).

8.5 Development of Haploids

Doubled haploid (DH) approach can reduce the time span for development of true breeding lines. Plants having single set (gametophytic) of chromosome in their sporophyte are termed as haploids. Spontaneous or induced duplication of haploid chromosome resulted in doubled haploid (DH) plant. The first landmark observation on spontaneous haploid was in *Datura stramonium* L. (Blackslee et al. 1922), which followed the use of anther culture in *Datura innoxia* (Guha and Maheshwari 1964), and wide crossing in barley and tobacco (Kasha and Kao 1970; Burk et al. 1979) for haploid generation. Androgenesis is found highly successful in Solanaceae, Gramineae and Brassicaceae families, but not in all the angiosperms (Forster et al. 2007). Haploids offered the advantages of generating complete homozygous lines from a highly heterozygous parent in a single step. The genotypes with complete homozygosity have increased heritability of quantitative characters, which further enhanced the selection efficiency (Chen et al. 2010). The presence of a single set of chromosomes eases the isolation of mutants with recessive gene also. Doubled haploids can increase the efficiency of genome mapping by generating valuable information regarding the location of genes and QTLs that regulate economically important traits (Winzeler et al. 1987; Khush and Virmani 1996).

Brinjal is highly responsive to androgenesis and the first anther culture was reported by Raina and Iyer in 1973, but the first haploids were obtained by the Chinese Research Group of Haploid Breeding in 1978. Later many researchers reported successful induction of haploids and doubled haploids in brinjal (Isouard et al. 1979; Dumas de Vaulx and Chambonnet 1982; Yadava et al. 1989; Rotino 1996; Rotino et al. 2005). Although microspore culture is considered the best for haploid induction, its response varies with the genotype, growing conditions, season, temperature, light intensity, age of plant, irrigation, fertilization, chemicals used for plant protection, development stage of microspore, pretreatment of flower buds or anthers, type and composition of culture medium and cultural conditions in eggplant (Rotino et al. 2005; Khatun et al. 2006; Segui-Simarro et al. 2011; Corral-Martinez and Segui-Simarro 2014; Rivas-Sendra et al. 2015). The healthier the donor plant is grown, the greater the chance of success in obtaining haploid plants.

Plantlets obtained from uninucleate microspore from early bud stage were found to be mostly haploids (Vural et al. 2019). Anthers taken from short- or long-styled flowers did not show significant difference for production of androgenic embryos, and 4′-6-diamidino-2-phenylindole (DAPI) staining method is commonly used to determine the bud stage for the culture (Salas et al. 2012). The MS (Murashige and Skoog 1962), GD (Gresshoff and Doys 1972), B5, V3 and R medium containing various growth regulators like 2,4-D, BA, NAA and IAA are commonly used for callus induction and plant regeneration, but MS medium is considered the best in brinjal (Corral-Martınez and Segui-Simarro 2012). Embryogenic callus was initiated from the anthers on medium containing 2.0 mg/l 2,4-D and shoot from the callus on MS medium containing 0.2–0.5 mg/l NAA, which was further multiplied on 1.0 mg/l BAP with 0.2 mg/l IAA, elongated on hormone-free MS medium and rooted on half-strength MS medium (Kumar et al. 2003). Recently, regeneration process of DH plants from microspore-derived calli in brinjal suggested repeated subculture of the calli on MS + 0.2 mg/l IAA + 4 mg/l zeatin for production of shoots and roots of excised shoots on MS basal for their conversion into full plantlets. It increased the haploid efficiency to 7.6 plants/100 cultured calli with 70% regenerated DH plants (Rivas-Sendra et al. 2015). Pretreatment of cultured microspores with higher temperature shifted the same from gametophytic stage to sporophytic stage in this crop (Ratino 1996). Sucrose starvation in culture medium and high temperature (35 °C) treatment for 3 days resulted into spontaneous development of di-haploids along with haploid, triploids and tetraploid (Dumas de Vaulx and Chambonnet 1982; Miyoshi 1996). A modified protocol of tobacco microspore culture using mannitol, maltose and high temperature (33 °C) treatment can also be used (Vural et al. 2019). PEG significantly increased the induction efficiency of microspore embryogenesis in brinjal (Corral-Martınez and Segui-Simarro 2012). For chromosome doubling, haploid plants can be subjected either in vitro or in vivo to antimitotic chemicals such as colchicine or oryzalin or trifluralin. Lanolin treatment carrying 0.5–1% colchicine to axillary buds for 24–48 h in darkness resulted in 50–70% doubling. One to two hours incubation of micro shoots in 0.5 or 1% colchicine solution under in vitro conditions and one to two hours treatment of axillary buds through absorption of same concentration with wet cotton under in vivo conditions also created 100% di-haploid shoots in brinjal. However, in vitro treatment saved the time for growth and flowering of doubled haploids (Ellialtioglu et al. 2006; Rotino 2016).

8.6 Development and Use of Male Sterility

Male sterility relies on the non-functional male gametophyte. Genic male sterility is due to recessive nuclear genes while interactions of mitochondrial and nuclear genes lead to cytoplasmic male sterility in brinjal (Phatak et al. 1991). The involvement of open reading frame (ORF) from mitochondrial genes has been well documented for occurrence of CMS system (Hanson and Bentolila 2004). Further,

advancements revealed that structural and functional variations in *atp* and *cox* genes in mitochondria of wild *Solanum* species caused the male sterility (Yoshimi et al. 2013). On the other hand, *Rf* genes located in the nucleus play a vital role for restoring of fertility of the plant in the presence of male-sterile genes (Bentolila et al. 2002).

The male-sterile lines in brinjal were developed through interspecific hybridization with *S. gilo*, *S. kurzii*, *S. violaceum*, *S. virginianum*, *S. aethiopicum*, *S. anguivi* and *S. grandifolium* (Fang et al. 1985; Isshiki and Kawajiri 2002; Khan and Isshiki 2008, 2009, 2010, 2011, 2016; Saito et al. 2009a; Hasnunnahar et al. 2012a; Garcha and Dhatt 2017; Garcha et al. 2017). Male sterility is further categorised as anther indehiscent and pollen non-formation types. Anther indehiscent type of male sterility is developed from the cytoplasms of S. *violaceum*, *S. virginianum* and *S. kurzii* and pollen non-formation type from *S. aethiopicum*, *S. anguivi* and *S. grandifolium*. All male-sterile lines were female fertile and produce normal seed upon pollination with fertile counterpart (Hasnunnahar et al. 2012b). Male sterility is controlled by single recessive gene (*ms*), while fertility is restored by two different dominant (*Rf)* genes. The segregation pattern of male-sterile/male-fertile plants in F_2 generation was 3:1 (Hasnunnahar et al. 2012a; Khan et al. 2013b, 2014; Dhatt-unpublished data). At Punjab Agricultural University, Ludhiana, male-sterile lines were developed from interspecific hybridization with *S. aethiopicum* as female parent, while the fertility restorers were selected from the reciprocal crosses. Since brinjal has different fruit size, shape and colour segments, male-sterile gene was transferred in diverse genetic backgrounds through backcrossing. Scheme for development of male sterile and fertility restorer lines (Figs. 8.3 and 8.4) and salient characteristics of male-sterile and their resppective restorer lines of brinjal (Table 8.4) developed at PAU are given below:

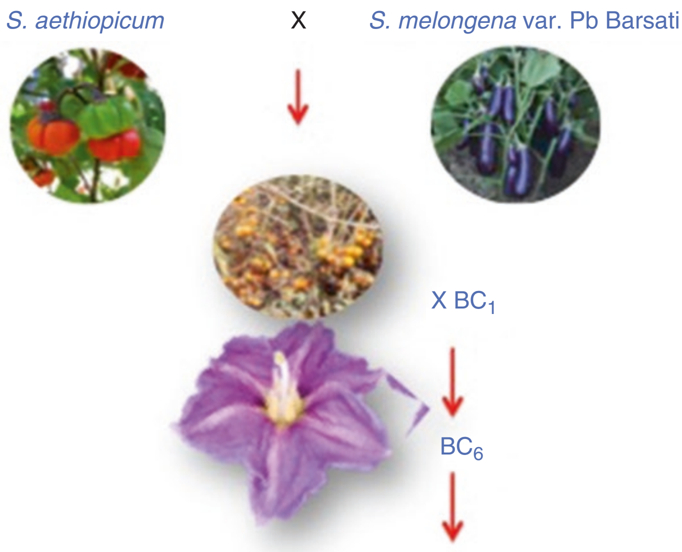

Fig. 8.3 Scheme for development of male-sterile lines (*ms*) in brinjal

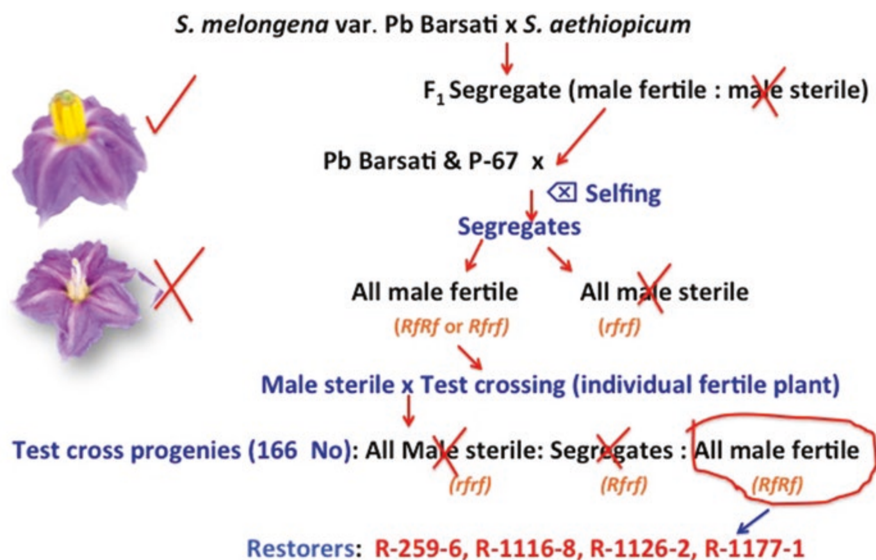

Fig. 8.4 Scheme for development of fertility restorer (*Rf*) lines in brinjal

Molecular marker tightly linked to *Rf* genes in fertility restorer plants has been found by Khan et al. (2014). This RAPD marker (OPAB10) was further converted into SCAR marker (SCAB101900) and has potential application in the selection of fertility restorer plants at seedling stage, simultaneously eliminating the development and screening of test cross progenies for the same. Its use would trigger the development of A (male-sterile), B (maintainer) and R (restorer) lines in diverse genetic backgrounds for commercial exploitation in heterosis breeding of brinjal.

8.7 Parthenocarpy

Parthenocarpy is the development of fruit without fertilization. Brinjal fruit produces large number of seeds (400–800) and has exerted stigma with apical dehiscence of pollen from the anthers. Therefore, parthenocarpy has significance in reducing the seed number and cultivation of brinjal under Net/Poly-houses. Genetic parthenocarpy was first observed in European cultivar ‘Talina’, which was used by Japanese for development of cultivar *Anominori* (Saito et al. 2009b). Later, more elaborative work for developing new parthenocarpic lines and testing their production potential was conducted (Kikuchi et al. 2008a, b; Saito et al. 2009b; Dhatt and Sidhu 2019). The Punjab Agricultural University, Ludhiana, has also developed new parthenocarpic lines, and PC-123 has been approved for cultivation under protected structures.

Table 8.4 Salient featuresof male sterile (A) and their respective maintainer (B) lines in brinjal

Trait/CMS line	D-CMS 104 A&B	D-CMS 67 A&B	D-CMS 201 A&B	D-CMS 213 A&B	D-CMS 214 A&B	D-CMS 219 A&B	D-CMS 5 A&B	D-CMS 7-2 A&B	D-CMS 291 A&B	D-CMS 93 A&B
Fruit colour	Violet blue	Purple	Black-purple	Purple	Purple	Black-purple	Black-purple	Black-purple	Purple	Purple
Fruit shape	Round	Oval	Long	Medium-long	Medium-long	Long	Small-round	Long	Small-oval	Small-oblong
Leaf colour	Grey-purple	Violet-blue	Green	Green	Green	Green	Green	Green	Green	Green
Fruiting pattern	Solitary	Cluster	Solitary	Solitary and double	Solitary and double	Cluster	Cluster	Solitary and double	Cluster	Cluster
Fruit Wt. (g)	190.4	72.3	88.6	62.8	65.2	63.5	40.4	65.5	43.4	35.8

Expression of parthenocarpic gene in brinjal during the fruit development stage under natural low-temperature conditions revealed involvement of two major genes expressing at 12.8 °C for occurrence of 100% parthenocarpy. The adverse low temperature with only 1.88–7.29% pollen germination did not affect the development of parthenocarpic fruit (Zhang et al. 2009). AFLP marker (smpc77) associated with parthenocarpy has also been identified in brinjal (Liu et al. 2008). The practical utility of this marker was confirmed with DH lines and has the potential to be used in breeding of parthenocarpic brinjal (Shimomura et al. 2010). The physiological basis revealed that higher concentrations of IAA and ABA at early stage enhanced the parthenocarpic fruit development, whereas GA_4 and ZR seemed to have no relation with seedless fruits (Li et al. 2012, 2013). The cDNA library constructed from parthenocarpic and non-parthenocarpic ovaries identified five ESTs (expressed sequence tags) related to parthenocarpy, out of which two ESTs were for MADS-box family, one as promoter auxin growth protein, one for enzyme cytochrome P450 NADPH-reductase and one for MAPKK family. It also highlighted that auxin synthesis in parthenocarpic ovaries is responsible for the development of fruits. Blast analysis of 139 differentially expressed genes and RT-PCR of 10 unigenes in parthenocarpic and non-parthenocarpic fruits partitioned two unigenes (Z569 and Z707) expressing for parthenocarpy under low-temperature conditions, (Zhou et al. 2010; Zhang et al. 2011). The transcriptomic profiles of natural parthenocarpic and non-parthenocarpic lines through RNA-sequencing technology revealed two upregulated genes, PBP1 (calcium-binding protein) and E2FB (transcription factor), involved respectively in auxin distribution and auxin-mediated cell division in parthenocarpic ovaries. Also the genes for gibberellin and cytokinin signalling as well as for flower development were differentially expressed between parthenocarpic and non-parthenocarpic lines. These up-regulated genes can further be used for identification of key genes involved in genomics-assisted breeding for parthenocarpy in brinjal (Chen et al. 2017).

8.8 Genetic Transformation

Many diseases and insect pests (*Fusarium* wilt, *Bacterial* wilt, *Phomopsis* blight, nematodes, shoot and fruit borer, whitefly, jassid, aphid, red spider mite and thrip) attack brinjal. Though resistant sources are available with wild relatives, cross-fertilization barrier and inadvertent linkage drag of undesirable genes are the limitations in introgression. We can overcome the cross-fertilization barriers through embryo rescue and somatic hybridization approach (Collonnier et al. 2001), but undesirable genetic drag is difficult to tackle. This issue can be solved with genetic transformation of targeted gene(s). Brinjal is highly amenable to in vitro regeneration and foreign DNA transfer (Kumar et al. 1998b; Kaur et al. 2015). The first successful transformation through *Agrobacterium tumefaciens* was reported in brinjal by Guri and Sink (1988). Most of the transgenics have been developed through *Agrobacterium* and a few through gene gun approach

Table 8.5 Gene introgression through genetic transformation

Character	Gene of interest	Mode of action	References
Shoot and fruit borer	*Cry2Aa*, Cry1Ab, *Cry1C*, *Cry1Ac*	Crystal proteins in insect gut	Kumar et al. (1998a) and Pal et al. (2009)
Colorado potato beetle	*Cry3B*, mutant version of *Cry3B*, *Cry3A*	Crystal proteins in insect gut	Arpaia et al. (1997) and Hamilton et al. (1997)
Myzus persicae (Sulzer) and *Macrosiphum euphorbiae* (Thomas)	*Oryzacystatin*	Increase mortality rate	Ribeiro et al. (2006)
Nematode	*Mi-1*, Mi-1.2 gene from tomato	Signalling the defensive pathways	Goggin et al. (2006)
Seedless fruits	*DefH9-iaaM*gene	Ovule and placenta-specific auxin production	Donzella et al. (2000), Rotino et al. (1997), and Acciarri et al. (2002)
Abiotic stresses	Mannitol-1-phophate dehydrogenase encoded by the *mtlD*gene	Improved morphological and physiological characteristics associated with tolerance	Prabhavati et al. (2002)

(Magioli and Mansur 2005; Sidhu et al. 2014). Although the roots, seedlings and hypocotyl can be used as explants for transformation, but cotyledonary leaves are found most effective due to their high regeneration ability (Fari et al. 1995; Blay and Oakes 1996; Franklin and Sita 2003; Sidhu et al. 2014). The introgression of targeted genes through genetic transformation has been given in Table 8.5.

Barnase gene expression in male gametophyte was used to induce male sterility in brinjal (Cao et al. 2010). Two sets of transgenic plants using *Cre* and *Barnase* gene were created through *Agrobacterium* transformation, separately with stable inheritance and expression. The *Barnase*-coding region was flanked by loxP recognition sites for *Cre*-recombinase. All the plants with *Barnase* gene were male-sterile with non-viable pollen and could be maintained with non-transformed plants. On the other side, *Cre* gene restores the fertility and helped in setting of fruits in F_1 plants. Plastid transformation method has also been tried in brinjal (Singh et al. 2010). The green stem segments were bombarded with pPRV111A plastid expression vector carrying aadA gene (aminoglycoside 300-adenylyltransferase). Out of the 525 bombarded explants, 2 transplastomic plants were confirmed with RT-PCR for expression of aadA gene. A successful attempt on development and field testing of transgenic brinjal carrying *Cry-1*Ac gene was made by Monsanto. However, release of these hybrids was not matured due to restrictions by the Genetically Engineered Approval Committee of India (Shelton 2010). Bangladesh became the first country to release *Bt* brinjal in the world, where fruit infestation in *Bt* brinjal was ranged between 0.0% and 2.27% as compared to 36.70–45.51% in non-transgenic brinjal (Prodhan et al. 2018). The genetic transformation of a single gene for insect resistance gave boost to brinjal cultivation by offering high returns to the farmers and pesticide-free brinjal to the consumers.

8.9 Future Prospects

Though yield remains a major focus in breeding programmes, attention on development of resistant stocks to biotic, abiotic and nutraceutical properties is also needed in brinjal. Resistant sources for biotic and abiotic stresses, for nutraceutical properties (anthocyanins and phenolics) and medicinal compounds (glycoalkaloids) are available in cultivated and wild species. Development of linked or gene-based markers for different traits of economic importance like male sterility, parthenocarpy, salinity tolerance, and resistance to bacterial wilt, virus, nematode, mite and jassid can further speed up the crop improvement programme. Transcriptomic sequencing of brinjal will facilitate comparison of parents, species and related crops and will help in intensifying the genomic resources. Intensification of genetic maps will help in locating the position of qualitative and quantitative traits. Focus on haploidy breeding will speed up the development of homozygous lines for use in genetic and genomic studies as well as heterosis breeding. Genetic transformation, cloning and characterization of major genes from wild species and other sources for resistance against insect pest and abiotic stresses and their site-specific expression will open new dimensions in transgenic research. The use of CRISPR/Cas genome editing tools offers great potential for targeted modifications of genes for valuable traits in commercial varieties of brinjal.

In conclusion, brinjal is native to India and has great natural reserve of genetic diversity in cultivated and wild forms. The wild relatives possess resistance against many biotic and abiotic stresses as well as carry high nutraceutical values. Hence, available genetic wealth needs attention for exploitation through conventional and biotechnological means. Though genomic studies on genetic diversity, gene introgressions, comparative mapping, gene tagging and marker-assisted selection gave impetus, their utilization for trait-specific selection in breeding programmes is required. Haploidy breeding can be helpful in development of homozygous inbred lines in shortest possible time. Their use in genomic studies will unfold new possibilities of understanding *Solanum* species and expedite the breeding programme of brinjal. Genetic tools like male sterility and parthenocarpy demand attention for improvement of yield and quality for diverse conditions of cultivation. Among insects, brinjal shoot and fruit borer is a cause of concern for indiscriminate use of pesticides and demands attention for introgression of resistant gene(s) from wild relatives and development of transgenics.

References

Acciarri N, Restaino F, Vitelli G, Perrone D, Zottini M, Pandolfini T, Spena A, Rotino GL (2002) Genetically modified parthenocarpic eggplants: improved fruit productivity under both greenhouse and open field cultivation. BMC Biotech 2(1) https://doi.org/10.1186/1472-6750-2-4

Adeniji OT, Kusolwa P, Reuben SOWM, Deo P (2012) Molecular diversity among seven Solanum (eggplant and relatives) species assessed by simple sequence repeats (SSRs) markers. African J Biotech 11(90):15643–15653

Afful NT, Nyadanu D 1, Akromah R 1, Amoatey HM, Annor C, Diawouh RG (2018) Evaluation of crossability studies between selected eggplant accessions with wild relatives *S. torvum*, *S. anguivi* and *S. aethopicum* (Shum group). J Plant Breed Crop Sci 10(1): 1–12

Ansari SF, Mehta N, Ansari S, Gavel JP (2011) Variability studies in brinjal (*Solanum melongena* L.) in Chhattisgarh plains. Electron J Plant Breed 2(2):275–281

Arpaia S, Mennella G, Onofaro V, Perri E, Sunseri F, Rotino GL (1997) Production of transgenic eggplant (*Solanum melongena* L.) resistant to Colorado potato beetle (*Leptinotarsa decemlineata* say). Theor Appl Genet 95:329–334

Banik S (2017) Gene tagging through association mapping for *Phomopsis* blight resistance in eggplant (*Solanum melongena L.*). Ph.D. dissertation, IARI, New Delhi

Barchi L, Lanteri S, Portis E, Stagel A, Vale G, Toppino L, Rotino GL (2010) Segregation distortion and linkage analysis in eggplant (*Solanum melongena* L.). Genome 53:805–815

Barchi L, Lanteri S, Portis E, Acquadro A, Vale G, Toppino L, Rotino GL (2011) Identification of SNP and SSR markers in eggplant using RAD tag sequencing. BMC Genomics 12:304. http://www.biomedcentral.com/1471-2164/12/304

Barchi L, Lanteri S, Portis E, Vale G, Volante A, Pulcini L, Ciriaci T, Acciarri N, Barbierato V, Toppino L, Rotino GL (2012) A RAD tag derived marker based eggplant linkage map and the location of QTLs determining anthocyanin pigmentation. PLoS One 7(8):e43740. https://doi.org/10.1371/journal.pone.0043740

Barchi L, Delledonne M, Lanteri S, Dal Molin A, Minio A, Ferrarini A, Rotino GL (2016) A high quality eggplant genome sequence: a new tool for the analysis of the Solanaceae family evolution and for the molecular deciphering of complex traits. In: Kölliker R, Boller B (eds) Plant breeding: the art of bringing science to life 20th Eucarpia general congress, Zurich, p 122

Barchi L, Pietrella M, Venturini L, Minio A, Toppino L, Acquadro A et al (2019) A chromosome-anchored eggplant genome sequence reveals key events in Solanaceae evolution. Sci Rep 9:11769. https://doi.org/10.1038/s41598-019-47985-w

Behera TK, Sharma P, Singh BK, Kumar G, Kumar R, Mohapatra T, Singh NK (2006) Assessment of genetic diversity and species relationships in eggplant (*Solanum melongena* L.) using STMS markers. Sci Hortic 107(4):352–357

Bentolila S, Alfonso AA, Hanson MR (2002) A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants. Proc Natl Acad Sci USA 99:10887–10892

Bhaduri PN (1951) Inter-relationship of non-tuberiferous species of S*olanum* with some consideration on the origin of brinjal. Indian J Genet 2:75–86

Bhanushree N , Saha P, Tomar BS, Lyngdoh YA, Gopala Krishnan S , Gurung B, Meena RD, Munshi AD, Saha ND, Ghoshal C (2018) Genetic analysis and identification of molecular marker linked to the gene for fruit skin colour in eggplant (Solanum melongena L.). Veg Sci 45(2):149–153

Blackslee AF, Belling J, Farnham ME, Bergner AD (1922) A haploid mutant in the Jimson weed, *Datura stramonium*. Science 55:646–647

Blay E, Oakes JV (1996) *Agrobacterium tumefasciens*-mediated transformation of *Solanum gilo* Raddi as influenced by explant type. Plant Cell Rep 15:582–585

Boyaci HF, Topcu V, Tepe A, Yildirim IK, Otem M, Aktas A (2015) Morphological and Molecular Characterization and Relationships of Turkish Local Eggplant Heirlooms. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 43(1):100–107

Burk LG, Gerstel DU, Wernsman EA (1979) Maternal haploids of *Nicotiana tabacum* L. from seed. Science 206:585

Cao B, Lei J, Wang Y, Chen G (2009) Inheritance and identification of SCAR marker linked to bacterial wilt-resistance in eggplant. Afr J Biotechnol 8:5201–5207

Cao B, Huanga Z, Chena G, Lei J (2010) Restoring pollen fertility in transgenic male-sterile eggplant by *Cre/lox* p-mediated site-specific recombination system. Genet Mol Biol 33:298–207

Chen JF, Cui L, Malik AA, Mbira KG (2010) *In vitro* haploid and dihaploid production via unfertilized ovule culture. Plant Cell Tissue Organ Cult 104:311–319

Chen X, Zhang M, Tan J, Huang S, Wang C, Zhang H, Tan T (2017) Comparative transcriptome analysis provides insights into molecular mechanisms for parthenocarpic fruit development in eggplant (*Solanum melongena L.*). PLoS One 12(6):e0179491. https://doi.org/10.1371/journal.pone.0179491

Chiarini FE, Mentz LA (2012) A new species of *Solanum* sect. Acanthophora (Solanaceae) from Argentina and Brazil. PhytoKeys 18:1–10
Choudhury B (1976) Brinjal (*Solanum melongena*). In: Simmonds NW (ed) Evolution of crop plants. Longman, New York, pp 278–279
Collonnier C, Fock I, Kashyap V, Rotino GL, Daunay MC, Lian Y, Mariska IK, Rajam MV, Servaes A, Ducreux G, Sihachakr D (2001) Applications of biotechnology in eggplant. Plant Cell Tissue Organ Cult 65:91–07
Corral-Martinez P, Segui-Simarro JM (2012) Efficient production of callus-derived doubled haploids through isolated microspore culture in eggplant (*Solanum melongena* L.). Euphytica 187(1):47–61
Corral-Martinez P, Segui-Simarro JM (2014) Refining the method for eggplant microspore culture: effect of abscisic acid, epibrassinolide, polyethylene glycol, naphthaleneacetic acid, 6-benzylaminopurine and arabinogalactan proteins. Euphytica 95(3):369–382
Daunay MC, Maggioni L, Lipman E (2003) Solanaceae genetic resources in Europe. Report of two meetings – 21 Sept 2001, Nijmegen and 22 May 2003, Skierniewice. International Plant Genetic Resources Institute, Rome
De Candolle A (1886) Origin of cultivated plants. Haffner, New York
Demir K, Bakır M, Sarıkamıs G, Acunalp S (2010) Genetic diversity of eggplant (*Solanum melongena*) germplasm from Turkey assessed by SSR and RAPD markers. Genet Mol Res 9(3):1568–1576
Dhatt AS, Sidhu MK (2019) Development of parthenocarpic lines for net house cultivation in brinjal (*Solanum melongena* L.). In: Proceedings; first vegetable science congress on emerging challenges in vegetable research and education, 1–3 Feb 2019 at Agriculture University, Jodhpur, p 7
Dhatt AS, Kaur G, Sidhu MK, Kaur S (2017) Morphological and molecular characterization of *Solanum melongena* and related species. Veg Sci 44:1–11
Doganlar S, Frary A, Daunay MC, Lester RN, Tanksley SD (2002) A comparative genetic linkage map of eggplant (*Solanum melongena*) and its implications for genome evolution in the Solanaceae. Genetics 161:1697–1711
Doganlar S, Frary A, Daunay M, Huvenaars K, Mank R, Frary A (2014) High resolution map of eggplant (Solanum melongena) reveals extensive chromosome rearrangement in domesticated members of the Solanaceae. Euphytica 198, 231–241. https://doi.org/10.1007/s10681-014-1096-2
Donzella G, Spena A, Rotino GL (2000) Transgenic parthenocarpic eggplants: superior germplasm for increased winter production. Mol Breed 6:79–86
Dumas De Vaulx R, Chambonnet D (1982) Culture *in vitro* d'anthères d'aubergine (*Solanum melongena* L.): stimulation de la production de plantes au moyen de traitments à 35°C associés à de faibles teneurs en substances de croissance. Agronomie 2(10):983–988
Ellialtioglu S, Basay S, Kuşvuran S (2006) Comparison of *in vitro* and *in vivo* colchicine treatments for chromosome doubling in haploid eggplants obtained from anther culture. In: VI. National vegetable growing symposium, 19–22 Sept, Kahramanmaras, pp 386–90
Fang M, Mao R, Xie W (1985) Breeding of cytoplasmically inherited male sterile lines of eggplant (Solanum melongena L.). Acta Hortic Sin 12:261–266 Chinese with English abstract).
Fari M, Nagy I, Csanyi M, Mityko J, Andrasfalvy A (1995) Agrobacterium mediated genetic transformation and plant regeneration via organogenesis and somatic embryogenesis from cotyledon leaves in eggplant (*Solanum melongena* L. cv.'Kecskemeti lila'). Plant Cell Rep 15(1–2):82–86
Forster BP, Heberle-Bors E, Kasha KJ, Touraev A (2007) The resurgence of haploids in higher plants. Trends Plant Sci 12:368–375
Franklin G, Sita GL (2003) Agrobacterium tumefaciens-mediated transformation of eggplant (*Solanum melongena* L.) using root explants. Plant Cell Rep 21(6):549–554
Frary A, Doganlar S, Daunay MC, Tanksley SD (2003) QTL analysis of morphological traits in eggplant and implications for conservation of gene function during evolution of Solanaceous species. Theor Appl Genet 107:359–370
Frary A, Frary A, Daunay MC, Huvenaars K, Mank R, Doganlar S (2014) QTL hotspots in eggplant (*Solanum melongena*) detected with a high resolution map and CIM analysis. Euphytica 197(2):211–228

Fukuoka H, Miyatake K, Nunome T, Negoro S, Shirasawa K, Isobe S, Asamizu E, Yamaguchi H, Ohyama A (2012) Development of gene-based markers and construction of an integrated linkage map in eggplant by using Solanum orthologous (SOL) gene sets. Theo Appl Genet 125 (1):47–56

Garcha KS, Dhatt AS (2017) Evaluation of cytoplasmic male sterile (CMS) and maintainer lines for yield and horticultural traits in brinjal (Solanum melongena L.). Vegetable Science, 44(2): 101–106

Garcha KS, Dhatt AS, Sidhu MK (2017) Genetic variability in CMS backcross generations and their maintainers in eggplant (*Solanummelongena* L.). J Appl Nat Sci 9(3):1854–1860

Goggin FL, Jia LL, Shah G, Hebert S, Williamson VM, Ullman DE (2006) Heterologous expression of the *Mi-1.2* gene from tomato confers resistance against nematodes but not aphids in eggplant. Mol Plant-Microbe Interact 19:383–388

Gramazio P, Prohens J, Plazas M, Andujar I, Herraiz FJ, Castillo E, Knapp S, Meyer RS, Vilanova S (2014) Location of chlorogenic acid biosynthesis pathway and polyphenol oxidase genes in a new interspecific anchored linkage map of eggplant. BMC Plant Biol 14(1):350. https://doi.org/10.1186/s12870-014-0350-z

Gramazio P, Blanca J, Ziarsolo P, Herraiz FJ, Plazas M, Prohens J, Vilanova S (2016) Transcriptome analysis and molecular marker discovery in *Solanum incanum* and *S. aethiopicum*, two close relatives of the common eggplant (*Solanum melongena*) with interest for breeding. BBC Genomics 17:300. https://doi.org/10.1186/s12864-016-2631-4

Gramazio P, Prohens J, Plazas M, Mangino G, Herraiz FJ, Vilanova S (2017) Development and genetic characterization of advanced backcross materials and an introgression line population of *Solanum incanum* in a *Solanum melongena* background. Front Plant Sci 8:1477. https://doi.org/10.3389/fpls.2017.01477

Gramazio P, Yan H, Hasing T, Vilanova S, Prohens J, Bombarely A (2019) Whole-genome resequencing of seven eggplant (Solanum melongena) and one wild relative (S. incanum) accessions provides new insights and breeding tools for eggplant enhancement. Front Plant Sci 10:1220. https://doi.org/10.3389/fpls.2019.01220

Gresshoff PM, Doy CH (1972) Development and differentiation of haploid Lycopersicon esculentum (tomato). Planta 107(2):161–170

Guha S, Maheshwari SC (1964) *In vitro* production of embryos from anthers of Datura. Nature 204:497

Guri A, Sink KC (1988) Agrobacterium transformation of eggplant. J Plant Physiol 133:52–55

Hall CA, Hobby T, Cipollini M (2006) Efficacy and mechanisms of solasonine- and solamargine induced cytolysis on two strains of *Trypanosoma cruzii*. J Chem Ecol 32:2405–2416

Hamilton GC, Jelenkovic GL, Lashomb JH, Ghidiu G, Billings S, Patt JM (1997) Effectiveness of transgenic eggplant (*Solanum melongena* L.) against the Colorado potato beetle. Adv Hortic Sci 11:189–192

Hanson MR, Bentolila S (2004) Interactions of mitochondrial and nuclear genes that affect male gametophyte development. Plant Cell 16:154–169

Hasnunnahar M, Khan MMR, Isshiki S (2012a) Inheritance of male fertility restoration of a male sterility system in eggplant with the cytoplasm of *S. grandifolium*. Aust J Crop Sci 6:475–479

Hasnunnahar M, Khan MMR, Isshiki S (2012b) Pollen and seed fertility of three functional male-sterile lines of eggplant with the wild *Solanum* cytoplasms. Sci Hortic 139:58–61

Hirakawa H, Shirasawa K, Miyatake K, Nunome T, Negoro S, Ohyama A, Yamaguchi H, Sato S, Isobe S, Tabata S, Fukuoka H (2014) Draft genome sequence of eggplant (*Solanum melongena* L.): the representative *Solanum* species indigenous to the old world. DNA Res 21:649–660

Isouard G, Raquin C, Demarly Y (1979) Obtention de plantes haploïdes et diploïdes par culture *in vitro* d'anthères d'aubergine (*Solanum melongena* L.). CR Acad Sci Paris 288:987–989

Isshiki S, Kawajiri N (2002) Effect of cytoplasm of *Solanum violaceum* Ort. on fertility of eggplant (*S. melongena* L.). Sci Hortic 93:9–18

Kasha KJ, Kao KN (1970) High frequency haploid production in barley (*Hordeum vulgare* L.). Nature 225:874–876

Kaur M, Dhatt AS, Sandhu JS, Gossal SS (2015) Effect of genotype, explant and culture media on direct plant regeneration in eggplant (*Solanum melongena* L.). Indian J Hortic 72:232–238

Kaushik P, Gramazioa P, Vilanovaa S, Raigonb MD, Prohens J, Plazas M (2017) Phenolics content, fruit flesh colour and browning in cultivated eggplant, wild relatives and interspecific hybrids and implications for fruit quality breeding. Food Res Int 102:392–301

Khan MMR, Hasnunnahar M, Iwayoshi M, Tsujita YO, Isshiki S (2015) Pollen degeneration in three functional male-sterile lines of eggplant with the wild Solanum cytoplasms. Horti Envir Biotech 56(3):350–357

Khan MMR, Isshiki S (2008) Development of a male sterile eggplant by utilizing the cytoplasm of *Solanum virginianum* and a biparental transmission of chloroplast DNA in backcrossing. Sci Hortic 117:316–320

Khan MMR, Isshiki S (2009) Functional male-sterility expressed in eggplant (*Solanum melongena* L.) containing the cytoplasm of *S. kurzii* Brace & Prain. J Hortic Sci Biotechnol 84:92–96

Khan MMR, Isshiki S (2010) Development of the male sterile line of eggplant utilizing the cytoplasm of *Solanum aethiopicum* L. *Aculeatum* Group. J Jpn Soc Hortic Sci 79:348–353

Khan MMR, Isshiki S (2011) Development of a cytoplasmic male-sterile line of eggplant (*Solanum melongena* L.) with the cytoplasm of *Solanum anguivi*. Plant Breed 130:256–260

Khan MMR, Isshiki S (2016) Cytoplasmic male sterility in eggplant. Hortic J 85:1–7

Khan MMR, Hasnunnahar M, Isshiki S (2013a) Production of amphidiploids of the hybrids between *Solanum macrocarpon* and Eggplant. HortScience 48(4):422–424

Khan MMR, Hasnunnahar M, Iwayoshi M, Isshiki S (2013b) Pollen and seed fertility of the male fertile lines having the fertility restorer gene in three CMS systems of eggplant. Sci Hortic 157:39–44

Khan MMR, Hasnunnahar M, Iwayoshi M, Isshiki S (2014) Fertility restoration in three CMS systems of eggplant by the *Rf* genes of each other's systems and their SCAR marker. Sci Hortic 172:149–154

Khatun F, Meah MB, Nasiruddin KM (2006) Regeneration of eggplant through anther culture. Pak J Biol Sci 9:48–53

Khush GS, Virmani SS (1996) Haploids in plant breeding. In: Jain SM, Sopory SK, Veilleux RE (eds) In vitro haploid production in higher plants, vol 1. Kluwer Academic Publisher, Dordrecht, pp 11–33

Kikuchi K, Honda I, Matsuo S, Fukuda M, Saito T (2008a) Stability of fruit set of newly selected parthenocarpic eggplant lines. Sci Hortic 115:111–116

Kikuchi K, Honda I, Matsuo S, Fukuda M, Saito T (2008b) Evaluation of fruit set and fruit development of new Japanese parthenocarpic eggplant lines. In: Cohen J, Lee JM (eds) XXVII international horticultural congress—IHC 2006: international symposium on endogenous and exogenous plant bioregulators. Acta Hortic 774:121–126

Kouassi B, Prohens J, Gramazio P, Kouassi AB, Vilanova S, Galan-Avila A, Herraiz FJ, Kouassi A, Segui-Simarro JM, Plazas M (2016) Development of backcross generations and new interspecific hybrid combinations for introgression breeding in eggplant (*Solanum melongena*). Sci Hortic 213:199–107

Kumar PA, Mandaokar A, Sreenivasu K, Chakrabarti SK, Bisaria S, Sharma SR, Kaur S, Sharma RP (1998a) Insect resistant transgenic brinjal plants. Mol Breed 4(1):33–37

Kumar PA, Mandaokar A, Sharma RP (1998b) Genetic engineering for the improvement of eggplant (*Solanum melongena* L.). AgBiotech News Inf 10(10):329–332

Kumar S, Singh M, Prabhavathi K, Mathews A (2003) *In vitro* induction of haploid in eggplant (*Solanum melongena* L.). Caps Eggp Newsl 22:147–150

Kumchai J, Wei YC, Lee CY, Chen FC, Chin SW (2013) Production of inter-specific hybrids between commercial cultivars of the eggplant (*Solanum melongena* L.) and its wild relative *S. torvum*. Genet Mol Res 12(1):755–764

Kuo KW, Hsu SH, Li YP, Lin WL, Liu LF, Chang LC, Lin CC, Lin CN, Sheu HM (2000) Anticancer activity evaluation of the *Solanum* glycoalkaloid solamargine: triggering apoptosis in human hematoma cells. Biochem Pharmacol 60:1865–1873

Lebeau A, Gouy M, Daunay MC, Wicker E, Chiroleu F, Prior P (2013) Genetic mapping of a major dominant gene for resistance to *Ralstonia solanacearum* in eggplant. Theor Appl Genet 126:143. https://doi.org/10.1007/s00122-012-1969-5

Li XJ, Liu FZ, Zhang Y, Li YW, Chen YH, Lian Y (2012) Research progress on eggplant parthenocarpy. China Veg 6:8–14

Li YW, Liu FZ, Chen YH, Zhang Y, Zhang WW, Lian Y (2013) Effect of temperature on endogenous hormones contents during parthenocarpic eggplant ovary (fruit) development. China Veg 22:32–38

Lincoln S, Daly M, Lander E (1992) Mapping genes controlling quantitative traits with MAPMAKER/QTL. Whitehead Institute technical report, 2nd edn. Cambridge, MA

Liu F, Wan X, Chen Y, Lian Y, Song M (2008) Inheritance of the eggplant parthenocarpy and AFLP molecular marker. Acta Hortic Sin 35:1305–1309

Liu J, Zheng Z, Zhou X, Feng C, Zhuang Y (2015) Improving the resistance of eggplant (*Solanum melongena* L.) to Verticillium wilt using wild species *Solanum linnaeanum*. Euphytica 201:463–469

Magioli C, Mansur E (2005) Eggplant (*Solanum melongena* L.): tissue culture, genetic transformation and use as an alternative model plant. Acta Bot Bras 19(1):139–148

Mahmoud MI, El-Mansy AB (2012) Molecular identification of eggplant cultivars (*Solanum melongena* L.) using ISSR markers. J Appl Sci Res 8(1):69–77

Mangal M, Upadhyay P, Kalia P (2016) Characterization of cultivated and wild genotypes of brinjal (*Solanum melongena L.*) and confirmation of hybridity using microsatellite markers. Vegetos 29(2). https://doi.org/10.5958/2229-4473.2016.00016

Mao W, Jinxin Y, Sihachakr D (2008) Development of core subset for the collection of Chinese cultivated eggplants using morphological-based passport data. Plant Genet Resour Characteriz Util 6:33–40

Mennella G, Rotino GL, Fibiani M, D'Alessandro A, Francese G, Toppino L, Cavallanti F, Acciari N, Lo Scalzo R (2010) Characterization of health-related compounds in eggplant (*Solanum melongena* L.) lines derived from introgression of allied species. J Agric Food Chem 58:7597–7503

Miyoshi K (1996) Callus induction and plantlet formation through culture of isolated microspores of eggplant (*Solanum melongena* L). Plant Cell Rep 15:391–395

Murashige T, Skoog FA (1962) Revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15:473–497

Mutlu N, Boyacı FH, Gocmen M, Abak K (2008) Development of SRAP, SRAP-RGA, RAPD and SCAR markers linked with a Fusarium wilt resistance gene in eggplant. Theor Appl Genet 117:1303. https://doi.org/10.1007/s00122-008-0864-6

Nishio T, Mochizuki H, Yamakawa K (1984) Inter-specific cross of eggplant and related species. Bull Veg Ornamental Crop Res Stn 12:57–64

Nunome T, Yoshida T, Hirai M (1998) Genetic linkage map of eggplant. In: Proceedings of the 10th Eucarpia meeting on genetics and breeding of *Capsicum*and eggplant, Avignon, pp 239–42

Nunome T, Ishiguro K, Yoshida T, Hirai M (2001) Mapping of fruit shape and color development traits in eggplant (*Solanum melongena* L.) based on RAPD and AFLP marker. Breed Sci 51:19–26

Nunome T, Suwabe K, Iketani H, Hirai M (2003) Identification and characterization of microsatellites in eggplant. Plant Breed 122:256–262

Nunome T, Negoro S, Kono I, Kanamori H, Miyatake K, Yamaguchi H, Ohyama A, Fukuoka H (2009) Development of SSR markers derived from SSR-enriched genomic library of eggplant (*Solanum melongena* L.). Theor Appl Genet 119:1143–1153

Pal JK, Singh M, Rai M, Satpathy S, Singh DV, Kumar S (2009) Development and bioassay of *Cry1Ac*-transgenic eggplant (*Solanum melongena* L.) resistant to shoot and fruit borer. J Hortic Sci Biotechnol 84:434–438

Phatak SC, Liu J, Jaworski CA, Sultanbawa AF (1991) Functional male sterility in eggplant: inheritance and linkage to the purple fruit color gene. J Hered 82:81–83

Plazas M, Vilanova S, Gramazio P, Rodrıguez-Burruezo A, Fita A, Herraiz FJ (2016) Interspecific hybridization between eggplant and wild relatives from different gene pools. J Am Soc Hortic Sci 141(1):34–44

Portis E, Barchi L, Toppino L, Lanteri S, Acciarri N, Felicioni N, Fusari F, Barbierato V, Cericola F, Vale G, Rotino GL (2014) QTL mapping in eggplant reveals clusters of yield-related loci and orthology with the tomato genome. PLoS One 9(2):e89499. https://doi.org/10.1371/journal.pone.0089499

Portis E, Lanteri S, Barchi L, Portis F, Valente L, Toppino L, Rotino GL, Acquadro A (2018) Comprehensive characterization of simple sequence repeats in eggplant (*Solanum melongena* L.) genome and construction of a web resource. Front Plant Sci 9:401. https://doi.org/10.3389/fpls.2018.00401

Prabhavati V, Yadav JS, Kumar PA, Rajam MV (2002) Abiotic stress tolerance in transgenic eggplant (*Solanum melongena* L.) by introduction of bacterial *mannitol phosphodehydrogenase* gene. Mol Breed 9:137–147

Prodhan MZH, Hasan MT, Chowdhury MMI, Alam MS, Rahman ML, Azad AK, Hossain MJ, Naranjo SE, Shelton AM (2018) Bt eggplant (*Solanum melongena* L.) in Bangladesh: fruit production and control of eggplant fruit and shoot borer (*Leucinodesorbonalis* Guenee), effects on non-target arthropods and economic returns. PLoS One 13(11):e0205713. https://doi.org/10.1371/journal.pone.0205713

Prohens J, Plazas M, Raigon MD, Seguı-Simarro JM, Stommel JR, Vilanova S (2012) Characterization of interspecific hybrids and first backcross generations from crosses between two cultivated eggplants (*Solanum melongena* and *S. aethiopicum* Kumba group) and implications for eggplant breeding. Euphytica 186:517–538

Prohens J, Whitaker BD, Plazas M, Vilanova S, Hurtado M, Blasco M, Gramazio P, Stommel JR (2013) Genetic diversity in morphological characters and phenolic acids content resulting from an interspecific cross between eggplant, *Solanum melongena*, and its wild ancestor (*S. incanum*). Ann Appl Biol 162:242–257

Ramesh KR, Hemalatha R, Vijayendra CA, Arshi UZ, Dushyant SB, Dinesh KB (2016) Transcriptome analysis of Solanum melongena L. (eggplant) fruit to identify putative allergens and their epitopes. Gene 576(1):64–71

Rao GR, Kumar A (1980) Some observations on interspecific hybrids of *Solanum melongena* L. Proc Indian Acad Sci (Plant Sci) 89:117–121

Ribeiro ADO, Pereira EJG, Galvan TL, Picanco MC, Picoli EDT, Da Silva DJH, Fari MG, Otoni WC (2006) Effect of eggplant transformed with oryzacystatin gene on *Myzus persicae* and *Macrosiphum euphorbiae*. J Appl Entomol 130(2):84–90

Rivas-Sendra A, Corral-Martinez P, Camacho-Fernandez C, Segui-Simarro JM (2015) Improved regeneration of eggplant doubled haploids from microspore-derived calli through organogenesis. Plant Cell Tissue Organ Cult 122(3):759. https://doi.org/10.1007/s11240-015-0791-6

Rotino GL (1996) Haploidy in eggplant. In: Jain SM, Sopory SK, Veilleux RE (eds) In vitro haploid production in higher plants. Kluwer Academic, Dordrecht, pp 115–141

Rotino GL (2016) Anther culture in eggplant (*Solanum melongena* L.). In: Germana MA, Lambardi M (eds) In vitro Embryogenesis in higher plants, methods in molecular biology, vol 1359. Springer Science+Business Media, New York, pp 453–466

Rotino GL, Perri E, Zottini M, Sommer H, Spena A (1997) Genetic engineering of parthenocarpic plants. Nat Biotechnol 15:1398–1301

Rotino GL, Sihachakr D, Rizza F, Valè G, Tacconi MG, Alberti P, Mennella G, Sabatini E, Toppino L, D'Alessandro A, Acciarri N (2005) Current status in production and utilization of dihaploids from somatic hybrids between eggplant (*Solanum melongena* L.) and its wild relatives. Acta Physiol Plant 27:723–733

Rotino GL, Sala T, Toppino L (2014) Eggplant. In: Pratap A, Kumar J (eds) Alien gene transfer in crop plants, vol 2. Springer, New York, pp 381–309

Saito T, Matsunaga H, Saito A, Hamato N, Koga T, Suzuki T, Yoshida T (2009a) A novel source of cytoplasmic male sterility and a fertility restoration gene in eggplant (*Solanum melongena* L.) lines. J Jpn Soc Hortic Sci 78:425–430

Saito T, Yoshida T, Monma S, Matsunaga H, Sato T, Saito A, Yamada T (2009b) Development of the parthenocarpic eggplant cultivar ‘Anominori’. JARQ 43:123–127

Salas P, Rivas-Sendra A, Prohens J, Segui-Simarro JM (2012) Influence of the stage for anther excision and heterostyly in embryogenesis induction from eggplant anther cultures. Euphytica 184:235–250

San Jose R, Plazas M, Sanchez-Mata MC, Camara M, Prohens J (2016) Diversity in composition of scarlet (*S. aethiopicum*) and gboma (*S. macrocarpon*) eggplants and of interspecific hybrids between *S. aethiopicum* and common eggplant (*S. melongena*). J Food Compos Anal 45:130–140

Sanchez-Mata MC, Yokoyama WE, Hong YJ, Prohens J (2010) α–solasonine and α–solamargine contents of gboma (*Solanum macrocarpon* L.) and scarlet (*Solanum aethiopicum* L.) eggplants. J Agric Food Chem 58:5502–5508

Sarkinen T, Knapp S (2016) Two new non-spiny *Solanum* (Solanaceae) from the Gran Chaco Americano and a key for the herbaceous glandular-pubescent solanums from the region. PhytoKeys 74:19–33

Sarkinen T, Gonzales P, Knapp S (2015a) Four new non-spiny *Solanum* (Solanaceae) species from South America. PhytoKeys 44:39–64

Sarkinen T, Knapp S, Nee M (2015b) Two new non-spiny *Solanum* species from the Bolivian Andes (Morelloid Clade). PhytoKeys 47:97–109

Schaff DA, Jelenkovic G, Boyer CD, Pollack BL (1982) Hybridization and fertility of hybrid derivatives of *Solanum melongena* L. and *Solanum macrocarpon* L. Theor Appl Genet 62:149–153

Segui-Simarro JM, Corral-Martinez P, Parra-Vega V, Gonzalez-Garcia B (2011) Androgenesis in recalcitrant Solanaceous crops. Plant Cell Rep 30:765–778

Shelton AM (2010) The long road to commercialization of Bt brinjal (eggplant) in India. Crop Prot 29:412–414

Shimomura K, Koga T, Sueyoshi T, Hamachi Y (2010) Development and suitability of DNA markers for breeding of parthenocarpic eggplant. Hortic Res (Japan) 9(1):13–17

Shiu LY, Chang LC, Liang CH, Huang YS, Sheu HM, Kuo KW (2007) Solamargine induces apoptosis and sensitizes breast cancer cells to cisplatin. Food Chem Toxicol 45:2155–2164

Sidhu MK, Dhatt AS (2019) Study on growth and fruit setting ability of interspecific hybrids between *S. melongena* and *S torvum*. In: Proceedings; first vegetable science congress on emerging challenges in vegetable research and education, 1–3 Feb 2019 at Agriculture University, Jodhpur, p 50

Sidhu MK, Dhatt AS, Sandhu JS, Gossal SS (2014) Biolistic Transformation of *Cry 1*Ac gene in Eggplant (*Solanum melongena* L.). Int J Agric Environ Biotechol 7(4):679–687

Singh AK, Verma SS, Bansal KC (2010) Plastid transformation in eggplant (*Solanum melongena* L.). Trans Res 19:113–119

Stagel A, Portis E, Toppino L, Rotino GL, Lanteri S (2008) Gene-based microsatellite development for mapping and phylogeny studies in eggplant. BMC Genomics 9(1):357. https://doi.org/10.1186/1471-2164-9-357

Stern SR (2014) A new species of spiny *Solanum* (Solanaceae) from Peru. PhytoKeys 39:27–34

Stern S, Bohs L (2010) Two new species of *Solanum* (Solanaceae) from the Amotape-Huancabamba Zone of southern Ecuador and northern Peru. PhytoKeys 1:53–65

Sunseri F, Sciancalepore A, Martelli G, Acciarri N, Rotino GL, Valentino D, Tamietti G (2003) Development of RAPD-AFLP map of eggplant and improvement of tolerance to *Verticillium* wilt. In: Hammerschlag FA, Saxena P (eds) XXVI international horticultural congress: biotechnology in horticultural crop improvement. Acta Hortic 625:107–115

Sunseri F, Polignano GB, Alba V, Lotti C, Bisignano V, Mennella G, D’Alessandro A, Bacchi M, Riccardi P, Fiore MC, Ricciard L (2010) Genetic diversity and characterization of African eggplant germplasm collection. African J Plant Sci 4(7):231–241

Taher D, Solberg S, Prohens J, Chou Y, Rakha M, Wu T (2017) World vegetable centre eggplant collection: origin, composition, seed dissemination and utilization in breeding. Front Plant Sci 8:1484. https://doi.org/10.3389/fpls.2017.01484

Toppino L, Valè G, Rotino GL (2008) Inheritance of *Fusarium* wilt resistance introgressed from *Solanum aethiopicum Gilo* and *Aculeatum* groups into cultivated eggplant (*S. melongena*) and development of associated PCR -based markers. Mol Breed 22:237–250

Toppino L, Acciarri N, Mennella G, Lo SR, Rotino GL (2009) Introgression breeding in eggplant (*Solanum melongena* L.) by combining biotechnological and conventional approaches. In: Proceedings of the 53rd Italian society of agricultural genetics annual congress, Torino

Vavilov NI (1951) Phytogeographical basis of plant breeding. The origin, variation, immunity and breeding of cultivated plants (Trans: Choster KJ). Chron Bot 13:366

Vorontsova MS, Knapp S (2012) A new species of *Solanum* (Solanaceae) from South Africa related to the cultivated eggplant. PhytoKeys 8:1–11

Vural GE, Ari E, Zengin S, Ellialtioglu SS (2019) Development of androgenesis studies on eggplant (*Solanum melongena* L.) in Turkey from past to present, Intech Open. https://doi.org/10.5772/intechopen. 88,299. https://www.intechopen.com/online-first/development-of-androgenesis-studies-on-eggplant-solanum-melongena-l-in-turkey-from-past-to-present

Weese TL, Bohs L (2010) Eggplant origins: out of Africa, into the Orient. Taxon 59:49–56

Wei Q, Du L, Wang W, Hu T, Hu H, Wang J, David Kand Bao C (2019) Comparative transcriptome analysis in eggplant reveals selection trends during eggplant domestication. Int J Genomics:1–12. https://doi.org/10.1155/2019/7924383

Winzeler H, Schmid J, Fried PM (1987) Field performance of androgenetic doubled haploid spring wheat line in comparison with line selected by the pedigree system. Plant Breed 99:41–48

Wu FN, Eannetta NT, Xu Y, Tanksley SD (2009) A detailed synteny map of the eggplant genome based on conserved ortholog set II (COSII) markers. Theor Appl Genet 118:927–935

Yang L, Jue D, Li W, Zhang R, Chen M, Yang Q (2013) Identification of MiRNA from eggplant (Solanum melongena L.) by small RNA deep sequencing and their response to Verticillium dahlia infection. PLoS One 8:e72840

Yang X, Cheng YF, Deng C, Ma Y, Wang ZW, Chen XH, Xue LB (2014) Comparative transcriptome analysis of eggplant (*Solanum melongena* L.) and turkey berry (*Solanum torvum* Sw.): phylogenomics and disease resistance analysis. BMC Genomics 15:412

Yoshimi M, Kitamura Y, Isshiki S, Saito T, Yasumoto K, Terachi T, Yamagishi H (2013) Variations in the structure and transcription of the mitochondrial *atp* and *cox* genes in wild *Solanum* species that induce male sterility in eggplant (*S. melongena*). Theor Appl Genet 126:1851–1859

Yadava NR, Verghese TM, Sharma DR (1989) Morphogenetic studies in androgenic callus of Solanum melongena L. Current Science 58:637–639

Zeven AC, Zhukovsky PM (1975) Dictionary of cultivated plants and their centres of diversity. PUDOC, Wageningen, p 219

Zhang Y, Liu FZ, Chen YH, Lina Y (2009) Characteristics of eggplant parthenocarpy at low temperature. China Veg 2:16–20

Zhang Y, Chen Y, Zhang Z, Fang Z, Lian Y, Liu F (2011) Construction and analysis of eggplant thermo-sensitive parthenocarpy SSH cDNA library. Sci Agric Sin 44:3368–3376

Zhou Y, Chen Y, Liu F, Zhang Y, Lian Y (2010) Isolation of parthenocarpy related ESTs in parthenocarpic eggplant using suppression subtraction hybridization (SSH). Acta Hortic Sin 37:1944–1952

Zhou X, Bao S, Liu J, Zhuang Y (2016) De novo sequencing and analysis of the transcriptome of the wild eggplant species Solanum aculeatissimum in response to Verticillium dahliae. Plant Mol Biol Rep 34:1193–1103

Zhou X, Liu J, Bao S, Yang Y, Zhuang Y (2018) Molecular cloning and characterization of a wild eggplant *Solanum aculeatissimum NBS-LRR* gene, involved in plant resistance to *Meloidogyne incognita*. Int J Mol Sci 19:583. https://doi.org/10.3390/ijms19020583

Chapter 9
Conventional and Contemporary Approaches to Enhance Efficiency in Breeding Chilli/Hot Pepper

A. Mohan Rao and C. Anilkumar

9.1 Introduction

Hot pepper (*Capsicum annuum* L; 2n = 2x = 24) is commonly known as chilli or chilli pepper (Dasgupta and Reshmi 2011). It is known by different names, viz. *Capsicum*, paprika, pimento, hot pepper, red pepper, bird pepper, etc., in different parts of the world. It is one of the most important economic spice cum vegetable crops worldwide (Poulos 1992) and is ranked second among solanaceous vegetable crops after tomato (Bosland 1995). The genus *Capsicum* originated in Mexico with its centre of diversity in South America (Gonzalez and Bosland 1991) and spread to Asian countries by Portuguese travellers. It is believed that the crop was introduced to India by Portuguese in the sixteenth century (Singh et al. 2004).

The diploid genus *Capsicum* comprise more than 31 species (Table 9.1). Among them, only five species, (Fig. 9.1) viz. *Capsicum annuum*, *C. frutescens*, *C. pubescens*, *C. chinense* and *C. baccatum*, originated from at least three domestication events are cultivated in different parts of the world (Moscone et al. 2007). Among these, *Capsicum annum* is cultivated across the world (Tong and Boseland 2003).

Chilli is used as a part of the human diet as spice, condiment and vegetable for its appealing colour, flavour and pungency. The value of the crop is significant due to its culinary and medicinal qualities (Fattori et al. 2016). Chilli fruits are a rich source of carbohydrates, proteins, minerals, ascorbic acid and vitamins C, A and E. Peppers are also a rich source of calcium, magnesium, iron and potassium (Pawar et al. 2011). A volatile alkaloid called ‘capsaicin’ present in the placenta of fruit causes pungency and has diverse prophylactic and therapeutic uses in allopathic and Ayurvedic medicines (Saleh et al. 2018). Its fruits also contain carotenoid pigments such as lycopene and zeaxanthin which are known to possess anticancer properties (Bosland and Votava 2000; Ghasemnezhad et al. 2011; Kim et al. 2014). Extract

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Table 9.1 List of 31 identified *Capsicum* species with some elite features and geographical distribution (Moscone et al. 2007)

Species and variety	Growth form	Corolla shape and colour	Fruit shape and colour	Seed colour	$2n^{b}$	Geographic distribution
C. annuum L. var. *annuum*	Herb or subshrub (1–2 m)	Stellate; white or cream (exceptionally violet)	Highly variable shape; violet, red, orange, yellow or green	Yellowish	$_{24}$1–3, 10–13 48^{2}	Cultivated worldwide
C. annuum L. var. *glabriusculum* (Dunal) Heiser & Pickersgill [syn. = var. *minimum* (Mill.) Heiser]	Herb or subshrub (1–2 m)	Stellate; white or cream	Ovoid or spherical; red	Yellowish	$_{24}$1–3	S USA, Mexico, Antilles, Belize, Honduras, El Salvador, Panama, Costa Rica, Guatemala, Suriname, Venezuela, Colombia, Ecuador, Peru, N and NE Brazil
C. baccatum L. var. *baccatum*	Shrub (0.6–3.5 m)	Stellate; white with greenish spots in the throat	Ovoid or elliptic; red	Yellowish	$_{24}$2–3, 13–14	Colombia, Peru, Bolivia, Paraguay, S and SE Brazil, N Argentina
C. baccatum L. var. *pendulum* (Willd.) Eshbaugh	Shrub (0.6–2 m)	Stellate; white with greenish spots in the throat	Elliptic or fusiform; red or yellow	Yellowish	$_{24}$2–3, 10–13	Cultivated in the USA, Mexico, Costa Rica, Colombia, Ecuador, Peru, Brazil, Bolivia, Paraguay, Chile, Argentina, India

(continued)

Table 9.1 (continued)

Species and variety	Growth form	Corolla shape and colour	Fruit shape and colour	Seed colour	$2n$[b]	Geographic distribution
C. baccatum L. var. *umbilicatum* (Vellozo) Hunz. & Barboza	Herb or subshrub (1.6–2 m)	Stellate; white with greenish spots in the throat	Umbonate umbilicate; red	Yellowish	$_{24}$9, 13	Cultivated in the USA, Mexico, Jamaica, Peru, Bolivia, Brazil, Paraguay, Argentina
C. caballeroi M. Nee[a]	Herb, shrub or tree (1–7 m)	Campanulate; lemon yellow	Spherical; red	Yellowish	–	Bolivia: COCH, SC (endemic)
C. campylopodium Sendtn.	Shrub (0.7–1.1 m)	Stellate; white with golden spots in the throat	Spherical compressed; yellowish green	Blackish	$_{26}$10–11, 13, 17	Brazil: ES, MG, RJ (endemic)
C. cardenasii Heiser & Smith	Shrub (1 m)	Campanulate; violet lobules with azure throat	Spherical; red	Brownish	$_{24}$2–3, 15	Bolivia: LP (endemic)
C. ceratocalyx M. Nee[a]	Shrub (1.5 m)	Rotate; yellow with green spots in the throat	Spherical; red	–	–	Bolivia: LP (endemic)
C. chacoense Hunz.	Shrub (0.4–0.8 m)	Stellate; white	Ovoid or elliptic; red	Yellowish	$_{24}$2–3, 6, 10–11,13	S Bolivia, Paraguay, N and C Argentina
C. chinense Jacq.	Herb or shrub (0.5–2 m)	Stellate; white or cream	Spherical or conical; red, orange, yellow or white	Yellowish	$_{24}$2–3, 11–13, 17	Cultivated in the USA, Mexico, Central America, Ecuador, Peru, Bolivia, Brazil, Argentina, China, Japan
C. coccineum (Rusby) Hunz.	Herb or climbing subshrub (1.5–3 m)	Stellate; yellowish white with purplish spots in the throat	Spherical; orange or red	Brownish	–	Peru, Bolivia

(continued)

Table 9.1 (continued)

Species and variety	Growth form	Corolla shape and colour	Fruit shape and colour	Seed colour	$2n^{b}$	Geographic distribution
C. cornutum (Hiern.) Hunz.	Shrub (1.2–1.8 m)	Rotate; white with violet or brownish spots in the throat, green in the tube	Spherical depressed; yellowish green	Blackish	$_{26}$17	Brazil: RJ, SP (endemic)
C. dimorphum (Miers) Kuntze	Shrub (1.5–2 m)	Stellate; yellow, sometimes with violet spots in the throat	Spherical; orange or red	Brownish	–	Colombia, Ecuador
C. eximium Hunz.	Herb, shrub or three (0.6–4 m)	Stellate; white with violet greenish in the tube	Spherical; red	Brownish	$_{24}$2–3, 13	S Bolivia, N Argentina
C. flexuosum Sendtn.	Shrub (0.5–2 m)	Stellate; white with greenish spots in the throat	Spherical depressed; red	Blackish	$_{24}$7, 17	Paraguay, S and SE Brazil, NE Argentina
C. friburgense Bianchetti & Barboza	Shrub (0.8–2.5 m)	Campanulate urceolate; pink or lilac	Spherical depressed; yellowish green	Blackish	$_{26}$17	Brazil: MG, RJ (endemic)
C. frutescens L.	Herb or shrub (1–2 m)	Stellate; white or cream	Elongate; red	Yellowish	$_{24}$2–3, 11–13	Cultivated in the USA, Mexico, Central and South America, Africa, India, China, Japan
C. galapagoense Hunz.	Shrub (1–4 m)	Stellate; white	Spherical; red	Yellowish	$_{24}$2–3	Ecuador: Galapagos Islands (endemic)
C. geminifolium (Dammer) Hunz.	Shrub (0.7–4 m)	Rotate; white or yellowish with violet spots in the throat	Spherical; red	Brownish	–	Colombia, Ecuador, Peru
C. hookerianum (Miers) Kuntze	Shrub (1–3 m)	Stellate; ochre	Spherical; colour unknown	Brownish	–	S Ecuador, N Peru (endemic)

(continued)

Table 9.1 (continued)

Species and variety	Growth form	Corolla shape and colour	Fruit shape and colour	Seed colour	$2n^b$	Geographic distribution
C. hunzikerianum Barboza & Bianchetti	Shrub (1–3 m)	Stellate; white with purple spots in lobules and throat, yellowish in the tube	Spherical; yellowish green	Blackish	–	Brazil: SP (endemic)
C. lanceolatum (Greenm.) Morton & Standley	Herb or shrub (1–5 m)	Stellate campanulate; white or yellowish	Spherical; pale orange or red	Brownish	$_{26}$16	México, Guatemala
C. mirabile Mart. (syn. = *C. buforum* Hunz.)	Herb or shrub (0.5–3 m)	Stellate; white with purple spots in the lobules, greenish in the throat and tube	Spherical; yellowish green	Blackish	$_{26}$11, 17	Brazil: MG, RJ, SP (endemic)
C. parvifolium Sendtn.	Shrub or tree (1.5–5 m)	Rotate; white with purple spots in the lobules, greenish in the throat and tube	Spherical; orange or red	Brownish	$_{24}$8, 10–11, 13, 17	Colombia, Venezuela, NE Brazil
C. pereirae Barboza & Bianchetti	Shrub (0.5–3 m)	Stellate; white with purple spots in the lobules, yellowish in the throat and tube	Spherical; yellowish green	Blackish	$_{26}$5, 17	Brazil: ES, MG, SP (endemic)
C. praetermissum Heiser & Smith [syn. = *C. baccatum* var. *praetermissum* (Heiser & Smith) Hunz.]	Herb or shrub (0.8–1.8 m)	Rotate; white with purple lobule margins and greenish spots in the throat	Spherical or elliptic; orange or red	Yellowish	$_{24}$2–3, 14, 17	Central and SE Brazil, Paraguay
C. pubescens Ruiz & Pav.	Shrub (0.8–2 m)	Rotate; purple or violet in the lobules, white or yellowish in the tube	Turban-shaped, spherical or elongate; red, orange or yellow	Blackish	$_{24}$2–3, 10–13	Cultivated in Mexico, Central and South America

(continued)

Table 9.1 (continued)

Species and variety	Growth form	Corolla shape and colour	Fruit shape and colour	Seed colour	$2n$[b]	Geographic distribution
C. recurvatum Witas.	Herb or shrub (0.5–3 m)	Stellate; white with greenish spots in the throat	Spherical; yellowish green	Blackish	26[c]	Brazil: RJ, SP, PN, SCA (endemic)
C. rhomboideum (Dunal) Kuntze [syn. = *C. ciliatum* (Kunth) Kuntze]	Shrub or small tree (0.8–4 m)	Rotate; yellow	Spherical; red	Brownish	$_{26}$2–3	Mexico, Guatemala, Honduras, Colombia, Venezuela, Ecuador, Peru
C. schottianum Sendtn.	Shrub (1.2–3 m)	Stellate; white with violet or brownish spots in the throat, greenish in the tube	Spherical; yellowish green	Blackish	$_{26}$5, 17	Brazil: MG, RJ, SP (endemic)
C. scolnikianum Hunz.	Shrub (ca. 1.5 m)	Campanulate; yellowish white	Spherical depressed; red	Brownish	–	Ecuador: EO, Peru: PI (endemic)
C. tovarii Eshbaugh, Smith & Nickrent	Shrub (1 m)	Stellate; variable colour (purple or cream, cream with greenish spots in the lobules)	Spherical; red	Brownish	$_{24}$2–4, 15	Peru: AY, HU, JUN (endemic)
C. villosum Sendtn.	Subshrub or shrub (1–3 m)	Stellate; white with violet or brownish spots in the throat, greenish in the tube	Spherical; yellowish green	Blackish	$_{26}$17	Brazil: MG, RJ, SP (endemic)

[a]Newly described species (Nee et al. 2006)

[b]Main chromosome report references: 1 = Pickersgill (1971), 2 = Pickersgill (1977), 3 = Pickersgill (1991), 4 = Eshbaugh et al. (1983), 5 = Moscone (1989), 6 = Moscone (1990), 7 = Moscone (1992), 8 = Moscone (1993), 9 = Moscone (1999), 10 = Moscone et al. (1993), 11 = Moscone et al. (1995), 12 = Moscone et al. (1996), 13 = Moscone et al. (2003), 14 = Cecchini and Moscone (2002), 15 = Cecchini et al. (2003), 16 = Tong and Bosland (1997), 17 = Pozzobon et al. (2006)

[c]First chromosome report

Country subdivisions (states, Brazil; departments, Peru, Bolivia; provinces, Ecuador): *AY* ayacucho, *COCH* cochabamba, *EO* el oro, *ES* espirito santo, *HU* huancavelica, *JUN* junín, *LP* la paz, *MG* minas gerais, *PN* paraná, *PI* piura, *RJ* rio de janeiro, *SCA* santa catarina, *SC* santa cruz, *SP* são paulo

Fig. 9.1 Five cultivated chilli species. A. *Capsicum annuum*, B. *Capsicum baccatum*, C. *Capsicum chinense*, D. *Capsicum frutescens* and E. *Capsicum pubescens*

from pepper has antimicrobial properties that are important to human health (Perucka and Materska 2007).

Multipurpose uses of chilli fruits have created huge demand in the world spice trade. Annual trade of chilli is approximately 17% of world spice trade owing to its high cash values and consumption (Ahmed et al. 2000). India is the foremost producer and exporter of chilli followed by China, accounting for 26% of global pepper production. Chillies are grown in all parts of the country, both under rain-fed and irrigated conditions covering an area of 0.84 m ha, with an annual production of 21.06 mt with productivity of 2.5 t ha^{-1} (Anon 2017). Andhra Pradesh, Karnataka, Orissa, Maharashtra, Rajasthan and Tamil Nadu are the major chilli-growing states in India accounting for 80% in area and 84% in total production (Spice Board 2017).

Though India stands first in production and export followed by China, productivity in India (2.5 t ha^{-1}) is much lower than that in China (6.82 t ha^{-1}) and that of global productivity (1.86 t ha^{-1}) (Chakrabarty and Aminul Islam 2017; Geetha and Selvarani 2017). Genetic interventions are considered as the best options for maximizing productivity of chilli.

9.2 Botany

Chilli plant is an erect growing sub-herb with variable height and branched, ovate leaves with a sharp tapering point; small white flowers are borne singly or in clusters of 2 or 3 in the axils of the leaves. Fruit size and shape vary in different varieties, and fruit colour and pungency differ depending on species and variety. It is semi-perennial but usually cultivated as an annual (Hussain and Abid 2011).

Floral structure, biology and mode of pollination: Knowledge on the floral structure and biology of the crop is a prelude for any crop improvement programme. The plant produces perfect and complete flowers with calyx, corolla and male and female sex organs. *Capsicum* flower is bisexual and hypogynous with capitate stigma. Anthesis and dehiscence, pollen viability and germination and stigma receptivity change over different chilli-growing locations (Kalloo 1994). Anther dehiscence starts early morning at 5 AM and extends up to 10 AM. Stigma receptivity ranges from 2 to 4 days after flowering; these timings vary with climatic conditions prevailing in cropping location and genetic architecture of the plant. Pollen grains in chilli are light yellow to cream in colour, subspherical, pitted and tricolpate with

longitudinal grooves. Each flower produces 12,000 to 16,000 pollen grains per anther.

Basically, *Capsicum* is a self-pollinated crop, but cross-pollination does occur by entomophily. Pollination occurs after the flower opens, which allows some extent of natural cross-pollination. The extent of cross-pollination varies from 10% to over 50% (Kim et al. 2009); thus, several researchers suggested to consider chilli as a facultative cross-pollinated species (Franceschetti 1972). However, this variation depends on the genotype, the region and environment within the region.

9.3 Origin, Evolution and Domestication

Evolutionary history of any well-domesticated crop has been of interest not only for evolutionary biologists but also for plant breeders (Scaldaferro et al. 2018). By tracing back the ancestry of any domesticated plant, we can better understand the genetic evolution of that species (Gepts 2014). Advances in the phylogeographical analysis resulted in the effective investigation of sites of origin of domesticated crops (Londo et al. 2007).

Chilli has been a part of the human diet since 7500 BC in southern regions of the Americas (Bosland 1998). Archaeologists traced the sites of origin of today's most common type of chilli, *Capsicum annuum*, to have been domesticated earlier in Tehuacán Valley of Mexico at least 6000 years ago from the wild bird pepper (*C. annuum* var. *glabriusculum*) (Kraft et al. 2014). These domesticated species spread to different parts of the world by different means. They reached Europe along with Christopher Columbus, while they were introduced to Asia by Portuguese traders (Bosland and Votava 2000). Other forms/species of chilli are domesticated in different parts of the globe, such as *C. chinense*, in northern lowland Amazonia; *C. pubescens*, Andes Mountains; *C. baccatum*, lowland Bolivia (Scaldaferro et al. 2018); and *C. frutescens*, the Caribbean (Kraft et al. 2014).

9.4 Genetic Resource

A strong base of plant genetic resources is the prerequisite to initiate any crop improvement programme. Genetic resources are the treasure of any country for continuous genetic improvement of economically important crops to cater to the needs of present and future generations. Several institutes are tendering their efforts to collect, conserve, characterize, evaluate and catalogue the genetic resources in chilli considering its importance in improving different qualitative and quantitative traits. Collection and conservation of genetic resources are essential to maintain the genetic diversity of the crop.

To acquire and conserve chilli accessions from different parts of the globe is a mandate of plant genetic resources conservation and utilization division of the United States Department of Agriculture (USDA). As of 2019, it has conserved about 4878 *Capsicum* accessions belonging to 5 different cultivated species and wild relatives. AVRDC – World Vegetable Center, Taiwan, a major vegetable breeding station, plays an important role in the conservation and distribution of *Capsicum* germplasm (Table 9.2). The Center holds the world's largest collection (a total of 8165) accessions of *Capsicum* comprising 11% of all accessions held globally

Table 9.2 The list of countries/islands/territories that received pepper seeds from AVRDC – The World Vegetable Center (Lin et al. 2013)

Geographical region	Name of the country (germplasm accessions/breeding lines/total)
East-South Africa	Angola (0/60/60), Botswana (0/129/129), Ethiopia (55/241/296), Kenya (0/190/190), Madagascar (0/9/9), Malawi (0/95/95), Mauritius (1/256/257), Mozambique (0/20/20), Namibia (0/50/50), Reunion (0/18/18), Rwanda (0/20/20), Lesotho (0/95/95), Somalia (0/4/4), Sudan (0/43/43), Uganda (4/288/292), Zambia (3/110/113), Seychelles (0/49/49), South Africa (82/307/389), Suriname (0/12/12), Swaziland (0/20/20), Zimbabwe (0/81/81)
West-Central Africa	Benin (0/31/31), Burkina Faso (0/55/55), Cameroon (0/55/55), Cote d'Ivoire (0/40/40), Democratic Republic of Congo (0/25/25), Gambia (0/15/15), Ghana (13/380/393), Liberia (0/10/10), Mauritania (0/23/23), Niger (0/156/156), Nigeria (11/43/54), Senegal (0/30/30), Sierra Leone (0/10/10), Togo (0/40/40)
Europe	Austria (46/0/46), Belgium (0/10/10), the Czech Republic (1/0/1), Denmark (0/5/5), Finland (110/0/110), France (107/55/162), Germany 25/194/219), Greece (0/31/31), Hungary (3/0/3), Italy (163/82/245), Poland (5/0/5), Serbia (48/0/48), Slovenia (15/0/15), Spain (140/3/143), Sweden (60/0/60), Switzerland (0/1/1), Turkey (36/172/208), the UK (20/35/55)
Central-West Asia and North Africa	Armenia (20/83/103), Azerbaijan (0/41/41), Bahrain (0/10/10), Egypt (0/82/82), Georgia (0/23/23), Iran (61/28/89), Israel (1/21/22), Jordan (4/5/9), Kazakhstan (223/18/241), Kyrgyzstan (0/15/15), Oman (0/95/95), Qatar (0/19/19), Saudi Arabia (0/57/57), Tajikistan (0/70/70), Turkmenistan (0/37/37), Tunisia (0/47/47), United Arab Emirates (0/3/3), Uzbekistan (0/56/56)
South-East-Southeast Asia	Afghanistan (0/83/83), Bangladesh (81/270/351), Bhutan (19/84/103), Cambodia (9/349/358), North Korea (0/81/81), Japan (75/281/356), Hong Kong (0/378/378), Indonesia (23/1041/1064), Lao PDR (32/192/224), Myanmar (0/154/154), Malaysia (13/186/199), Mongolia (0/24/24), Nepal (9/34/43), Pakistan (17/433/450), the Philippines (129/353/482), Singapore (0/27/27), Sri Lanka (0/373/373)
Australia-Oceania	Australia (118/0/118), Fiji (0/164/164), Guam (0/20/20), Kiribati (0/10/10), Palau (0/8/8), Papua New Guinea (0/26/26), Samoa (0/2/2), Solomon Islands (0/25/25), Tonga (0/6/6), Vanuatu (0/15/15)
North-Central-South America	Belize (0/4/4), Bolivia (0/51/51), Barbados (0/31/31), Canada (19/0/19), El Salvador (0/33/33), Guatemala (219/50/269), Honduras (0/91/91), Mexico (0/52/52), Nicaragua (0/148/148), Panama (0/25/25), Bahamas (0/10/10), Saint Kitts and Nevis (0/25/25), Saint Vincent and the Grenadines (0/20/20), Trinidad and Tobago (4/28/32), Venezuela (0/79/79)

(Ebert 2013). The Center is also utilizing these accessions to incorporating pest resistance, developing male sterile lines and heat tolerance. As of 2019, the National Bureau of Plant Genetic Resources (NBPGR), a supreme body of plant genetic resource collection, conservation, evaluation, characterization and distribution in India, holds about 4974 accessions in its base collection. The majority of accessions conserved in NBPGR are exotic (Kalloo et al. 2005). It is the mandate of this institute to procure and provide experimental material to researchers in the country. Many horticulture and vegetable research institutes in the world and the country have been collecting and conserving chilli genetic resources based on their temporal and spatial availability. As reported by UN-FAO (1996), over 53,000 total accessions of *Capsicum* germplasm collections are held in several countries worldwide.

Diversity and characterization: The genus *Capsicum* is highly diverse, having more than 31 species in it (Moscone et al. 2007; Barboza 2011). Among them, only five species are domesticated and cultivated all over the world. Among these, *Capsicum annuum* is cultivated across the world (Tong and Boseland 2003). The species *C. annuum* comprise vast diverse types that are cultivated in different parts of the globe.

Traditionally, the diversity in chilli varieties has been established by combined characterization and evaluation of morphological and agronomic traits. To enhance the efficiency of breeding programmes, detailed characterization of *Capsicum* diversity by combining morphological, geographical and molecular data is required. Cytological or biochemical markers have also been used to evaluate genetic diversity within germplasm (Kaur and Kapoor 2001). Smith et al. (1987) classified the pepper types with 7 main categories and a total of 13 groups based on fruit type. Addition of small hot group by Bosland and Votava (2000) to the scheme is the only major change till today. The descriptors developed by the International Plant Genetic Resources Institute (IPGRI) and the Germplasm Resources Information Network (GRIN), United States Department of Agriculture are used as standardized descriptors of *Capsicum* (IPGRI AVRDC, CATIE 1995).

With the evolution and developments in DNA marker technology, several efforts to assess the overall genetic diversity of the *Capsicum* breeding germplasm have been carried out (Tam et al. 2009; Akbar et al. 2010). The advances in the high-throughput genome-wide studies using SNP markers have helped in understanding the relatedness and diversity of *Capsicum* germplasm accessions (Hill et al. 2013).

9.5 Cytogenetics

Evolutionary genetics and karyotypic stability of a species can be elucidated using cytogenetic and molecular techniques. By the studies of somatic and meiotic chromosome counts, it has been reported that all the domesticated species are composed of $n = 12$ chromosomes (Eshbaug 1964; Moscone 1992). Chromosome number of $n = 13$ was also reported in some of the wild species (Moscone et al. 2003).

Interspecies crosses between domesticated species were attempted, and partial sterility among interspecies hybrids was reported by Aniel Kumar et al. (1987). Cytological study of these interspecies hybrids revealed the genomic differences between different cultivated species leading to irregular meiosis. The *Capsicum* species has morphologically different chromosomes, viz. median, sub-median and sub-telocentric chromosomes (Cheema and Pant 2013). Based on the partial homology and partial cross-compatibility, three species, viz. *C. annuum, C. chinense* and *C. frutescens*, were grouped under a single complex called *C. annuum* complex. The other two species (*C. baccatum* and *C. pubescens*) were independently domesticated. These shreds of evidence reinforced the hypothesis of three independent lines leading to the domesticated peppers: the *C. annuum* complex, *C. baccatum* and *C. pubescens* (Moscone et al. 2007). Breeding behaviour of these *Capsicum* species is presented in Table 9.3.

As technology advanced, techniques used to study the cytology of *Capsicum* were also upgraded. Initially, researchers started using staining techniques to study karyotype; later development of chromosomal markers helped to generate more refined and detailed karyotype. Association of classical staining techniques with tools like flow cytometry and fluorescent in situ hybridization allowed researchers to confirm the evolutionary path of the species. Park et al. (1999) was the first to use the FISH technique in *Capsicum* and was able to compare and distinguish the karyotypes among domesticated species. Cytogenetic studies suggested that karyotypes in all 5 species were similar with 10 or 11 metacentric/submetacentric chromosome pairs along with a pair of acrocentric chromosomes. Recently, using gamma ray in *C. annuum* (Kumar and Raja Rao 2003) and X-ray induction in *C. baccatum* var. *pendulum* (Scaldaferro et al. 2013), polyploids were obtained and cytogenetic and somatic effects evaluated.

9.6 Genetics

9.6.1 Qualitative Traits

Qualitative traits are controlled by major genes, are less influenced by external environment and can easily be observed and characterized. Reports are available on studies of genetics of major qualitative traits, number of genes involved and their interactions. Qualitative traits such as fruit peduncle attachment, fruit orientation, fruit node^{-1}, fruit shape, fruit colour and pepper leaf curl virus resistance are well studied. The gene actions of these traits reported by researchers are presented in Table 9.4.

The association of qualitative traits will lead to joint inheritance of traits. Hence, understanding the inheritance pattern and association among these traits is important. Anilkumar and Mohan Rao (2018) performed joint segregation analysis to understand the association between fruit orientation and the number of fruit node^{-1}.

Table 9.3 Breeding behaviour of *Capsicum* species (Visalakshi and Pandiyan 2018)

Sl. No.	Crossing behaviour	Characteristics
1	*C. annuum* × *C. frutescens*	About 2% of the seeds were viable when *C. frutescens* was used as female parent The F_1 plants expressed complete pollen sterility to partial fertility Backcross to both parents had been obtained. Both F_2 and backcross plants showed higher degree of pollen sterility The L gene for tobacco mosaic resistance was successfully transferred from *C. frutescens* to *C. annuum* The transfer of resistance to the tobacco etch virus from *C. frutescens* has also been reported
2	*C. annuum* × *C. chinense*	Crosses can be made in either direction but are much easier when *C. annuum* is used as the female parent Only an occasional successful cross was obtained in the other direction The F_1 plants ranged from completely pollen sterile to moderately fertile and backcross to both the parents was obtained Considerable sterility occurred in F_2 and backcross plants. However, gene interchanges could be made with ease
3	*C. annuum* × *C. pendulum*	No viable F_1 seeds were obtained. However, F_1 plants could be obtained by culture of partially developed embryos The F_1 plants were highly self-sterile. However, an occasional F_2 plant had been obtained It appears probable that genes can be transferred from one species to the other but with difficulty
4	*C. annuum* × *C. pubescens*	Completely cross-sterile Crosses rarely resulted in fruit set, and those obtained were without seed
5	*C. frutescens* × *C. chinense*	Crosses would be obtained in either direction although with considerable difficulty when *C. chinense* was used as the female parent The F_1 plants were completely to moderately self-sterile Backcrosses to both the parents were obtained
6	*C. frutescens* × *C. pendulum*	Crosses were made in either direction, but more readily when *pendulum* was used as the female parent The F_1 plants were highly sterile, and viable F_2 seeds were very rarely formed Backcrosses to each parent resulted in only an occasional viable seed
7	*C. frutescens* × *C. pubescens*	No fruit had ever been obtained from repeated pollination in both directions
8	*C. chinense* × *C pendulum*	Crosses were made with some difficulty with *C. pendulum* as the female parent A few F_2 seeds were obtained but none were viable A limited number of backcrosses were successful

(continued)

Table 9.3 (continued)

Sl. No.	Crossing behaviour	Characteristics
9	*C. chinense* × *C. pubescens*	Fruits were formed when *C. chinense* was used as the seed parent The fruit at maturity contained many seeds with fully developed embryos but without endosperm. Such embryos could be grown only on artificial media The F_1 plants were completely sterile and could not be backcrossed to either parent
10	*C. pendulum* × *C. pubescens*	No successful crosses could be obtained

Table 9.4 Summary of genetics of qualitative traits in chilli

Traits and its different forms	Number of genes and F_2 ratio	Mode of action	References
Peduncle attachment Fleshy, strongly attached/thin, easily removed	Monogenic 3 fleshy, strongly attached/1 thin, easily removed	Fleshy, strongly attached > thin, easily removed	Uzo (1984)
Fruit colour Red/yellow Purple/non-purple	Monogenic 3 red/1 yellow Monogenic 3 purple/1 non-purple	Red > yellow Purple > non-purple	Uzo (1984), Peterson (1959)
Fruit shape Round/elongate	Monogenic 3 round/1 elongate	Round > elongate	Peterson (1959)
Fruit orientation Downward/upward	Monogenic 3 downward/1 upward	Downward > upward	Anilkumar and Mohan Rao (2018)
Fruits node^{-1} Single/cluster	Monogenic 3 single/1 cluster	Single > cluster	Anilkumar and Mohan Rao (2018)
Pepper leaf curl resistance Susceptible/resistance	Monogenic 3 susceptible/1 resistance	Susceptible > resistance	Rai et al. (2014)

Results suggest the independent segregation of genes controlling these traits. On the contrary, genes controlling fruit colour and fruit shape were found to be linked with a negligible number of recombinants (Peterson 1959). Thorough knowledge of the genetics of qualitative traits could also be used in the confirmation of true hybridity, to rule out self-pollination.

9.6.2 Quantitative Traits

Most economically important traits in chilli are reported to be quantitatively inherited. These traits are measured instead of categorized. A few examples of quantitative traits in chilli and their units of measures (in parenthesis) are yield (tons hectare^{-1}), fruit length (centimetres), fruit size (centimetres), fruits per plant,

average fruit weight (grams), capsaicin/pungency (Scoville Heat Units), oleoresin (percentage), etc. Since these traits are controlled by several genes, knowledge on the mode of action of genes controlling these traits is prerequisite for conceiving well-defined breeding strategies to develop improved cultivars. To unravel the genetics of these quantitative traits, first- or second-degree statistics and very rarely both are used (Kearsey and Pooni 1996).

Inheritance of quantitative traits in chilli varies with genetic background, environment and trait under study. First- and second-degree statistics are more commonly used to understand the mode of inheritance of these traits. Hasanuzzaman and Golam (2011) reported the complex genetic behaviour of yield and related traits and prevalence of the high magnitude of nonadditive gene effects. Anilkumar et al. (2019) reported the importance of genetic background in the inheritance of quantitative traits. They also highlighted the varied role of the magnitude of additive genetic effect and dominance genetic effect and the magnitude of additive genetic variance and dominance genetic variance in the inheritance of quantitative traits under different genetic backgrounds. Fruit size and its attributing traits are significantly influenced by both additive and dominance gene effects with predominance of dominance effect (Dhall and Hundal 2010). Fruit pericarp thickness was also reported to be a metric trait with significant influence of additive × additive and dominance × dominance interactions (Yunandra et al. 2018). Naresh et al. (2016) reported the importance of both dominance and additive gene effects in the inheritance of biochemical traits. Carotenoid content in chilli is predominantly under the control of dominance gene action, whereas capsaicinoid content is predominantly influenced by additive gene action.

9.7 Breeding Objectives

Breeding objectives in chilli depend on the available market segments (Table 9.5). Therefore breeding objectives are more specific to particular market and define the profitability of target traits. The most common and important breeding objectives/researchable issues are listed below (Bosland 1993; Yuen and Hoffman 1993; Poulos 1994; Rêgo et al. 2009, 2012; Manzur et al. 2014).

(i) *High yielding*: As in the other vegetable crops, the major breeding objective in chilli is to produce higher yields to meet the increased demand
(ii) *Early maturity*: Being an annual crop, early maturing varieties/hybrids are more preferred than late maturing types
(iii) *Fruit quality*: Visual appearance, shape, size, number, flavour, pericarp thickness, fruit firmness, endocarp/seeds ratio, provitamin A, vitamins C, E, B1, B2 and B3
(iv) *Biochemicals*: Quantity and quality of extractable oleoresin content and pungency

Table 9.5 Breeding objectives for major quality traits for specific market segment (Visalakshi and Pandiyan 2018)

S. No	Market type	Important fruit quality traits
1	Fresh market (green, red)	Colour, pungency, shape, size, flavour, exocarp thickness
2	Fresh processing (sauce, paste, canning, pickling)	Colour, pungency, flavour, pericarp thickness
3	Dried spice (whole fruit, powder)	Colour, pungency, flavour, dry matter, low crude fibre
4	Oleoresin extraction	Essential oil (colour, pungency)

(v) *Resistance to diseases*: Developing resistant cultivars against anthracnose, leaf spot, powdery mildew, root rot, bacterial leaf spot and leaf curl virus
(vi) *Resistance to insects*: Developing resistant cultivars against thrips, mites, aphids and fruit borers
(vii) *Resistance to abiotic stress*: Developing resistant cultivars against heat, water logging, chilling injury and salinity
(viii) *Ornamental quality*: Breeding for aesthetic traits like plant type, plant firmness, fruit colour, fruiting type, foliage colour, foliar type, stem anthocyanin content, flower type, flower colour, etc.

9.8 Breeding Methods

Several breeding methods can be utilized for the development of new chilli cultivars. The breeding method or strategy depends on the targeted breeding objective and availability of variability in the breeding material. The most common and widely used breeding methods in developing new cultivars can be categorized into (1) conventional methods and (2) nonconventional methods.

9.8.1 Conventional Methods

These include classical breeding methods used for crop improvement (Table 9.6). Depending upon the type of variability and objective of the breeder, these methods can be employed more effectively.

9.8.1.1 Mass Selection

Selection is purely based on the phenotype of the targeted trait; therefore, this method should be used in populations with high genetic variability. This method must be practiced in the environment where the target trait is expressed to its

Table 9.6 Main classical techniques used in *Capsicum* breeding programmes (Visalakshi and Pandiyan 2018)

Technique name	Principle	References
Mass selection	Seeds of the best plants are saved for the next growing season; oldest method	Nsabiyera et al. (2013)
Pedigree method	Keeping records of matings and their progeny. This includes making single plant selections and self-pollination	Oliveira et al. (2015)
SSD (single seed descent)	This method involves advancing the segregating of generations without selection; it is also utilized in the development of recombinant inbred lines	Ulhoa et al. (2014)
Recurrent selection	Selecting individuals from a population followed by intercrossing to form a new population	Singh et al. (2014)
Backcross	Used particularly for traits controlled by one or few genes, which involves selection of individual plants and successive crosses to a recurrent parent	Lin et al. (2015)
Hybridization	Genes of one species or variety move into another through the process of crossing	Hundal and Dhall (2005)
Mutation	Spontaneous or artificially induced variations followed by selection for desired types	Neil O. Anderson (2006)

complete potential. It is more advantageous to use for improvement of multiple traits which have high heritability. The efficiency of this method can be enhanced by rouging off types prior to flowering. Earlier, this method was used to improve landraces or open-pollinated cultivars of peppers. This technique was also used to purify pure-line varieties. In this approach, characters with high heritability are fixed, and a reasonable level of variability is also maintained. It is still used in Mexico to select seed for poblano, guajillo and other traditional pepper landraces.

9.8.1.2 Pure-Line Selection

This approach is more applicable to landraces/local cultivars being grown by farmers. In this method, superior plants from the space planted population are selected and are self-pollinated. Seeds from selected plants are harvested separately. Next season, superior performing individual plant progenies with no genetic variability within the family are bulk harvested. These seeds are used for further evaluation along with checks in replicated trails. Many varieties in India have been developed following this method. These varieties are G 1, G 2, G 3, G 4, NP 46A, K 1, Co 1, Musalwadi, Sindhur, Patna Red and Pant C 1.

9.8.1.3 Pedigree Method

This approach is based on selection of superior plants in the segregating generations upon hybridization between superior genotypes along with the maintenance of record of ancestry (Fehr 1987). Selection of superior genotypes as parental cultivars is crucial in this approach. Some chilli cultivars developed in India through this method are Andhra Jyoti, Pusa Jwala, Pusa Sadabahar, K2, Punjab Lal and Jawahar 218.

Selected superior segregants at F_2 generation should be advanced. This process of generation advancement and selection continues until desired level of homozygosity is attained. Selection is based on phenotype; it demands an environment which allows complete potential expression of traits. Selection skills of the breeder to evaluate large F_2 population to select superior genotypes with low heritability or for polygenic traits are more important.

9.8.1.4 Single Seed Descent Method

This method involves the use of single seed from each genotype to advance the generations without selection. Since no selection is performed, greenhouses can be utilized for accelerated generation advancement. This method is widely employed to generate large number of inbred lines to be used for development of hybrids. Villalon (1986) used this approach to fix the potyvirus resistance recessive genes. The method was also used to fix genes responsible for resistance to bacterial leaf spot and high yields (Moreira et al. 2009).

9.8.1.5 Backcross Breeding

This method is more effective to rectify specific drawbacks in an otherwise elite cultivar. It is more effective when the breeder aims to transfer one or few major genes. It is normally practiced to transfer genes from wild forms/primitive cultivars to leading/elite cultivar. This approach is more common in CMS conversion, for development of A, B and R lines, and is also most effectively employed to transfer disease resistance/abiotic stress resistance genes from primitive forms. A successful case of its use was the transfer of virus resistance from the species *C. chinense* to *C. frutescens* (Greenleaf 1986). The genes for TMV resistance and bacterial leaf spot resistance were transferred through backcross approach.

9.8.1.6 Recurrent Selection

This method involves interpopulation mating of two base populations to develop new improved base population. This approach is more effectively used for accumulating gene/alleles for a quantitative trait and more efficiently utilized for improving

quantitative trait with low heritability. Palloix et al. (1990a, b) used this approach in the improvement of two lines of pepper (*C. annuum*) resistant to *Verticillium dahliae* and *Phytophthora capsici*.

9.8.1.7 Heterosis Breeding

Combining genes from two contrasting parents leads to expression of hybrid vigour in the F_1. The success of hybrid development in chilli is attributed to sufficiently large number of flowers, easy emasculation and abundant pollen availability in each flower. Higher yield potential in hybrid progeny under better management and inputs prompted the farmers to shift to hybrid cultivars from OPVs. Availability of male sterility helped accelerate heterosis breeding programmes, resulting in large number of heterotic hybrids in the market. In India, private sector seed companies are aggressive in heterotic hybrid development and are ruling the chilli market in the country. Advantages of hybrids and methods of development are discussed under hybrid development section.

9.8.2 Nonconventional Breeding Methods

These are not exactly the breeding methods; these are tool to generate the new variability/new alleles when natural variability for a targeted trait is exhausted. Most often these methods produce random results, thereby demanding more attention and resources to yield expected outcome.

9.8.2.1 Distant Hybridization

This approach involves mating between individuals belonging to two different species of the same genus or two different genera of same family. Method is mainly utilized to introgress desirable genes into cultivated plants from related species. These wide crosses are more successful if the species are more closely related. Interspecific hybridization is employed to transfer genes controlling resistance to many common diseases in *C. annuum*. For example *C. baccatum* and *C. pubescens* are extremely low/nil cross compatible with *C. annuum* (Yoon et al. 2004), but they harbour resistance genes for several diseases like anthracnose (Kim et al. 2008), bacterial wilt (Matsunaga and Monma 1995), YMV (Bento et al. 2013) and tomato spotted wilt virus (Soler et al. 2015). Chilli breeding programmes utilizing the distant crosses with related species are reportedly limited (Mongkolporn and Taylor 2011). This limitation has been mainly due to presence of pre-zygotic and post-zygotic barriers which prevents fertile hybrid development leading to embryo/endosperm abortion or sterility (Yoon et al. 2004).

9.8.2.2 Mutation Breeding

This is a novel approach that generates the new alleles for a target trait by altering the base sequence through addition or deletion in the original sequence. The first mutation experiment in chilli was undertaken by Raghavan and Venkatasubban (1940). They used X-ray treatment on dry seed and noticed altered plant types. Thereafter, mutation breeding was frequently used to create new variability for selected traits. Bhargava and Umalkar (1989) used chemical and ionizing radiation to generate mutants in pericarp colour and selected stable individuals in subsequent generations. Upon chemical treatment, *C. praetermissum* produced mutants which were resistance to streptomycin (Venkataiah et al. 2005). Nascimento et al. (2015) found different forms of fruit in mutated plants and determined ethyl methanesulphonate (EMS) as an ideal mutagen. They also standardized appropriate exposure time to obtain pepper mutants with higher frequency.

9.8.2.3 Biotechnology Tools

Plant tissue culture or micropropagation and recombinant DNA technologies are powerful tools that can complement conventional breeding and expedite *Capsicum* improvement.

9.8.2.3.1 Tissue Culture

Exploiting the totipotent nature of plant cells to produce propagating material is a key biotechnology tool conceptualized by Haberlandt (1902). This tool in combination with molecular techniques is used to incorporate desirable genes in chilli. Recalcitrant nature of chilli restricted the progress rate of regeneration of plant tissues (Benson 2000). It is hard to work with *Capsicum* unlike other model crops such as tobacco, tomato and potato. Rosette shoot formation is the most important limiting factor for *Capsicum* in vitro regeneration (Arroyo and Revilla 1991). Despite these difficulties, there are several reports with relative success rates (Ochoa-Alejo and Ireta-Moreno 1990; Valera-Montero and Ochoa-Alejo 1992; Ramírez-Malagón and Ochoa-Alejo 1996; Husain et al. 1999; Venkataiah et al. 2003) available on shoot morphogenesis. Several researchers aimed to find inroads to overcome difficulties of regeneration in different species of chilli, with different combinations of growth regulators (Table 9.7).

9.8.2.3.2 Genetic Transformation

Genetic manipulation is an effective tool which combines efficient tissue culture regeneration system with recombinant DNA technology. The tool is efficient in transfer of specific genes across taxa to modify the expression of native genes.

Table 9.7 In vitro plant regeneration in chilli (Kothari et al. 2010)

Sl. No.	Species	Tissue	Mode of regeneration	PGR's + medium	References
1	*Capsicum annuum* *C. frutescens*	Cotyledon, hypocotyl	Organogenesis	BA (8.88 μM) + IAA (2.85–5.71 μM)	Gunay and Rao (1978)
2	*C. annuum*	Zygotic embryo	Organogenesis	BA (22.2 μM)	Agrawal and Chandra (1983)
3	*C. annuum*	Seedling explants	Organogenesis	BA (2.22–44.4 μM)	Phillips and Hubstenberger (1985)
4	*C. annuum*	Hypocotyl, cotyledon, stem, leaf, root, shoot tip, embryo	Organogenesis	BA (22.2 μM) + IAA (2.85–5.71 μM)	Agrawal et al. (1989)
5	*C. annuum*	Cotyledon, hypocotyl	Organogenesis	BA(2.22, 4.40, 8.90 μM) + IAA (0.57, 5.71 μM)	Arroyo and Revilla (1991)
6	*C. annuum*	Seedling explants	Organogenesis	BA (22.2 μM) + NAA (0.54 μM)	Ebida and Hu (1993)
7	*C. annuum*	Mature seeds	Organogenesis	MS without PGRs	Ezura et al. (1993)
8	*C. annuum*	Immature zygotic embryos	Direct somatic embryogenesis	2–10% sucrose, 10% CW, 2,4-D (4.53–22.6 μM)	Harini and Lakshmi Sita (1993)
9	*C. annuum*	Mature zygotic embryo	Indirect somatic embryogenesis	3% sucrose 2,4-D (4.52 μM)	Buyukalaca and Mavituna (1996)
10	*C. annuum*	Shoot tip	Axillary meristem	BA (8.88 μM)	Madhuri and Rajam (1993)
11	*C. annuum C. praetermissum*	Shoot tip	Axillary meristem	BA (66.6, 88.8 μM)	Christopher and Rajam (1994)
12	*C. annuum*	Immature zygotic embryos	Direct somatic embryogenesis	6–10% sucrose, 2,4-D (9 μM) TDZ (10 μM)	Binzel et al. (1996)
13	*C. annuum*	Cotyledon	Organogenesis	BA (8.88 μM) + IAA (2.85 μM) + $AgNO_3$ (5.85 μM)	Hyde and Phillips (1996)
14	*C. annuum* *C. praetermissum* *C. baccatum*	Hypocotyl, cotyledon, leaf	Organogenesis	BA (13.3 μM) + IAA (5.71 μM) BA (44.4 μM) BA (22.2 μM)	Christopher and Rajam (1996)
15	*C. annuum*	Wounded hypocotyls	Organogenesis	MS without PGRs	Ramírez-Malagón and Ochoa-Alejo (1996)

(continued)

Table 9.7 (continued)

Sl. No.	Species	Tissue	Mode of regeneration	PGR's + medium	References
16	*C. annuum*	Cotyledon	–	BA (13, 35 μM) + IAA (3.4–5.9 μM) + EBr (0.1 μM)	Franck-Duchenne et al. (1998)
17	*C. annuum*	Cotyledon	Organogenesis	BA (22.2–31 μM) + PAA (14.7 μM)	Husain et al. (1999)
18	*C. annuum*	Zygotic embryos	Organogenesis	BA (22.2 μM) + NAA (5.37 μM)	Arous et al. (2001)
19	*C. annuum*	Seedling explants, embryonal explants	Organogenesis	TDZ (4.5–9 μM)	Dabauza and Pena (2001)
20	*C. annuum*	Leaf, cotyledon	Organogenesis	TDZ (4.5–13.5 μM)	Venkataiah et al. (2003)
21	*C. frutescens*	Shoot tip	Axillary proliferation	BA (22.2 μM) + Kin (4.6 μM)	Sanatombi and Sharma (2007a, b)
19	*C. annuum*	Cotyledon	Organogenesis	BA (22.2 μM) + PAA (14.7 μM)	Joshi and Kothari (2007)
20	*C. annuum* *C. frutescens* *C. chinense*	Leaf, cotyledon, hypocotyl	Organogenesis	BA (8.8 μM) + IAA (11.4 μM)	Sanatombi and Sharma (2008)

Generally, *Agrobacterium tumefaciens* has been used as the vector for genetic transformation of diverse dicotyledonous species. However, biolistic bombardment has been a very useful technique to introduce foreign DNA into plant cells of monocotyledons and dicotyledonous plants. In the case of chilli, *Agrobacterium tumefaciens* is used as transformation tool to transfer genes for disease resistance from different sources. Due to very low efficiency of plant regeneration in chilli, advances made in the area of transformation are limited. Only cotyledons and leaf tissues were quite successful in producing callus (Liu et al. 1990). Some advances have been made in recent years to standardize transformation protocols in chilli with different transformation systems and varying compositions of growth regulators (Table 9.8). To overcome the poor regeneration problem, Kumar et al. (2009a) developed a tissue culture-free *Agrobacterium*-mediated *In planta* transformation in *C. annuum*. The main aim of transformation in chilli is to incorporate genes conferring disease resistance. Although advances have been made to an extent in this area, the efficiency of recovering transformed plants using *Agrobacterium tumefaciens* is very low. Other physical systems (biolistic, electroporation, etc.) still remain incomplete or inexistent to obtain transformed plants of pepper.

Table 9.8 Genetic transformation in chilli (Kothari et al. 2010)

Species	Transformation system	Tissue	Results	References
C. annuum	*Agrobacterium* strains A282 and C58; *A. tumefaciens* strain LBA 4404,	Hypocotyl, cotyledon and leaf	GUS positive	Liu et al. (1990)
C. annuum	p5T35 AD (acetolactate synthase (ALS) gene, gus, pSLJ 1911, (npt II, gus), pWTT2039 (hpt, gus))	Cotyledon	Transformed tissues, GUS positive	Engler et al. (1993)
C. annuum	*A. tumefaciens* strain LBA 4404, pROK1/105 (CMV I_{17}N-satellite RNA)	Cotyledon	Transformed plants (4% transformation frequency)	Lee et al. (1993)
C. annuum	*nptII A. tumefaciens* strain GV 3III-SE, (CMV-CP, *nptII*)	Young leaves	Transformed plants expressing (CMVCP) gene	Zhu et al. (1996)
C. annuum	*A. tumefaciens*	Young leaves	Transgenic lines	Kim et al. (1997)
C. annuum	pROK1/105 (CMV satellite RNA, nptII)	Cotyledon	Transgenic plants	Manoharan et al. (1998)
C. annuum	*A. tumefaciens* strain EHA 105, (pBI 121)(nptII, gus)	Wounded	Hairy roots	Jayashankar et al. (1997)
C. annuum	*A. rhizogenes* strain K599(gus)	Hypocotyl Cotyledon	40.8% transgenic plants	Li et al. (2003)
C. annuum	*A. tumefaciens* strain LBA 4404(nptII) *A. tumefaciens* strain EHA 105, LBA	Cotyledon Hypocotyl	Transformed plants involving callus phase	Lee et al. (2004)
C. annuum	4404,pCAMBIA 2300(TMV-CP,PPI1) *A. tumefaciens* strain	Hypocotyl	Transformed shoots	Delis et al. (2005)
C. annuum	LBA4404, pBI121(nptII) PEG-mediated transformation, pCAMBIA::Ac(hpt, gus)	Mesophyll protoplast	Efficient transient expression and transformation	Jeon et al. (2007)
C. frutescens	EHA 101 harbouring binary vector pTCL5	Hypocotyl segments	Transformation	Hasnat et al. (2008)

9.8.2.3.3 Doubled Haploids

The main goal of doubled haploid technology in breeding is to develop monoploid lines with single set of chromosomes, to develop homozygous doubled haploid pure lines and to isolate meiotic recombinants in F_1 with new genomic constitutions (Caranta et al. 1996). A shed-microspore culture to develop doubled haploids in chilli is reported as a potential method with higher per cent of DH recovery compared to other protocols (Supena et al. 2006). Shmykova et al. (2014) characterized doubled haploids produced through microsporogenesis of interspecies hybrids and reported the utility of these DHs for breeding. Anther culture stands atop in chilli DH production techniques due to abundance of experimental results and detailed

experimental protocols. The double haploid production in chilli is more prominently focused towards development of homozygous lines to be utilized in breeding.

9.9 Major Cultivar Options in Chilli

Chilli, being one of the most indispensable adjuncts in every home of the tropical world, provides a spicy taste and pungency and adds appealing colour to the food preparation. It is cultivated in different forms. Ancient chilli growers preferred to use self-harvested seed bulks to raise their next crop. This kind of chilli is called heirloom peppers since these are created naturally not commercially. Chilli is basically a self-pollinated crop; homogeneous varieties were more common cultivar choice for early day breeders. However, it is also a facultative cross-pollinated crop due to pollination by insects (Franceschetti 1972). Thus, maintaining varietal purity is quite difficult. To resolve this problem of maintaining varietal purity, breeders evolved with the idea of developing open-pollinated varieties (OPVs). Due to comparative yield levels and ease in seed production, the OPVs captured the large growing area in a short time. Many public and private breeding institutes developed a large number of OPVs to reach out to the farmers' demand.

Knowledge of heterosis encouraged breeders to develop hybrids in chilli to exploit hybrid vigour. Two categories of chilli hybrids can be developed and made available: (1) non-CMS-based hybrids and (2) CMS-based hybrids. Initially, non-CMS-based hybrids were developed and utilized as a cultivar option. Off late, CMS-based hybrids occupy extensive chilli cultivation area. Advances in the use of DNA markers in the identification and development of CMS line and restorer lines helped breeders to evolve more number of CMS-based hybrids. The time and resources required for manual emasculation and pollination in case of non-CMS-based hybrid development are overcome by the CMS system. These advantages over non-CMS-based hybrids made CMS-based hybrids to rule the present chilli market.

Even though OPVs and high-performing hybrids are available in the market, it is quite imperative that few farmers in Karnataka and Andhra Pradesh regions sample ripened fruit of best hybrids in the market and use the bulked seed for raising the next crop. This crop segregates like F_2 in the field, but theoretically, they produce very less proportion of recessive homozygotes for all the yield-attributing traits. Consciously or unconsciously farmers are exploiting the residual heterosis by following this method.

Arka Khyati, Arka Sweta, Arka Meghana and Arka Harita are few examples of hybrids released by IIHR Bengaluru. KBCH 1 is the hybrid released by University of Agricultural Sciences, Bengaluru. Byadagi Kaddi, Byadagi Dabbi, bird eye chilli, Ellachipur Sannam-S4 Type, Guntur Sannam-S4 Type, Hindupur-S7, Jwala, Kanthari white, Kashmir chilli, Madhya Pradesh G.T. Sannam, Phule Jyoti, Pusa Sadabahar and Utkal Awa are examples of released varieties across the country. Indam 5, Tejaswini, Garuda, etc. are few examples of private bred hybrids ruling the chilli market. A large number of hybrids and varieties are developed and released for farmers' cultivation across the country.

9.10 Hybrid Development

Since chilli is a seed propagated crop, F_1 hybrid development involves three major steps, viz. (i) development, maintenance and multiplication of parental lines, (ii) assessment of combining ability among the parental lines and (iii) hybrid seed production utilizing selected parental combination(s). All the three steps are equally important even though the first two steps require more attention to develop heterotic hybrids; the third step is more important in ensuring commercial viability of hybrid. Over time several mechanisms have evolved in hybrid seed production in chilli. Among them, hand emasculation with hand pollination, male sterility with hand pollination and male sterility with natural bee pollination are most commonly exploited. Development of hybrids by manual emasculation and pollination is also called as non-CMS-based hybrid development, and utilization of male sterility to develop hybrids is commonly termed as CMS-based hybrid development.

9.10.1 Non-CMS-Based Hybrid Development

The hybrids are developed by manually emasculating flowers of the designated female parent and pollinated with pollen from the desired/designated male parent. Homozygosity and purity of parental lines are maintained by self-pollination. Selected lines contrasting for yield traits are designated as male and female parents for hybridization. These selected lines are subjected to combining ability by developing experimental hybrids. Based on the performance of experimental hybrids, best-combining parents are used in commercial hybrid production. Since single emasculation and pollination produces large number of hybrid seeds, this method is economically feasible and has been successful in hybrid seed production.

9.10.2 Male Sterility-Based Hybrid Development

Use of male sterility system brings down the cost of hybrid seed production in two ways. Sharp reduction in labour expenditure of hybrid seed production can be achieved by the elimination of manual emasculation process, as it accounts for approximately 40% of the total expenditure (Yordanov 1983). Since considerable amount of natural cross-pollination can take place on the plants of female parent (Kumar et al. 2002), the expenditure on manual pollination can be saved. Two types of male sterility systems are commonly used: (1) genetic male sterility and (2) cytoplasmic-genetic male sterility system.

9.10.2.1 Genetic Male Sterility (GMS)

As the name suggests, this type of male sterility is under the control of recessive genes residing in nucleus. More than 12 recessive genes controlling male sterility have been reported (Shifriss 1997). However, only two genes, MS-12 and ms-3, are commercially utilized in India and Hungary, respectively (Kumar et al. 2000). The genes responsible for male fertility are sensitive to temperature. Threshold temperatures induce male sterility. This kind of male sterility is termed as temperature-sensitive male sterility. However, stability of such male sterility system is influenced by environmental fluctuations and genetic backgrounds. Temperature-sensitive genetic male sterility in chilli was also reported by Daskalov (1972); when temperature drops below 25 °C/17 °C day/night, breakdown of sterility was observed (Kim et al. 2013).

Genetic male sterile lines can be developed by transferring the recessive male sterile gene to elite genotype through backcrossing programme. The male sterility is under the control of recessive gene (ms ms); heterozygous male fertile (Ms ms) isogenic line should be used to maintain the male sterility. Since the male sterility is maintained through backcrossing, in hybrid seed production, 50% of fertile segregants need to identified and removed before they flower. Phenotypic marker genes which express at early seedling stage linked tightly with male sterile genes are good proposition to identify male fertile/sterile plants in the field. For example, ms-10 gene is linked with taller plant height, erect growth and dark purple anther (Dash et al. 2001) which can used as phenotypic marker. The segregation of male sterile and male fertile lines in generation of backcrossing consumes more resource and labour for identification and removal; this feature of GMS system limits its commercial exploitation.

9.10.2.2 Cytoplasmic Male Sterility (CMS)

This mechanism of male sterility was first reported in an Indian accession (Peterson 1958). The expression of male sterility in plants is the result of incompatibility between recessive nuclear gene (*rf*) and male sterile specific cytoplasmic genome. The expression of male sterility trait is under the control of mitochondrial genome (Kumar et al. 2000). It is required to identify a dominant fertility restorer gene residing in the nuclear genome for commercial exploitation of this system. Therefore, those cytoplasmic male sterile lines for which *Rf* gene(s) have been identified are widely known as genic-cytoplasmic male sterility. Since this mechanism is a combination of genes in cytoplasm and nucleus, this system is popularly known as cytoplasmic-genetic male sterility (CGMS), or in more common words it is CMS system. Based on the mode of action of restorer and maintainer alleles, CMS are of two types: (1) gametophytic and (2) sporophytic. In gametophytic system, expression of restorer allele is pollen specific. Therefore, a plant heterozygous at that locus produces 50% sterile and 50% fertile individuals upon crossing with sterile plant. In

contrast, sporophytic system produces all sterile or all fertile pollen grains (Pearson 1981).

The CMS line or A line is developed by backcrossing of a selected B line on to already available A line for six to seven generations. This will generate a pair of isogenic lines (A and B lines) on different genetic backgrounds. This CMS line or A line is maintained by crossing with isogenic maintainer line (B line), and progeny is 100% male sterile. This reduces the hybrid seed production costs and ensures the purity of F_1 Seed (Yang et al. 2008). Restorer line is developed by introgressing *Rf* allele into a identified male parent, or if restorer gene is already available in homozygous state, such male parents are directly used in hybrid seed production.

9.11 Stress Resistance Breeding

Similar to other crops, chilli production is constrained by several biotic and abiotic factors. Chilli being a sessile plant has developed several mechanisms to face adverse conditions (Abuqamar et al. 2009). Biotic and abiotic stresses are associated such that biotic stresses might be triggered by abiotic stresses (Fujita et al. 2006).

9.11.1 Biotic Stress Breeding

Chilli is affected by large number of fungal, bacterial and viral diseases and insect pests, causing major economic loss to the farmers. To mitigate the losses due to biotic agents, cultivation of resistant varieties is the best remedy. Development of resistance varieties against biotic stresses has been a part of breeder's choice for a long time (Table 9.9). Conventional phenotype-based selection is effectively utilized for development of resistance cultivars. However, it is labour intensive, requires lot of time and is expensive.

Anthracnose: Among the fungal diseases, anthracnose is a seed-borne disease caused by *Colletotrichum gloeosporioides* and *C. capsici*. Different pathotypes of this pathogen affect the chilli plant in different parts of the globe (Table 9.10). This pathogen has a wide host range of more than 460 associated plant species (Farr and Rossman 2016). In the USA, *C. acutatum* is considered to be the most destructive species as it affects both ripe and unripe pepper fruits, while *C. gloeosporioides* only affects ripe pepper fruit (Harp et al. 2008). Several agronomic, chemical control measures and combinations were attempted to control this pathogen. However, it was found expensive and highly risky to environment (Setiawati et al. 2011). Thus, one of the most economic and significant strategies to reduce crop losses is to cultivate resistant/tolerant cultivars or hybrids.

Only phenotype-based selection for resistant cultivar development is ineffective and labour intensive. Thus, utilizing molecular approaches for identifying

Table 9.9 Identified resistant/tolerant source against major insect pests diseases of chilli in India, since 2000 (Vishalakshi and Pandiyan 2018)

S. No	Biotic stress and causal organism	Name of line and reference
1	Anthracnose (*Colletotrichum* spp.)	Bhut Jolokia; PBC80 (VI046804), PBC81 (VI046805), PBC932 (VI047018); LLS, Breck-1, Breck-2, Jaun (Kaur et al. 2011)
2	Bacterial wilt (*Pseudomonas solanacearum*)	AVPP0102 (PP0107–7011), PBC66 (VI037518), PBC67 (VI037519), PBC384 (VI037548), PBC385 (VI039374), PBC535 (VI037556), MC-4
3	Phytophthora blight (*Phytophthora capsici*)	GKC29, PI201234, IC364063
4	Chilli/pepper leaf curl virus (ChiLCV)	BS35, GKC29, Bhut Jolokia (Kumar et al. 2006), CHUH-4 (Mondal et al. 2013)
5	Chilli veinal mottle virus (ChiVMV)	Individual plant selections from PBC495 (VI037455), PBC521, PBC370 (VI037453), PBC569 (VI046889), PBC371 (VI039369), Tiwari (Erect), 9852–131 (AVPP9807), Punjab Gucchedar, Perennial, Punjab Surkh, Pusa Sadabahar, Pant C1, Perennial HDV (Reddy and Reddy 2010)
6	Cucumber mosaic virus (CMV)	Perennial, PBC495 (VI037455), VC246, VR42, VR55 (Reddy and Reddy 2010), AVPP9812 (PP9852–10), Aparna, Phule Jyoti
7	Peanut bud necrosis virus (PBNV)	EC121490, IC119611 (Kalloo et al. 2005)
8	Nematode (*Meloidogyne javanica*)	EC402105, EC402113, EC405253, NIC19969, IC214965, IC214985, IC215012, EC391083, EC391087, EC378632, EC378688 (Pandravada et al. 2010)
9	Powdery mildew (*Leveillula taurica*)	Arka Harita, Arka Suphal, PBC167 (VI046819)
10	Thrips	Caleapin Red, Chamatkar, P46-A, X1068, X743, X1047, BG4, X226, X230, X233 (Kalloo et al. 2005)
11	Yellow mites (*Polyphagotarsonemuslatus*)	Jwala, RHRC Erect, AEG77 (Desai et al. 2007)
12	Aphid	LEC-28, LEC-30, LEC-34, 'Kalyanpur Red', x 1068 (Tewari et al.1985)
13	Southern root knot	Charleston Hot (Dukes and Fery 1997)

resistance source and introgressing into elite cultivar is most fruitful strategy. No commercial cultivar with resistance to anthracnose is developed in *Capsicum annuum* due to lack of resistance source in *C. annuum* gene pool (Temiyakul et al. 2012). Using the population developed from interspecies cross, Voorips et al. (2004) reported the inheritance pattern of tolerance to *Colletotrichum gloeosporioides* and *C. capsici.* The study revealed one major QTL controlling tolerance against both the pathogen species and three minor QTLs against *C. gloeosporioides.* Kim et al. (2010) identified a total of 18 resistant QTLs for *C. baccatum* out of which 2 were major and 16 were minor QTLs, respectively. Following single marker analysis,

Table 9.10 Reported causal agents of chilli anthracnose (Than et al. 2008)

Countries and regions	Causal agent	References
Australia	*Colletotrichum acutatum*, *C. atramentarium*, *C. dematium*, *C. gloeosporioides* var. *minor*, *C. gloeosporioides* var. *gloeosporioides*	Simmonds (1965)
India	*C. capsici*	Maiti and Sen (1979) and Paul and Behl (1990)
Indonesia	*C. acutatum*, *C. capsici*, *C. gloeosporioides*	Voorrips et al. (2004)
Korea	*C. acutatum*, *C. gloeosporioides*, *C. coccodes*, *C. dematium*	Park and Kim (1992)
Myanmar (Burma)	*Gloeosporium piperatum* E. and E., *C. nigrum* E. and Hals	Dastur (1920)
Papua New Guinea	*C. capsici*, *C. gloeosporioides*	Pearson et al. (1984)
New Zealand	*C. coccodes*	Johnston and Jones (1997)
Taiwan	*C. acutatum*, *C. capsici*, *C. gloeosporioides*	Manandhar et al. (1995)
Thailand	*C. acutatum*, *C. capsici*, *C. gloeosporioides*	Than et al. (2008)
UK	*C. acutatum*, *Glomerella cingulata*	Adikaram et al. (1983)
USA	*C. acutatum*	Roberts et al. (2001)

Jayaram et al. (2016) tagged SSR marker HpmsE047 to genomic region controlling anthracnose tolerance. A successful attempt was made to introgress resistant allele to *C. acutatum* by using linked SSR marker (Suwor et al. 2017). The amount of success in developing resistance to this disease through conventional and molecular approaches is still limited. Therefore, studies using PCR-based markers will be useful for breeding cultivars with enhanced resistance to anthracnose and for pyramiding resistant loci to *Colletotrichum* spp.

Powdery mildew: Another most devastating fungal disease on chilli is powdery mildew caused by *Leveillula taurica.* Incidence of this disease causes yield losses up to 80% with severe defoliation and reduced photosynthesis resulting in low fruit set and affecting marketable quality of fruits (Sivaprakasam et al. 1976; Gohokar and Peshney 1981). Inheritance of resistance to powdery mildew is complex and least understood. Daubeze et al. (1995) reported resistance to this disease to be under the control of few major genes. In contrast to this, Lefebvre et al. (2003) and several others reported resistance to this disease to be under the control of several genes. Jo et al. (2017) investigated the novel single locus controlling powdery mildew resistance (PMR1); this region is suspected as an alien introgression, and genotype sequencing-derived SNP markers indicated that *C. baccatum* is a probable source of this introgression. Wankhade and Wadikar (2018) identified two SCAR markers linked to the region controlling resistance to powdery mildew in chilli.

Bacterial leaf spot: Bacterial leaf spot caused by *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) is a major problem across the globe (Jones and Stall 1998). Three independent dominant genes (*Bs1*, *Bs2* and *Bs3*) are reported to control qualitative resistance to specific races of *X. campestris* pv. *vesicatoria* (Xcv) in peppers (Kim and Hartmann 1985; Hibberd et al. 1987). The prevalence of host-differentiated races makes it difficult to manage through conventional breeding. Further, shifts in races can also nullify the effect of resistance breeding efforts (Kousik and Ritchie 1996). AFLP markers linked to genomic regions controlling resistance to bacterial leaf spot were identified and mapped by Pierre et al. (2000). Research efforts to develop resistant chilli cultivars to bacterial leaf spot are still limited.

Bacterial blight: *Phytophthora capsici*, considered as an oomycete, is among the most destructive pathogens, which hamper potential yield of pepper worldwide (Quirin et al. 2005; Bosland 2008). Management practices against this disease are not much effective. Infestation of this pathogen was first reported in New Mexico (Garcia 1908). Adapting classical breeding approaches to transfer resistance to this pathogen is the goal of many plant breeding programmes. Different inheritance models have been reported with different sources of resistance to *P. capsici.* Thabuis et al. (2003) and Sy et al. (2005) reported resistance to this pathogen to be conferred by at least two genes or more than two genes. In contrast to this, Kim and Hur (1990) reported monogenic-biallelic control of resistance. According to Truong et al. (2012) and Curtis (2014), resistance to *P. capsici* has polygenetic inheritance based on multimodal distributions and higher-order epistasis effect. Efforts have been made to identify and transfer QTLs linked to *P. capsici* resistance into elite pepper genotypes (Kim et al. 2008; Truong et al. 2012; Liu et al. 2014; Naegele et al. 2014). Even though several molecular markers are reported to be associated with resistance to *P. capsici* (Wang et al. 2016; Xu et al. 2016), there is some level of phenotype-genotype mismatch that limits selection efficiency (Barchenger et al. 2018). Lack of R genes against different races is the major drawback of resistance breeding against this pathogen.

Leaf curl virus: Among the 65 viruses reported to be infecting chilli, chilli leaf curl virus (ChLCV) belonging to begomoviruses is the most destructive throughout the world (Nigam et al. 2015). It is the most destructive virus disease and in severe cases causes 100% marketable yield losses (Senanayake et al. 2012). This virus is transmitted by the whitefly (*Bemisia tabaci*) in persistent manner. Development of resistant cultivars is a more effective control measure than other methods against this virus. Resistance to ChLCV is controlled by single recessive gene (Kumar et al. 2009; Rai et al. 2010, 2014; Anandhi and Khader 2011). However, Jindal et al. (2018) reported that resistance is controlled by single dominant gene. These differences could be attributable to the genetic background used to study the inheritance of resistance to ChLCV. The disease can be partially controlled by managing the vectors (Kumar et al. 2009). Host plant resistance is more preferred to control the virus diseases. Out of five cultivated species in chilli, *C. frutescens* shows resistance to ChLCV (Anandhi and Khader 2011). Among the *C. annuum* species, sev-

eral germplasm lines and breeding lines showed varied levels of resistance or tolerance to ChLCV (Table 9.11).

Several other diseases and insects like *Alternaria* blight, *Cercospora* leaf spot, *Fusarium* wilt, frogeye leaf spot, CMV, whiteflies, thrips, aphids, nematodes, etc. occur in chilli. Many disease and insects can be managed by adopting appropriate agronomic practice or by applying suitable chemical measures. Developing cultivars with multiple disease and insect resistance in chilli has attracted less research attention.

Table 9.11 Chilli germplasm and breeding lines showing resistance/tolerance to leaf curl viruses in India (Thakur et al. 2018)

S. No.	Sources of resistance	References
1	Puri Red, Puri Orange	Mishra et al. (1963) and Chattopadhyay et al. (2008)
2	Jwala	Tewari and Ramanujam (1974)
3	Surjamani, Perennial, S 118, S 114 (derived from Perennial 9 Long red)	Sooch et al. (1976)
4	Perennial, S 5–4, S 20–1, S 41–1, S 118–2 – also resistant to *Tobacco mosaic virus* (TMV) and *Cucumber mosaic virus* (CMV)	Singh and Thakur (1977)
5	Pant C-1, Pant C-2 – tolerant to leaf curl virus	Mathai et al. (1977)
6	Delhi Local – tolerant to leaf curl virus, also tolerant to TMV, immune to CMV and PVX	Konai and Nariani (1980) and Tewari and Viswanath (1986)
7	Cross 218, EC 121490, IC 18253, IC 18885, JCA 196, Karanja, Pant C-I – less than 30% leaf curl incidence in the field	Bhalla et al. (1983)
8	CA-960, G-4, Jwala	Dhanju (1983)
9	Lorai, Longi, Pant C-I, Perennial, S 118–2 – resistant/tolerant to leaf curl virus, also resistant/tolerant to CMV and TMV	Sharma and Singh (1985)
10	JCA 196, JCA 218, JCA 248, NP-46-A, Pant C-I, Pusa Jwala	Sangar et al. (1988) and Brar et al. (1989)
11	Bangla Green (BG-1), CH-1, Indonesian Selection, Laichi-1, Laichi-2, Lorai, LS-l, MF41–1, MS- 13, Pant C-I, Perennial, Punjab Lal, S 20–1, Surjamani – field resistant to leaf curl virus, also field resistant to CMV	Singh and Kaur (1990)
12	Surajmukhi, Japani Loungi, Pant Chilli-1, Pusa Jwala and PBC-473	Awasthi and Kumar (2008)
13	Punjab Sindhuri and Punjab Tej – moderate resistant to leaf curl virus	Dhaliwal et al. (2013)
14	CH-27 – F_1 hybrid highly resistant to leaf curl virus	Dhaliwal et al. (2015)
15	Saurian 2010, Perennial and Japani Loungi	Ahmad et al. (2016)
16	DLS-Sel-10, WBC-Sel-5 and PBC-142	Srivastava et al. (2017)

9.11.2 Abiotic Stress

Climate change and global warming have been well-accepted facts. Changing climate influences the crop production as the plants need to adapt to challenges posed by the climate change. Also, development of cultivars that can cope with adverse climatic conditions like heat, cold, drought and other climate extremes due to global warming may be the single most important step one can take to adapt to today's changes and in the future. Plants respond to stresses differentially at different physiological growth stages for varied types of stress (Bray 2002).

Drought stress: Inadequate and uneven distribution of rainfall leading to unexpected drought is a biggest challenge for chilli productivity worldwide. Inadequate water availability to plant hampers several physiological processes and directly or indirectly affects the productivity. Chilli plants are more sensitive to drought at vegetative state than flowering and fruiting stage (Okunlola et al. 2017). Cultivars with higher pungency have high water retention capacity and are relatively tolerant to drought stress (Phimchan and Techawongstien 2012). Photosynthetic efficiency and root volume are the traits well associated with drought in pepper (Kevin 2007). Since the root volume trait is quantitative with poor heritability, it is a challenge to breeders to breed varieties with higher root volume (Chaitra et al. 2006). Tolerance index (TI), mean productivity (MP) and *per cent* injury (%I) are considered as drought indices for selecting tolerant breeding lines (Showemimo and Olarewaju 2007). However, research reports on breeding resistance to drought stress in chilli are scanty.

Heat stress: As a consequence of global warming, heat stress is gaining importance in all the crops. Heat stress associated with drought and other abiotic stresses lead severe loses in agricultural production (Mittler 2006). The optimal temperature range for growing pepper is 20–30 °C. Temperature higher than this range can affect pollination and fertilization significantly (Guo et al. 2014). Under heat stress, heat shock proteins are produced, and the protein homoeostasis is maintained (Li et al. 2015). Heat shock transcription factors (TEs) induce production of chaperones or heat shock proteins which help in conformational changes in the protein structure and allow to maintain physiological interactions (Ahrman et al. 2007). Heat stress tolerance breeding is still in its juvenile stage in chilli. There is an acute need to screen available germplasm including different species in appropriate artificial screening conditions to identify heat stress-tolerant accessions.

Other abiotic stress resistance breeding research efforts, viz. salinity tolerance, cold tolerance, ion stress tolerance, freezing tolerance, etc., are limited in chilli.

9.12 Quality Breeding

There are three important quality-determining characters in chilli, viz. pungency or capsaicin content, oleoresin content and ascorbic acid or vitamin C content. Consideration of quality parameters varies with users and uses of chilli. Therefore, it is difficult to produce a single definition for quality in chilli. To plan breeding objectives for quality breeding, one should have sufficient knowledge on the pattern of inheritance of these traits. However, studies on nature and magnitude of gene actions controlling these quality traits in chilli are very limited. Therefore, very pertinent and highly valuable reports are reviewed and presented under corresponding quality trait.

Pungency: Pungency is defined as 'sharp, piercing, stinging, biting or penetrating quality' or 'power to stimulate' (Bosland 1995). The alkaloid capsaicinoids found only in genus *Capsicum* is the cause of pungency in chilli. Chemically, capsaicin is also known as n-vanillyl-8-methyl-6-(e)-nonenamide, which is a most pungent group. By relying on the organoleptic tests, Greenleaf (1952) and Dempsey (1960) reported that the pungency is dominant over non-pungency and inherited monogenically. As the technology developed, use of several techniques to quantify capsaicin content postulated that pungency is a polygenically controlled trait (Quagliotti and Ottaviano 1969). Dhall and Hundal (2005) reported the preponderance of additive gene effects in the control of capsaicin content, and it can be exploited using simple selection. Zewdie and Bosland (2000) reported different number of effective factors and varied number of genes with differential gene action in the inheritance of capsaicinoids.

Oleoresin content: Bosland and Votava (2000) identified more than 30 different colour pigments in chilli. Capsanthin and capsorubin components of oleoresin constitute more than 60% of the total carotenoids present in chilli fruits. As the fruit development progresses, capsanthin content increases gradually (Bosland 1996). Increased temperature at fruit ripening stage reduces capsanthin content (Orak and Demirchi 2005). Earlier studies reported single dominant gene controlling red fruit colour in chilli, and later it was reported that three genes controlled colour synthesis in chilli (Popovsky and Paran 2000). Ahmed et al. (1994) reported the importance of additive gene actions in the inheritance of red fruit colour in chilli. Bal and Singh (1995) indicated duplicate dominant genes to be controlling red fruit colour. There are no clear-cut evidences on the genetics of fruit colour in chilli.

Vitamin C content: Chilli is a rich source of vitamin C, and green chilli fruits contain more vitamin C than the citrus fruits (Chigoziri and Ekefan 2013). Vitamin C content gradually increases with fruit maturity and reduces as fruit ripens, and red dry fruits have almost negligible quantity (Martínez et al. 2005). Very limited reported evidences are available on vitamin C content in chilli, and available evidences are not sufficient to explain the genetics of the inheritance of this trait.

9.13 Genomics and Molecular Approaches

Similar to other economically important crops, there are several research attempts in chilli improvement using DNA markers. Marker systems like non-PCR-based (RFLP) and PCR-based makers (RAPD, AFLP, SSR, ISSR, SNP, etc.) have supplemented in enhancing the efficiency in chilli breeding. These marker systems are efficiently utilized from genetic diversity analysis to fine mapping of traits controlled by quantitative genes. The availability of large genetic resources has accelerated the development of high density genetic maps. Tanksley et al. (1988) reported one of the first genetic maps constructed using RFLP markers in interspecific cross between *C. annuum* and *C. chinense* with wide genome coverage. Thereafter, several researchers developed *Capsicum* genetic maps with different marker systems. Development of microsatellite markers accelerated the DNA marker-dependent breeding in chilli and paved the path for marker-assisted breeding. For the first time, Huang et al. (2001) used microsatellite markers in *Capsicum* species. Minamiyama et al. (2006) developed the first microsatellite-based genetic map in chilli. Sugita et al. (2013) developed 265 pairs of SSR primers that were used to build a precise genetic map. Most comprehensive DNA marker dense genetic maps have been published by Qin et al. (2014) and Kim et al. (2014). Even though more than 700 microsatellite markers have been developed and are available in *Capsicum* sp., still it is insufficient to cover the oversized genome size of 3.84 GB (Kong et al. 2012).

DNA markers have been extensively used by breeders in different breeding activities. The adoption of marker technology has enhanced the pace of breeding activities, especially breeding for disease resistance. Mimura et al. (2012) developed a linkage map with microsatellite markers placed into 12 linkage groups. It is believed that development of genetic maps for quantitative traits called QTL mapping facilitates the understanding of complex inheritance of complex trait. Several studies in pepper involving QTL mapping for important traits have been reported (Alimi et al. 2013). Most of these studies focused on commercial characters like fruit quality and production (Wang et al. 2004; Lee et al. 2011). In addition to this, several reports are available about the use of molecular markers for mapping QTLs governing disease resistance (Sugita et al. 2006; Kim et al. 2011). In contrast to this, very limited reports are available on identification of QTL related to vegetative parts (Mimura et al. 2010). The QTL related to fruit length explaining about 27% of total fruit length variation was mapped on to linkage group three by Lee et al. (2011).

Development of molecular markers and identification of genes/QTLs linked to commercially important traits paved the path for marker-assisted breeding. Jeong et al. (2015) performed marker-assisted backcrossing to incorporate gene controlling higher capsaicinoid content from *C. chinense* to *C. annuum*. Tanaka et al. (2014) employed marker-assisted selection to develop new cultivars with high capsaicinoid content and low pungency. Two major recessive genes controlling anthracnose resistance in chilli were introgressed from *C. baccatum* and *C. chinense* to *C. annuum* through marker-assisted backcross breeding (Suwor et al. 2017).

For mining new genes from population with effective utilization of historical linkage disequilibrium, genome-wide association mapping is most effective. Nimmakayala et al. (2016) employed genome-wide association mapping to identify markers associated with fruit weight and capsaicinoids. They were able to identify 16 SNPs which are strongly associated with fruit weight in diverse collection of *C. annuum* accessions. Nimmakayala et al. (2014) demonstrated strong association of eight genome-wide SSRs to fruit weight that contributed an average effect of 15%.

An upgraded version of MAS selection to predict and select the best individuals from the population is genomic selection (GS). Selection is based on genomic estimated breeding values (GEBVs) which are computed effect of all the loci genome utilizing genome-wide markers. Oversized genome of chilli has limited development of genome-wide markers, utilizing them in genomic prediction and selection based on GEBVs. There are only few reports on the genomic selection/predictions in chilli.

9.14 Future Prospectus

Even though extensive chilli improvement research on different aspects have been conducted, there are few areas demanding extensive research. Developing hybrids as commercial cultivars utilizing male sterility systems is a boon for seed industry. However, it is largely required to diversify male sterile cytoplasm to avoid the probable outbreak of diseases. Utilizing biotechnological tools like doubled haploid production and transformation techniques to accelerate the *Capsicum* breeding research is need of the hour. Efforts should be made in the direction of development and use of DNA marker resources to deploy MAS in breeding for disease or pest resistance/tolerance through GWS. Focusing towards identifying best individuals with accurate genomic estimated breeding values to develop best parents for hybrid breeding is the thirst area of research.

References

Abuqamar S, Luo H, Laluk K, Mickelbart MV, Mengiste T (2009) Crosstalk between biotic and abiotic stress responses in tomato is mediated by the AIM1 transcription factor. Plant J 58:347–360

Adikaram NKB, Brown A, Swinburne TR (1983) Observations on infection of *Capsicum annuum* fruit by *Glomerella cingulata* and *Colletotrichum capsici*. Trans Br Mycol Soc 80:395–401

Agrawal S, Chandra N (1983) Differentiation of multiple shoot buds and plantlets in cultured embryos of *Capsicum annuum* L. var. *Mathania*. Curr Sci 52:645–646

Agrawal S, Chandra N, Kothari SL (1989) Plant regeneration in tissue cultures of pepper (*Capsicum annuum* L. cv. Mathania). Plant Cell Tissue Organ Cult 16:47–55

Ahmad A, Sharma A, Zehra SB, Kang SS, Bhat M, Hussain A (2016) Evaluation of chilli genotypes against *Chilli leaf curl virus*. Indian J Ecol 43:144–147

Ahmed N, Bhat MY, Tanki M (1994) Genetics of red and purple fruit colour in chilli (*Capsicum annuum* L.). Veg Sci 21:151–153

Ahmed J, Shivhare US, Raghavan GSV (2000) Rheological characteristics and kinetics of color degradations of green chilli puree. J Food Eng 44:239–244

Ahrman E, Lambert W, Aquilina JA, Robinson CV, Emanuelsson CS (2007) Chemical cross-linking of the chloroplast localized small heat-shock protein, Hsp21, and the model substrate citrate synthase. Protein Sci 16(7):1464–1478

Akbar N, Ahmad H, Ghafoor S, Begum K, Afridi SG, Muhammad I, Khan IA (2010) Estimation of genetic diversity in *Capsicum* germplasm using randomly amplified polymorphic DNA. Asian J Agric Sci 2:53–56

Alimi NA, Bink MCAM, Dieleman JA, Nicolai M, Wubs M, Heuvelink E, Magan J, Voorrips RE, Jansen J, Rodrigues PC, Van der Heijden GWAM, Vercauteren A, Vuylsteke M, Song Y, Glasbey C, Barocsi A, Lefebvre V, Palloix A, Eeuwijk AF (2013) Genetic and QTL analyses of yield and a set of physiological traits in pepper. Euphytica 190:181–201

Anandhi K, Khader KM (2011) Gene effects of fruit yield and leaf curl virus resistance in interspecific crosses of chilli (*Capsicum annuum* L. and C. frutescens L.). J Trop Agric 49:107–109

Anderson NO (2006) In: Anderson NO (ed) *Flower breeding and genetics: issues, challenges and opportunities for the 21st century*. Springer Science and Business media

Aniel Kumar O, Panda RC, Raja Rao KG (1987) Cytogenetic studies of the F_1 hybrids of *Capsicum annuum* with *C. chinense* and *C. baccatum*. Theor Appl Genet 74:242–246

Anilkumar C, Mohan Rao A (2018) Genetics of fruiting habit traits in chilli (*Capsicum annuum* L.). Mysore J Agric Sci 52(2):293–299

Anilkumar C, Mohan Rao A, Ramesh S, Bhavani B, Pranesh (2019) Genetics of fruit yield and its component traits under different fruiting habit backgrounds in chilli (*Capsicum annuum* L.). J Genet 98(3):84

Anonymous (2017) Ministry of agriculture, Government of India. www.indiastat.com

Arous S, Boussaid M, Marrakchi M (2001) Plant regeneration from zygotic embryo hypocotyls of Tunisian chili (*Capsicum annuum* L.). J Appl Hortic 3:17–22

Arroyo R, Revilla MA (1991) *In vitro* plant regeneration from cotyledon and hypocotyl segments in two bell pepper cultivars. Plant Cell Rep 10:414–416

Awasthi LP, Kumar P (2008) Response of chilli genotypes/cultivars against viral diseases. Indian Phytopathol 61:282–284

Bal SS, Singh J (1995) Inheritance of qualitative characters in chilli (*Capsicum annuum* L.). J Res Punjab Agric Univ 32:39–45

Barboza GE (2011) Lectotypifi cations, synonymy, and a new name in *Capsicum* (Solanoideae, Solanaceae). PhytoKeys 2:23–38

Barchenger DW, Lamour KH, Bosland PW (2018) Challenges and strategies for breeding resistance in *Capsicum annuum* to the multifarious pathogen, *Phytophthora capsici*. Front Plant Sci 9:628

Benson EE (2000) Special symposium: in vitro plant recalcitrance do free radicals have a role in plant tissue culture recalcitrance? In Vitro Cell Dev Biol Plant 86:163–170

Bento CS, Rodrigues R, Gonçalves LS, Oliveira HS, Santos MH, Pontes MC (2013) Inheritance of resistance to Pepper yellow mosaic virus in *Capsicum baccatum var. pendulum*. Genet Mol Res 12:1074–1082. https://doi.org/10.4238/2013.April.10.3. PMID: 23661433

Bhalla PL, Baghel BS, Krishana A (1983) A screening of chilli cultivars/lines against leaf curl and anthracnose. In: Proceedings of national seminar production technology of tomato and chillies. Tamil Nadu Agricultural University, Coimbatore, p 147

Bhargava YR, Umalkar GV (1989) Productive mutations induced in *Capsicum annuum* by physical and chemical mutagens. Acta Hortic 253:233–238

Binzel ML, Sankhla N, Joshi S, Sankhla D (1996) Induction of direct embryogenesis and plant regeneration in pepper (*Capsicum annuum* L.). Plant Cell Rep 15:536–540

Bosland PW (1993) Breeding for quality in *Capsicum*. Capsicum Eggplant Newsl 12:25–31

Bosland PW (1995) *Capsicum*: history, cultivation and uses. Species, herbs and edible fungi. Elsevier Publ, New York, pp 347–366
Bosland PW (1996) *Capsicums* innovative uses of an ancient crop. In: Janick J (ed) Progress in new crops. ASHS Press, Arlington, pp 479–487
Bosland PW (1998) *Capsicums*: innovative uses of an ancient crop. In: Janick J (ed) Progress in new crops. ASHS Press, Arlington, pp 479–487. Retrieved 23 Dec 2010
Bosland PW (2008) Think global, breed local: specificity and complexity of *Phytophthora capsici*. In: 19th international pepper conference, Atlantic City
Bosland PW, Votava EJ (2000) Peppers: vegetable and spice *Capsicums*. CABI Publishing, Wallingford, pp 1–16
Brar SS, Rewal HS, Singh D, Singh H, Hundal JS (1989) Screening of indigenous germplasm of chillies against virus disease in the south western region of Punjab. Plant Dis Res 4:180
Bray EA (2002) Abscisic acid regulation of gene expression during water-deficit stress in the era of the Arabidopsis genome. Plant Cell Environ 25(2):153–161
Buyukalaca S, Mavituna F (1996) Somatic embryogenesis and plant regeneration of pepper in liquid media. Plant Cell Tissue Organ Cult 46:227–235
Caranta C, Pallo A, Gebre-S K, Lefebvre V, Daubeze AM (1996) A complementation of two genes originating from susceptible *Capsicum annuum* lines confers a new and complete resistance to pepper veinal mottle virus. Phytopathology 86:739–743
Cecchini NM, Moscone EA (2002) Análisis cariotípico en dos variedades de *Capsicum baccatum* (Solanaceae) mediante bandeos cromosómicos AgNOR y de fluorescencia. Actas XXXI Congreso Argentino de Genética, La Plata, Argentina, 17–20 September. J Basic Appl Genet 15(Suppl):86
Cecchini NM, Scaldaferro MA, Barboza GE, Moscone EA (2003) Estudio de cromosomas mitóticos en dos ajíes silvestres de flores púrpuras (*Capsicum* – Solanaceae) mediante bandeo de fluorescencia. Actas IV Jornadas Argentino-Chilenas de Genética, Huerta Grande, Argentina, 21–24 September. J Basic Appl Genet 15(Suppl. 2):91
Chaitra J, Vinod MS, Sharma N, Hittalmani S, Shashidhar HE (2006) Validation of markers linked to maximum root length in rice (*Oryza sativa* L.). Curr Sci 6:835–838
Chakrabarty S, Aminul Islam AKM (2017) Selection criteria for improving yield in chili (*Capsicum annuum* L.). Adv Agric 2017:1–10
Chattopadhyay B, Singh AK, Yadav T, Fauquet CM, Sarin NB, Chakraborty S (2008) Infectivity of the cloned components of a begomovirus: DNA beta complex causing chilli leaf curl disease in India. Arch Virol 153:533–539
Cheemaa SK, Pant MR (2013) Karyotype analysis of seven cultivated varieties of *Capsicum annuum* L. Caryologia Int J Cytol Cytosyst Cytogenet 66(1):70–75
Chigoziri E, Ekefan EJ (2013) Seed-borne fungi of Chilli pepper (*Capsicum frutescens*) from pepper producing areas of Benue state, Nigeria. Agric Biol J Am 4:370–374
Christopher T, Rajam MV (1994) *In vitro* clonal propagation of *Capsicum* spp. Plant Cell Tissue Organ Cult 38:25–29
Christopher T, Rajam MV (1996) Effect of genotype, explant and medium on *in vitro* regeneration of red pepper. Plant Cell Tissue Organ Cult 46:245–250
Curtis MR (2014) QTL Mapping of *Phytophthora capsici* resistance and horticultural traits in pepper (*Capsicum annuum*) recombinant inbred line population. MS thesis, University of California, Davis
Dabauza M, Pena L (2001) High efficiency organogenesis in sweet pepper (*Capsicum annuum* L.) tissues from different seedlings explants. Plant Growth Regul 33:221–229
Dasgupta, Reshmi R (2011) Indian chilli displacing jalapenos in global cuisine – The Economic Times. The Times of India
Dash SS, Sanjeet K, Singh JN (2001) Cytomorphological characterization of a nuclear male sterile line of chilli pepper (*Capsicum annuum* L.). Cytologia 66:365–371
Daskalov S (1972) Male sterile pepper (*C. annuum* L.) mutants and their utilisation in heterosis breeding. In: Proceedings of Eucarpia meeting. Capsicum 7: 202210

Dastur JF (1920) *Glomerella cingulata* (Stoneman) Spald and its conidial form, *Gloeosporium piperatum* E and E and *Colletotrichum nigrum* E and Hals on chillies. Ann Appl Biol 6(4):245–268. https://doi.org/10.1111/j.1744-7348.1920.tb06472.x

Daubeze AM, Hennart JW, Palloix A (1995) Resistance to *Leveillula taurica* in pepper (*Capsicum annuum*) is oligogenically controlled and stable in Mediterranean regions. Plant Breed 114(4):327–332

Delis M, Garbaczewska G, Niemirowicz-Szczytt K (2005) Differentiation of adventitious buds from *Capsicum annuum* hypocotyls after co-culture with *Agrobacterium tumefaciens*. Acta Biol Cracov 17:193–198

Dempsey AH (1960) Inheritance studies of certain fruit and plant characters in *Capsicum frutescens*. Doctoral dissertation, The Ohio State University

Desai HR, Bandania KA, Rai AB, Patel AJ (2007) Assessment of yield loss and resistance to yellow mite Polypagotarsonemus latus (Bank) in chilli. Veg Sci 34:46–50

Dhaliwal MS, Jindal SK, Cheema DS (2013) Punjab Sindhuri and Punjab Tej: new varieties of chilli. J Res Punjab Agric Univ 50:79–81

Dhaliwal MS, Jindal SK, Cheema DS (2015) CH-27: a multiple disease resistant chilli hybrid. Agric Res J 52(4):127–129

Dhall RK, Hundal JS (2005) Genetic analysis of capsaicin content of red ripe fruits in chilli (*Capsicum Annuum* L.). J Res 42:38-43

Dhall RK, Hundal JS (2010) Genetics of fruit size in chilli. Indian J Hortic 67(1):117–121

Dhanju BS (1983) Response of different chilli *(Capsicum annuum* L.) genotypes to split application of nitrogen. Thesis Abstract. 91(7): 02

Dukes PD, Fery RL (1997) Charleston Hot: a southern root knot nematode resistant yellow fruited cayenne pepper with a compact plant habit. Hortic Sci 32:947–948

Ebert AW (2013) *Ex Situ* conservation of plant genetic resources of major vegetables. In: Normah MN, Chin HF, Reed BM (eds) Conservation of tropical plant species. Springer Science-Business Media, New York, pp 373–417

Ebida AIA, Hu CY (1993) *In vitro* morphogenetic responses and plant regeneration from pepper (*Capsicum annuum* L. cv. Early California Wonder) seedling explant. Plant Cell Rep 13:107–110

Engler DE, Guri AZ, Lauritis JA, Schloemer LMP (1993) Genetically transformed pepper plants and method for their production. USA Patent 5:262–316

Eshbaugh LH (1964) A numerical taxonomic and cytogenetic study of certain species of the genus *Capsicum.* Ph.D. Thesis, Indiana University

Eshbaugh WH, Smith PG, Nickrent DL (1983) *Capsicum tovarii* (Solanaceae), a new species of pepper from Peru. Brittonia 35:55–60

Ezura H, Nishimiya S, Kasumi M (1993) Efficient regeneration of plants independent of exogenous growth regulators in bell pepper (*Capsicum annuum* L.). Plant Cell Rep 12:676–680

FAO (1996) Report on the state of the world's plant genetic resources for food and agriculture. Rome

Farr DF, Rossman AY (2016) Fungal databases, systematic mycology and microbiology laboratory. USDA: ARS. Available online: https://nt.ars-grin.gov/fungaldatabases/

Fattori V, Hohmann MS, Rossaneis AC, Pinho-Ribeiro FA, Verri WA (2016) Capsaicin: current understanding of its mechanisms and therapy of pain and other pre-clinical and clinical uses. Molecules 21(7):844

Fehr WR (1987) Principles of cultivar development: theory and technique, vol-1. Macmillan, New York, p 736

Franceschetti U (1972) Natural cross-pollination in pepper (*Capsicum annuum* L.). In: Belletti P, Nassi MO, Quagliotti L (eds) Proceeding Eucarpia meeting on genetic and breeding of *Capsicum*, Turin, pp 346–353

Franck-Duchenne M, Wang Y, Ben Tahar S, Beachy RN (1998) *In vitro* stem elongation of sweet pepper in media containing 24-epi-brassinolide. Plant Cell Tissue Organ Cult 53:79–84

Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki K (2006) Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr Opin Plant Biol 9:436–442

Garcia F (1908) Chile culture. New Mexico College of Agriculture and Mechanic Arts

Geetha R, Selvarni K (2017) A study of chilli production and export from India. Int J Adv Res Innov Ideas Educ 3(2):205–210

Gepts P (2014) Birthplace of the domesticated chilli pepper identified in Mexico. ScienceDaily, 21 Apr 2014

Ghasemnezhad M, Sherafati M, Payvas GA (2011) Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicum annuum* L.) fruits at two different harvest times. J Funct Foods 3:44–49

Gohokar RT, Peshney NI (1981) Chemical control of powdery mildew of chilli. Indian J Agric Sci 51:663–665

Gonzalez M, Bosland P (1991) Strategies for stemming genetic erosion of *Capsicum* germplasm in the Americas. Diversity 7:52–53

Greenleaf WH (1952) Inheritance of pungency and of the deciduous character in chili (*Capsicum annuum*). Proc Assoc 8th Agric Wkrs 49:110–111

Greenleaf WH (1986) Pepper breeding. In: Basset MJ (ed) Breeding vegetable crops. AVI, Wesport, pp 69–127

Gunay AL, Rao PS (1978) In vitro plant regeneration from hypocotyl and cotyledon explants of red pepper (*Capsicum*). Plant Sci Lett 11:365–372

Guo W, Chen R, Du XZ, Yin Y, Gong Z, Wang G (2014) Reduced tolerance to abiotic stress in transgenic *Arabidopsis* overexpressing a *Capsicum annuum* multiprotein bridging factor. BMC Plant Biol 14:138

Haberlandt G (1902) Plant cell culture experiment with isolierten. SB Vienna Ways Sci 111:69–92

Harini I, Lakshmi Sita G (1993) Direct somatic embryogenesis and plant regeneration from immature embryos of chilli (*Capsicum annuum* L.). Plant Sci 89:107–112

Harp TL, Pernezny K, Lewis Ivey ML, Miller SA, Kuhn PJ, Datnoff L (2008) The etiology of recent pepper anthracnose outbreaks in Florida. Crop Prot 27:1380–1384

Hasanuzzaman M, Golam F (2011) Gene actions involved in yield and yield contributing traits of chilli (*Capsicum annuum* L.). Aust J Crop Sci 5(13):1868–1875

Hasnat R, Abbasi NA, Hafiz IA, Ahmad T, Chudhary Z (2008) Effect of different bacterial dilutions on transformation efficiency of hot chilli (*Capsicum frutescens* L.) varieties. Pak J Bot 40:2655–2662

Hibberd AM, Stall RE, Bassett MJ (1987) Different phenotypes associated with incompatible races and resistance genes in bacterial spot disease of pepper. Plant Dis 71:1075–1078

Hill TA, Ashrafi H, Reyes-Chin-Wo S, Yao J, Stoffel K, Truco MJ, Kozik A, Michelmore RW, Van Deynze A (2013) Characterization of *Capsicum annuum* genetic diversity and population structure based on parallel polymorphism discovery with a 30K unigene Pepper GeneChip. PLoS One 8(2):e56200. https://doi.org/10.1371/journal.pone.0056200

Huang S, Zhang B, Milbourne D, Cardle DL, Yang G, Guo J (2001) Development of pepper SSR markers from sequence databases. Euphytica 117:163–167

Hundal JS, Dhall RK (2005) Breeding for hybrid hot pepper. J New Seeds 6(2–3):31–50

Husain S, Jain A, Kothari SL (1999) Phenylacetic acid improves bud elongation and *in vitro* plant regeneration efficiency in *Capsicum annuum* L. Plant Cell Rep 19:64–68

Hussain F, Abid M (2011) Pest and diseases of Chilli crop in Pakistan: a review. Int J Biol Biotechnol 8(2):325–332

Hyde C, Phillips GC (1996) Silver nitrate promotes shoot development and plant regeneration on chile pepper (*Capsicum annuum* L.) via organogenesis. In Vitro Plant 32:72–80

IPGRI, AVRDC, CATIE (1995) Descriptors for *Capsicum* (*Capsicum* spp.). International Plant Genetic Resources Institute, Rome

Jayaram N, Rao AM, Ramesh S, Manjunath B, Mangalagowri N, Ashwini M (2016) Tagging ssr markers to genomic regions associated with anthracnose disease resistance and productivity *per se* traits in hot pepper (*Capsicum annuum* L.). Environ Ecol 34(4A):1440–1446

Jayashankar S, Bagga S, Phillips GC (1997) Sweet pepper (*Capsicum annuum*) transformation using *Agrobacterium rhizogenes*. Hortic Sci 32:454

Jeon JM, Ahn NY, Son BH, Kim CY, Han CD, Kim GD (2007) Efficient transient expression and transformation of PEG-mediated gene uptake into mesophyll protoplasts of pepper (*Capsicum annuum* L.). Plant Cell Tissue Organ Cult 88:225–232

Jeong HS, Jang S, Han K, Kwon JK, Kang BC (2015) Marker-assisted backcross breeding for development of pepper varieties (*Capsicum annuum*) containing capsinoids. Mol Breed 35(12):226

Jindal SK, Dhaliwal MS, Sharma A, Thakur H (2018) Inheritance studies for resistance to leaf curl virus disease in chilli (*Capsicum annuum* L.). Agric Res J 55:757–760

Jo J, Venkatesh J, Han K, Lee HY, Choi GJ, Lee HJ, Choi D, Kang BC (2017) Molecular mapping of PMR1, a novel locus conferring resistance to powdery mildew in pepper (*Capsicum annuum*). Front Plant Sci 8:2090

Johnston PR, Jones D (1997) Relationships among *Colletotrichum* isolates from fruit-rots assessed using rDNA sequences. Mycologia 89(3):420–430. https://doi.org/10.2307/3761036

Jones JB, Stall RE (1998) Diversity among xanthomonads pathogenic on pepper and tomato. Annu Rev Phytopathol 36:41–58

Joshi A, Kothari SL (2007) High copper levels in the medium improves differentiation and elongation from cultured cotyledons of *Capsicum annuum* L. Plant Cell Tissue Organ Cult 88:127–133

Kalloo G (1994) Vegetable breeding. Panima Educational Book Agency, New Delhi, pp 22–23

Kalloo G, Srivastava U, Singh M, Kumar S (2005) Solanaceous vegetables. In: Dhillon BS, Tyagi RK, Saxena S, Randhawa GJ (eds) Plant genetic resources: horticultural crops. Narosa Publishing House, New Delhi, pp 19–33

Kaur C, Kapoor HC (2001) Antioxidants in fruits and vegetables–the millennium's health. Int J Food Sci Technol 36(7):703–725

Kaur N, Singh DJ, Singh KD (2011) Physiological and biochemical traits analysis of *Capsicum annuum* L. germplasm for resistance *to Colletotrichum capsici*. J Cell Plant Sci 2:12–21

Kearsey MJ, Pooni HS (1996) The genetic analysis of quantitative traits. Chapman and Hall, London

Kevin MC (2007) Pepper. Handbook of plant breeding vegetables II. Fabaceae, Liliaceae, Solanaceae, and Umbelliferae. Springer, New York, pp 221–248

Kim BS, Hartmann RW (1985) Inheritance of a gene (Bs3) conferring hypersensitive resistance to *Xanthomonas campestris pv. vesicatoria* in pepper (*Capsicum annuum*). Plant Dis 69:233–235

Kim B, Hur J (1990) Inheritance of resistance to bacterial spot and to Phytophthora blight in pepper. J Korean Soc Hortic Sci 31:350–357

Kim SJ, Lee SJ, Kim BD, Paek KH (1997) Satellite-RNA mediated resistance to cucumber mosaic virus in transgenic plants of hot pepper (*Capsicum annuum* cv. Golden Tower). Plant Cell Rep 16:825–830

Kim HJ, Nahm SH, Lee HR, Yoon GB, Kim KT, Kang BC, Choi D, Kweon OY, Cho MC, Kwon JK, Han JH (2008a) BAC-derived markers converted from RFLP linked to *Phytophthora capsici* resistance in pepper (*Capsicum annuum* L.). Theor Appl Genet 118(1):15–27. https://doi.org/10.1007/s00122-008-0873-5

Kim SH, Yoon BJ, Park HG (2008b) Inheritance of anthracnose resistance in a new genetic resource, *Capsicum baccatum* PI594137. J Crop Sci Biotechnol 11:13–16

Kim CG, Kim DI, Kim HJ, Park JI, Lee B, Park KW, Jeong SC, Choi KH, An JH, Cho KH, Kim YS, Kim HM (2009) Assessment of gene flow from genetically modified anthracnose-resistant chili pepper (*Capsicum annuum* L.) to a conventional crop. J Plant Biol 52:251–258

Kim S, Kim KT, Kim DH, Yang EY, Cho MC, Jamal A, Chae Y, Pae DH, Oh DG, Hwang JK (2010) Identification of quantitative trait loci associated with anthracnose resistance in chili pepper (*Capsicum* spp.). Korean J Hortic Sci Technol 28(6):1014–1024

Kim HJ, Han JH, Kim S, Lee HR, Shin JS, Kim JH, Cho J, Kim YH, Lee HJ, Kim BD, Choi D (2011) Trichome density of main stem is tightly linked to PepMoV resistance in chili pepper (*Capsicum annuum* L.). Theor Appl Genet 122:1051–1058

Kim YM, Jo YD, Kwon JK, Kim IT, Kang BC (2013) Characterization and inheritance of a novel thermo-sensitive restoration of cytoplasmic male sterility in *Capsicum annuum*. Sci Hortic 164:512–520

Kim S, Park M, Yeom SI, Kim YM, Lee JM, Lee HA, Seo E, Choi J, Cheong K, Kim KT, Jung K (2014) Genome sequence of the hot pepper provides insights into the evolution of pungency in *Capsicum* species. Nat Genet 46(3):270

Konai M, Nariani TK (1980) Reaction of different chilli varieties and *Capsicum* spp. to mosaic and leaf curl viruses. Indian. Phytopathology 33:155

Kong Q, Zhang G, Chen W, Zhang Z, Zou X (2012) Identification and development of polymorphic EST-SSR markers by sequence alignment in pepper, *Capsicum annuum* (Solanaceae). Am J Bot 99:59–61

Kothari SL, Joshi A, Kachhwaha S, Ochoa-Alejo N (2010) Chilli peppers—a review on tissue culture and transgenesis. Biotechnol Adv 28(1):35–48

Kousik CS, Ritchie DF (1996) Race shift in *Xanthomonas campestris pv. vesicatoria* within a season in field grown peppers. Phytopathology 86:952–958

Kraft KH, Brown CH, Nabhan GP, Luedeling E, Luna Ruiz JD, Coppens d'Eeckenbrugge G, Hijmans RJ, Gepts P (2014) Multiple lines of evidence for the origin of domesticated chili pepper, *Capsicum annuum*, in Mexico. Proc Natl Acad Sci U S A 111(17):6165–6170

Kumar OA, Raja Rao KGR (2003) Cytomorphological studies in Gamma-ray induced autotriploids of *Capsicum annuum* L. Cytologia 68(1):45–50

Kumar S, Banerjee MK, Kalloo G (2000) Male sterility: mechanisms and current status on identification, characterization and utilization in vegetables. Veg Sci 27:1–24

Kumar S, Kumar S, Rai SK, Kumar V, Kumar R (2002) Considerable amount of natural cross pollination on male sterile lines of chilli (*Capsicum annuum* L.). Capsicum Eggplant Newsl 21:48–51

Kumar S, Kumar S, Singh M, Singh AK, Rai M (2006) Identification of host plant resistance to pepper leaf curl virus in chilli (*Capsicum* species). Sci Hortic 110:359–361

Kumar AM, Reddy KN, Sreevathsa R, Ganeshan G, Udaykumar M (2009a) Towards crop improvement in bell pepper (*Capsicum annuum* L.): transgenics (uid A:: hpt II) by a tissue-culture-independent Agrobacterium-mediated in planta approach. Sci Hortic Amsterdam 119:362–370

Kumar S, Kumar R, Kumar S, Singh M, Rai AB, Rai M (2009b) Reaction of pepper leaf curl virus field resistance of chilli (*Capsicum annuum* L.) genotypes under challenged condition. Veg Sci 36:230–232

Lee SJ, Kim BD, Paek KH (1993) *In vitro* plant regeneration and *Agrobacterium*-mediated transformation from cotyledon explants of hot pepper (*Capsicum annuum* cv. Golden Tower). Korean J Plant Tissue Cult 20:289–294

Lee YH, Kim HS, Kim JY, Jung M, Park YS, Lee JS (2004) A new selection method for pepper transformation: callus-mediated shoot formation. Plant Cell Rep 23:50–58

Lee HR, Kim KT, Kim HJ, Han JH, Kim JH, Yeom SI, Kim HJ, Kang WH, Shi J, Park SW, Bae IH, Lee S, Cho J, Oh D, Kim BD (2011) QTL analysis of fruit length using rRAMP, WRKY, and AFLP markers in chili pepper. Hortic Environ Biotechnol 52:602–613

Lefebvre V, Daubèze AM, van der Voort JR, Peleman J, Bardin M, Palloix A (2003) QTLs for resistance to powdery mildew in pepper under natural and artificial infections. Theor Appl Genet 107(4):661–666

Li D, Zhao K, Xie B, Zhang B, Luo K (2003) Establishment of a highly efficient transformation system for pepper (*Capsicum annuum* L.). Plant Cell Rep 21:785–788

Li T, Xu X, Li Y, Wang H, Li Z, Li Z (2015) Comparative transcriptome analysis reveals differential transcription in heat-susceptible and heat-tolerant pepper (*Capsicum annuum L.*) cultivars under heat stress. J Plant Biol 58:411–424

Lin S, Chou Y, Shieh H, Ebert AW, Kumar S, Mavlyanova R, Rouamba A, Tenkouano A, Afari-Sefa V, Gniffke PA (2013) Pepper (*Capsicum* spp.) germplasm dissemination by AVRDC–The World Vegetable Center: an overview and introspection. Chronica Hortic 53(3):21–27

Lin SW, Shieh HC, Wang YW, Tan CW, Schafleitner R, Yang WJ, Kumar S (2015) Restorer breeding in sweet pepper: introgressing Rf allele from hot pepper through marker-assisted backcrossing. Sci Hortic 197:170–175. https://doi.org/10.1016/j.scienta.2015.09.036

Liu W, Parrott WA, Hildebrand DF, Collins GB, Williams EG (1990) *Agrobacterium* induced gall formation in bell pepper (*Capsicum annuum* L.) and formation of shoot-like structures expressing introduced genes. Plant Cell Rep 9:360–364

Liu WY, Kang JH, Jeong HS, Choi HJ, Yang HB, Kim KT et al (2014) Combined used of bulk-segregated analysis of microarrays reveals SNP markers pinpointing a major QTL for resistance to *Phytophthora capsici* in pepper. Theor Appl Genet 127:2503–2513. https://doi.org/10.1007/s00122-014-2394-8

Londo JP, Chiang Y-C, Hung K-H, Chiang T-Y, Schaa BA (2007) Phylogeography of Asian wild rice, *Oryza rufipogon*, reveals multiple independent domestications of cultivated rice, *Oryza sativa*. Proc Natl Acad Sci USA 103:9578–9583

Madhuri V, Rajam MV (1993) Apical shoot meristem culture in red pepper (*Capsicum annuum* L.). J Plant Biochem Biotechnol 2:67–68

Maiti S, Sen C (1979) Fungal diseases of betel vine. Proc Natl Acad Sci USA 25:50–157

Manandhar JB, Hartman GL, Wang TC (1995) Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioides*. Plant Dis 79:380–383

Manoharan M, Sree Vidya CS, Lakshmi Sita G (1998) *Agrobacterium*-mediated genetic transformation in hot chilli (*Capsicum annuum* L. var. Pusa jwala). Plant Sci 131:77–83

Manzur JP, Oliva-Alarcón M, Rodríguez-Burruezo A (2014) *In vitro* germination of immature embryos for accelerating generation advancement in peppers (*Capsicum annuum* L.). Sci Hortic 170:203–210

Martínez S, López M, González-Raurich M, Bernardo Alvarez A (2005) The effects of ripening stage and processing systems on vitamin C content in sweet peppers (*Capsicum annuum* L.). Int J Food Sci Nutr 56(1):45–51

Mathai PJ, Dubey GS, Peter KV, Saklani VD, Singh NP (1977) Pant C-1 and Pant C-2 two new promising selections of chilli (*Capsicum annuum* L.). South Indian Hortic 25:123–125

Matsunaga H, Monma S (1995) Varietal differences in resistance to bacterial wilt in related species of *Capsicum annuum*. Capsicum Eggplant Newsl 14:60–61

Mimura Y, Minamiyama Y, Sano H, Hirai M (2010) Mapping for axillary shooting, flowering date, primary axis length, and number of leaves in pepper (*Capsicum annuum*). J Jpn Soc Hortic Sci 79:56–63

Mimura Y, Inoue T, Minamiyama Y, Kubo N (2012) An SSR-based genetic map of pepper (*Capsicum annuum* L.) serves as an anchor for the alignment of major pepper maps. Breed Sci 62:93–98

Minamiyama Y, Tsuro M, Hirai M (2006) An SSR-based linkage map of *Capsicum annuum*. Mol Breed 18:157–169

Mishra MD, Raychaudhuri SP, Jha A (1963) Virus causing leaf curl of chilli (*Capsicum annuum* L.) Indian. J Microbiol 3:73–76

Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends Plant Sci 11:15–19

Mondal CK, Acharyya O, Hazra P (2013) Biochemical basis of plant defense for leaf curl virus of chilli (*Capsicum annuum*). In: Proceeding XV EUCARPIA meeting on genetics genetics and breeding of *Capsicum* and eggplant, Turin, pp 315–322

Mongkolporn O, Taylor PWJ (2011) Capsicum. In: Kole C (ed) Wild crop relatives: genomic and breeding resources. Springer, Berlin/Heidelberg, pp 43–57. https://doi.org/10.1007/978-3-642-20450-0_4

Moreira SO, Rodrigues R, Araújo ML, Sudréc P, Riva-Souza EM (2009) Desempenho agronômico de linhas endogâmicas recombinadas de pimenta em dois sistemas de cultivo. Ciênc Rural 39(5):1387–1393

Moscone EA (1989) Estudios citotaxonómicos en las tribus Solaneae y Nicotianeae (Solanaceae) de América del Sur. Ph.D. thesis. University of Córdoba

Moscone EA (1990) Chromosome studies on *Capsicum* (Solanaceae) I. Karyotype analysis in *C chacoense*. Brittonia 42:147–154

Moscone EA (1992) Estudios de cromosomas meióticos en Solanaceae de Argentina. Darwin 31:261–297

Moscone EA (1993) Estudios cromosómicos en *Capsicum* (Solanaceae) II. Análisis cariotípico de *C parvifolium* y *C annuum* var *annuum*. Kurtziana 22:9–18

Moscone EA (1999) Análisis cariotípico en *Capsicum baccatum* var. *umbilicatum* (Solanaceae) mediante bandeos AgNOR y de fluorescencia. Kurtziana 27:225–232

Moscone EA, Lambrou M, Hunziker AT, Ehrendorfer F (1993) Giemsa C- banded karyotypes in *Capsicum* (Solanaceae). Plant Syst Evol 186:213–229

Moscone E, Loidl J, Ehrendorfer F, Hunziker AT (1995) Analysis of active nucleolus organizing regions in *Capsicum* (Solanaceae) by silver staining. Am J Bot 82:276–287

Moscone EA, Lambrou M, Ehrendorfer F (1996) Fluorescent chromosome banding in the cultivated species of *Capsicum* (Solanaceae). Plant Syst Evol 202:37–63

Moscone EA, Baranyi M, Ebert I, Greilhuber J, Ehrendorfer F, Hunziker AT (2003) Analysis of nuclear DNA content in *Capsicum* (Solanaceae) by flow cytometry and Feulgen densitometry. Ann Bot 92:21–29

Moscone EA, Scaldaferro MA, Grabiele M, Cecchini NM, Sanchez Garcia Y, Jarret R, Davina JR, Ducasse DA, Barboza GE, Ehrendorfer F (2007) The evolution of chili pepper (*Capsicum* – Solanaceae): a cytogenetic perspective. Acta Hortic 745:137–169

Naegele RP, Ashrafi H, Hill TA, Chin-Wo SR, Van Deynze AE, Hausbeck MK (2014) QTL mapping of fruit rot resistance to the plant pathogen *Phytophthora capsici* in a recombinant inbred line *Capsicum annuum* population. Phytopathology 104(5):479–483. https://doi.org/10.1094/PHYTO-05-13-0143-R

Naresh P, Rao VK, Reddy L, Reddy A, Venkatachalapathi V, Reddy M (2016) Genetic analysis for fruit biochemical traits (capsaicinoids and carotenoids) and dry fruit yield in chilli (*Capsicum annuum* L.). Ind Crop Prod 94:920–931

Nascimento KS, Rêgo MM, Nascimento AMM, Rêgo ER (2015) Ethyl methane sulfonate in the generation of genetic variability in *Capsicum*. Acta Hortic 1087:357–363

Nee M, Bohs L, Knapp S (2006) New species of Solanum and Capsicum (Solanaceae) from Bolivia, with clarification of nomenclature in some Bolivian Solanum. Brittonia 58:322–356

Nigam K, Suhail S, Verma Y, Singh V, Gupta S (2015) Molecular characterization of begomovirus associated with leaf curl disease in chilli. World J Pharm Res 4:1579–1592

Nimmakayala P, Abburi VL, Abburi L, Alaparthi SB, Cantrell R, Park M, Choi D, Hankins G, Malkaram S, Reddy UK (2014) Linkage disequilibrium and population-structure analysis among *Capsicum annuum* L. cultivars for use in association mapping. Mol Gen Genomics 289(4):513–521

Nimmakayala P, Abburi VL, Saminathan T, Alaparthi SB, Almeida A, Davenport B, Nadimi M, Davidson J, Tonapi K, Yadav L, Malkaram S (2016) Genome-wide diversity and association mapping for capsaicinoids and fruit weight in *Capsicum annuum* L. Sci Rep 6:38081

Nsabiyera V, Ochwo-ssemakula M, Sseruwagi P, Ojewo C, Gibson P (2013) Combining ability for field resistance to disease, fruit yield and yield factors among hot pepper (*Capsicum annuum* L.) Genotypes in Uganda. Int J Plant Breed 7(1):12–21

Ochoa-Alejo N, Ireta-Moreno L (1990) Cultivar differences in shoot-forming capacity of hypocotyl tissues of chilli pepper (*Capsicum annuum* L.) cultured in vitro. Sci Hortic Amst 42:21–28

Okunlola GO, Olatunji OA, Akinwale RO, Tariq A, Adelusi AA (2017) Physiological response of the three most cultivated pepper species (*Capsicum spp.*) in Africa to drought stress imposed at three stages of growth and development. Sci Hortic 224:198–205

Oliveira FA, Duarte SN, Medeiros JF, Mendes EM, Aroucha DNS (2015) Quality in the pepper under different fertigation managements and levels of nitrogen and potassium. Rev Ciênc Agron 46(4):764–773

Orak HH, Demirchi M (2005) Effect of different blanching methods and period of frozen storage on enzyme activities and some quality criterias of hot and sweet red peppers (*Capsicum annuum* L.). Pak J Biol Sci 8:641–648

Palloix A, Daubese AM, Phaly T, Pochard E (1990a) Breeding transgressive lines of pepper for resistance to Phytophthora capsici in a recurrent selection system. Euphytica 51:141–150

Palloix A, Pochard E, Phaly T, Daubese AM (1990b) Recurrent selection for resistance to Varticillium dahlia in pepper. Euphytica 47:79–89

Pandravada SR, Varaprasad KS, Reddy KJ, Rao ES (2010) Screening and identification of sources of resistance against root-knot nematode (*Meloidogyne javanica*) in chilli (*Capsicum annuum*) germplasm. Indian J Agric Sci 80:92–94

Park KS, Kim CH (1992) Identification, distribution, and etiological characteristics of anthracnose fungi of red pepper in Korea. Korean J Plant Pathol 8:61–69

Park YK, Kim BD, Kim BS, Kim K, Armstrong C, Kim NS (1999) Karyotyping of the chromosomes and physical mapping of the 5S rRNA and 18S-26S rRNA gene families in five different species in *Capsicum*. Genes Genet Syst 74(4):149–157

Paul YS, Behl MK (1990) Some studies on bell pepper anthracnose caused by *Colletotrichum capsici* and its control. Seed Res 1:656–659

Pawar SS, Bharude NV, Sonone SS, Deshmukh RS, Raut AK, Umarkar AR (2011) Chillies as food, spice and medicine: a perspective. Int J Pharm Biol Sci 1(3):311–318

Pearson OH (1981) Nature and mechanism of cytoplasmic male sterility in plants: a review. HortScience 16:482–487

Pearson MN, Bull PB, Speke H (1984) Anthracnose of *Capsicum* in Papua, New Guinea; varietal reaction and associated fungi. Trop Pest Manag 30:230–233

Perucka I, Materska M (2007) Antioxidant vitamin contents of *Capsicum annuum* fruit extract as affected by processing and varietal effects. Acta Sci Pol Technol Aliment 6(4):67–74

Peterson PA (1958) Cytoplasmically inherited male sterility in *Capsicum*. Am Nat 92:111–119

Peterson PA (1959) Linkage of fruit shape and color genes in *Capsicum*. Genetics 44(3):407

Phillips GC, Hubstenberger JF (1985) Organogenesis in pepper tissue cultures. Plant Cell Tissue Organ Cult 4:261–269

Phimchan P, Techawongstien S (2012) Impact of drought stress on the accumulation of capsaicinoids in *Capsicum* cultivars with different initial capsaicinoid levels. HortScience 47:1204–1209

Pickersgill B (1971) Relationships between weedy and cultivated forms in some species of chili peppers (genus *Capsicum*). Evolution 25:683–691

Pickersgill B (1977) Chromosomes and evolution in *Capsicum*. In: Pochard E (ed) *Capsicum* 77. Comptes Rendus 3ème Congrès Eucarpia Piment. Institut National de la Recherche Agronomique, Avignon-Montfavet, pp 27–37, 5–8 July 1977

Pickersgill B (1991) Cytogenetics and evolution of *Capsicum* L. In: Tsuchiya T, Gupta PK (eds) Chromosome engineering in plants: genetics, breeding, evolution, Part B. Elsevier, Amsterdam, pp 139–160

Pierre M, Noel L, Lahaye T, Ballvora A, Veuskens J, Ganal M, Bonas U (2000) High-resolution genetic mapping of the pepper resis-tance locus Bs3governing recognition of the *Xanthomonas campestris* pv *vesicatora* AvrBs3 protein. Theor Appl Genet 101:255–263

Popovsky S, Paran I (2000) Molecular analysis of the Y locus in pepper: its relation to capsanthin-capsorubin synthase and to fruit color. Theor Appl Genet 101:86–89

Poulos JM (1992) Problems and progress of chilli pepper production in the tropics. In: Hock CB, Hong LW, Rejab M, Syed AR (eds) Proceedings of the conference on chilli pepper production in the tropics, Kuala Lumpur, pp 98–129

Poulos JM (1994) Pepper breeding (*Capsicum* spp.): achievements, challenges and possibilities. Plant Breed Abstr 64(2):144–155

Pozzobon MT, Schifino-Wittmann MT, Bianchetti LB (2006) Chromosome numbers in wild and semidomesticated Brazilian *Capsicum* L. (Solanaceae) species: do x = 12 and x = 13 represent two evolutionary lines? Bot J Linn Soc 151:259–269

Qin C, Yu C, Shen Y, Fang X, Chen L, Min J, Cheng J, Zhao S, Xu M, Luo Y, Yang Y (2014) Whole-genome sequencing of cultivated and wild peppers provides insights into *Capsicum* domestication and specialization. Proc Natl Acad Sci 111(14):5135–5140

Quagliotti L, Ottaviano E (1969) Quantitative inheritance and capsaicin content in *Capsicum* Sp. Genetica Agraria 23:56–58

Quirin EA, Ogundiwin EA, Prince JP, Mazourek M, Briggs MO, Chlanda TS, Kim KT, Falise M, Kang BC, Jahn MM (2005) Development of sequence characterized amplified region (SCAR) primers for the detection of *Phyto.5.2*, a major QTL for resistance to *Phytophthora capsici* Leon. in pepper. Theor Appl Genet 110:605–612

Raghavan T, Venkatasubban K (1940) Studies in the South Indian chillies. I. A description of the varieties, chromosome numbers and the cytology of some X-ray derivatives in *Capsicum annuum* L. Proc Indian Acad Sci 12:29–46

Rai VP, Rai AC, Kumar S, Kumar R, Kumar S, Singh M, Rai AB, Singh SP (2010) Emergence of new variant of chilli leaf curl virus in north India. Veg Sci 37:124–128

Rai VP, Kumar R, Singh SP, Kumar S, Kumar S, Singh M, Rai M (2014) Monogenic recessive resistance to Pepper leaf curl virus in an interspecific cross of *Capsicum*. Sci Hortic 172:34–38

Ramírez-Malagón R, Ochoa-Alejo N (1996) An improved and reliable chilli pepper (*Capsicum annuum* L.) plant regeneration method. Plant Cell Rep 16:226–231

Reddy KM, Reddy MK (2010) Breeding for virus resistance. In: Kumar R, Rai AB, Rai M, Singh HP (eds) Advances in chilli research. Studium Press Pvt. Ltd., New Delhi, pp 119–132

Rêgo ER, Rego MM, Finger FL, Cruz CD, Casali VWD (2009) A diallel study of yield components and fruit quality in chilli pepper (*Capsicum baccatum*). Euphytica 168:275–287

Rêgo ER, Fortunato FLG, Nascimento MF, Nascimento NFF, Rêgo MM, Finger FL (2012) Inheritance of earliness in ornamental pepper (*Capsicum annuum*). Acta Hortic 961:405–410

Roberts PD, Pernezny K, Kucharek TA (2001) Anthracnose caused by *Colletotrichum* sp. on pepper [Online]. Journal of University of Florida/Institute of Food and Agricultural Sciences. Available from http://edis.ifas. ufl.edu/PP104. Accessed 25 Dec 2007

Saleh BK, Omer A, Teweldemedhin B (2018) Medicinal uses and health benefits of chili pepper (*Capsicum* spp.): a review. MOJ Food Process Technol 6(4):325–328

Sanatombi K, Sharma GJ (2007a) Micropropagation of *Capsicum annuum* L. Notulae Botanicae Hortic Agrobotanici Cluj-Napoca 35:57–64

Sanatombi K, Sharma GJ (2007b) Micropropagation of *Capsicum frutescens* L. using axillary shoot explants. Sci Hortic 113:96–99

Sanatombi K, Sharma GJ (2008) In vitro plant regeneration in six cultivars of *Capsicum* spp. using different explants. Biol Plant 52:141–145

Sangar RBS, Katwale TR, Saraf RK, Parihar MS (1988) Field screening of chilli varieties to viral diseases in Madhya Pradesh. Farm Sci J 3(1):69–71

Scaldaferro MA, Prina AR, Moscone EA, Kwasniewska J (2013) Effects of ionizing radiation on *Capsicum baccatum* var. *pendulum* (Solanaceae). Appl Radiat Isot 79:103–108

Scaldaferro MA, Barboza GE, Acosta MC (2018) Evolutionary history of the chili pepper *Capsicum baccatum* L. (Solanaceae): domestication in South America and natural diversification in the Seasonally Dry Tropical Forests. Biol J Linn Soc 124(3):466–478

Senanayake DMJB, Varma A, Mandal BJ (2012) Virus–vector relationships, host range, detection and sequence comparison of chilli leaf curl virus associated with an epidemic of leaf curl disease of chilli in Jodhpur. Indian Phytopathol 160:146–155

Setiawati W, Sutarya R, Sumiarta K, Kamandalu A, Suryawan IB, Latifah E, Luther G (2011) Incidence and severity of pest and diseases on vegetables in relation to climate change (with emphasis on East Java and Bali). In: Proceedings of the Prosiding Seminar Nasional Perhimpunan Hortikultura Indonesia, Balitsa, Lembang, pp. 88–99

Sharma OP, Singh J (1985) Reaction of different genotypes of pepper to cucumber mosaic and tobacco mosaic viruses. Capsicum Newsl 4:47

Shifriss C (1997) Male sterility in pepper (*Capsicum annuum* L). Euphytica 93:83–88

Shmykova NA, Pyshnaya ON, Shumilina DV, Dzhos EA (2014) Morphological characteristics of doubled haploid plants of pepper produced using microspore/anther in vitro culture of the interspecies hybrids of *Capsicum annum* L. and *C. chinense* Jacq. Russ Agric Sci 40(6):417–421. https://doi.org/10.3103/S1068367414060202

Showemimo FA, Olarewaju JD (2007) Drought tolerance indices in sweet pepper (*Capsicum annuum* L.). Int J Plant Breed Genet 1(1):29–33

Simmonds JH (1965) A study of the species of *Colletotrichum* causing ripe fruit rots in Queensland. Qld J Agric Anim Sci 22:437–459

Singh J, Kaur S (1990) Development of multiple resistance in chilli pepper. In: Proceedings of the 3rd international conference, pp 20–23

Singh J, Thakur MR (1977) Genetics of resistance to *Tobacco mosaic virus*, *Cucumber mosaic virus* and leaf curl virus in hot pepper (*Capsicum annuum* L.). In: *Capsicum* 77: 3rd Eucarpia Meet, pp 119–123

Singh PK, Dasgupta SK, Tripathi SK (2004) Hybrid vegetable development. International book distribution co., Lucknow

Singh P, Cheema DS, Dhaliwal MS, Garg N (2014) Heterosis and combining ability for earliness, plant growth, yield and fruit attributes in hot pepper (*Capsicum annuum* L.) involving genetic and cytoplasmic-genetic male sterile lines. Sci Hortic 168:175–188

Sivaprakasam K, Jaganathan R, Pillayarsamy K, Anavaradham K (1976) Control of powdery mildew of chillies. Madras Agric J 63:52–54

Smith PG, Villalon B, Villa PL (1987) Horticultural classification of peppers grown in the united-states. HortScience 22:11–13

Soler S, Debreczeni DE, Vidal E, Aramburu J, López C, Galipienso L (2015, In press) New *Capsicum baccatum* accession shows tolerance to wild-type and resistance-breaking isolates of tomato spotted wilt virus. Ann Appl Biol. https://doi.org/10.1111/aab.12229

Sooch BS, Thakur MR, Mayee CD (1976) Stability of some chilli (*Capsicum annuum* L.) genotypes against virus diseases. J Plant Dis Protection 83:514–518

Spice Board (2017) Agricultural statistics, Ministry of commerce, Government of India

Srivastava A, Mangal M, Saritha RK, Kalia P (2017) Screening of chilli pepper (*Capsicum* spp.) lines for resistance to the begomoviruses chilli leaf curl disease in India. Crop Prot 100:177–185

Sugita T, Yamaguchi K, Kinoshita T, Yuji K, Sugimura Y, Nagata R, Kawasaki S, Todoroki A (2006) QTL analysis for resistance to Phytophthora bright (*Phytophthora capsici* Leon.) using an intraspecific doubled-haploid population of *Capsicum annuum*. Breed Sci 56:137–145

Sugita T, Semi Y, Sawada H, Utoyama Y, Hosomi Y, Yoshimoto E, Maehata Y, Fukuoka H, Nagata R, Ohyama A (2013) Development of simple sequence repeat markers and construction of a high-density linkage map of *Capsicum annuum*. Mol Breed 31:909–920

Supena EDJ, Suharsono S, Jacobsen E, Custers JBM (2006) Successful development of a shed-microspore culture protocol for doubled haploid production in Indonesian hot pepper (*Capsicum annuum* L.). Plant Cell Rep 25(1):1–10. https://doi.org/10.1007/s00299-005-0028-y

Suwor P, Sanitchon J, Thummabenjapone P, Kumar S, Techawongstien S (2017) Inheritance analysis of anthracnose resistance and marker-assisted selection in introgression populations of chili (*Capsicum annuum* L.). Sci Hortic 220:20–26

Sy O, Steiner R, Bosland PW (2005) Inheritance of Phytophthora stem blight resistance as compared to Phytophthora root rot and Phytophthora foliar blight resistance in *Capsicum annuum* L. J Am Soc Hortic Sci 130:75–78

Tam SM, Lefebvre V, Palloix A, Sage-Palloix AM, Mhiri C, Grandbastien MA (2009) LTR-retrotransposons Tnt1 and T135 markers reveal genetic diversity and evolutionary relationships of domesticated peppers. Theor Appl Genet 119(6):973–989

Tanaka Y, Yoneda H, Hosokawa M, Miwa T, Yazawa S (2014) Application of marker-assisted selection in breeding of a new fresh pepper cultivar (*Capsicum annuum*) containing capsinoids, low-pungent capsaicinoid analogs. Sci Hortic 165:242–245

Tanksley SD, Bernatzky R, Lapitan NL, Prince JP (1988) Conservation of gene repertoire but not gene order in pepper and tomato. Proc Natl Acad Sci USA 85:6419–6423

Temiyakul P, Taylor PWJ, Mongkolporn O (2012) Differential fruit maturity plays an important role in chili anthracnose infection. J King Mongkut's Univ Technol North Bangkok 22(3):494–504

Tewari VP, Ramanujam S (1974) Grow Jwala, a disease resistant high-yield chilli. Indian Fmg 24:20

Tewari VP, Viswanath SM (1986) Breeding for multiple virus resistance in pepper (*C. annuum* L.). Capsicum Newsl 5:49

Tewari GS, Deshpande AA, Ananad N (1985) Chilli pepper genotypes resistant to thrips (*Scirtothrips dorsalis* Hoct). Capsicum Newsl 5:49

Thabuis A, Palloix A, Pflieger S, Daubeze AM, Caranta C, Legebvre V (2003) Comparative mapping of Phytophthora resistance loci in pepper germplasm: evidence for conserved resistance loci across Solanaceae and for a large genetic diversity. Theor Appl Genet 106:1473–1485. https://doi.org/10.1007/s00122-003-1206-3

Thakur H, Jindal SK, Sharma A, Dhaliwal MS (2018) Chilli leaf curl virus disease: a serious threat for chilli cultivation. J Plant Dis Protection 125(3):239–249

Than PP, Prihastuti H, Phoulivong S, Taylor PW, Hyde KD (2008) Chilli anthracnose disease caused by *Colletotrichum* species. J Zhejiang Univ Sci B 9(10):764

Tong N, Bosland PW (1997) Meiotic chromosome study of *Capsicum lanceolatum*. Another 13 chromosome species. Capsicum Eggplant Newsl 16:42–43

Tong N, Bosland PW (2003) *Capsicum tovarii*, a new member of the *Capsicum* complex. Euphytica 109:71–72

Truong HT, Kim KT, Kim DW, Kim S, Chae Y, Park JH, Oh DG, Cho MC (2012) Identification of isolate-specific resistance QTLs to phytophthora root rot using an intraspecific recombinant inbred line population of pepper (*Capsicum annuum*). Plant Pathol 61(1):48–56. https://doi.org/10.1111/j.1365-3059.2011.02483.x

Ulhoa AB, Telma NP, Raimundo NS, Carlos FR, Rodrigues R, Messias GP, Reifschneider FJB (2014) Molecular characterization of inbred lines of yellow Jalapeño pepper. Hortic Bras 32(1):35–40

Uzo JO (1984) Hybrid vigour and gene action of two qualitative traits of flavour peppers in Nigeria. Sci Hortic 22(4):321–326

Valera-Montero LL, Ochoa-Alejo NA (1992) novel approach for chili pepper (*Capsicum annuum* L.) plant regeneration: shoot induction in rooted hypocotyls. Plant Sci 84:215–219

Venkataiah P, Christopher T, Subhash K (2003) Thidiazuron induced high frequency adventitious shoot formation and plant regeneration in *Capsicum annuum* L. J Plant Biotechnol 5:245–250

Venkataiah P, Christopher T, Subhash K (2005) Induction and characterization of streptomycin resistant mutants in *Capsicum praetermissum*. J Appl Genet 46(1):19–24

Villalon B (1986) Tambel-2 bell pepper. HortScience 21:328

Visalakshi M, Pandiyan M (2018) Crop improvement in chillies: an overview. Int J Chem Stud 6(4):1736–1744

Voorips RE, Finkers R, Lia S (2004) QTL mapping of anthracnose (*Colletotrichum* spp.) resistance in a cross between. *Capsicum annuum* and *C. chinense*. Theor Appl Genet 109:1275–1282

Voorrips RE, Finkers R, Sanjaya L, Groenwold R (2004) QTL mapping of anthracnose (*Colletotrichum* spp.) resistance in a cross between *Capsicum annuum* and *C. chinense*. Theor Appl Genet 109(6):1275–1282. https://doi.org/10.1007/s00122-004-1738-1

Wang LH, Zhang BX, Lefebvre V, Huang SW, Daubeze AM, Palloix A (2004) QTL analysis of fertility restoration in cytoplasmic male sterile pepper. Theor Appl Genet 109:1058–1063

Wang P, Wang L, Guo J, Yang W, Shen H (2016) Molecular mapping of a gene conferring resistance to *Phytophthora capsici* Leonian race 2 in pepper line PI201234 (*Capsicum annuum* L.). Mol Breed 36(6):66. https://doi.org/10.1007/s11032-016-0464-0

Wankhade RR, Wadikar MS (2018) Identification of sequence-characterized amplified regions (SCARs) markers linking resistance to powdery mildew in chilli pepper (*Capsicum annuum* L.). Afr J Agric Res 13(49):2771–2779

Xu X, Chao J, Cheng X, Wang R, Sun B, Wang H, Luo S, Xu X, Wu T, Li Y (2016) Mapping of a novel race specific resistance gene to phytophthora root rot of pepper (*Capsicum annuum*) using bulked segregant analysis combined with specific length amplified fragment sequencing strategy. PLoS One 11(3):e0151401. https://doi.org/10.1371/journal.pone.0151401

Yang CJ, Chen BJ, Liu JG, Li S (2008) Study on the superiority of hybrid breeding by male sterility in hot pepper. J China Capsicum 1:36–38

Yoon JB, Do JW, Yang DC, Park HG (2004) Interspecific cross compatibility among five domesticated species of *Capsicum* genus. J Korean Soc Hortic Sci 45:324–329

Yordanov M (1983) Heterosis in the tomato. In: *Heterosis*. Springer, Berlin/Heidelberg, pp 189–219

Yuen CMC, Hoffman H (1993) New *Capsicum* varieties: storage suitability and consumer preference. Food Australia 45:184–187

Yunandra Y, Syukur M, Maharijaya A (2018) Inheritance study for yield components of pepper (*Capsicum annuum* L.). IOP Conf Ser Earth Environ Sci 196:012009

Zewdie Y, Bosland PW (2000) Capsaicinoid inheritance in an interspecific hybridization of *Capsicum annuum* × *C. chinense*. J Am Soc Hortic Sci 125(4):448–453

Zhu YX, Ou-Yang WJ, Zhang YF, Chen ZL (1996) Transgenic sweet pepper plants from *Agrobacterium*-mediated transformation. Plant Cell Rep 16:71–75

Chapter 10
Accelerated Breeding in Cucumber Using Genomic Approaches

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10.1 Introduction

Cucumber (*Cucumis sativus* var. *sativus* L.) is the most important vegetable crop in the family Cucurbitaceae and cultivated worldwide. It has a diploid chromosome number $2n = 2X = 14$. Because of relatively smaller estimated genome size (367 Mbp), this crop has been studied widely as a model crop for genomic research in cucurbitaceous vegetable crops (Huang et al. 2009a, 2011). India is considered as the primary centre of origin of cucumber (Sebastian et al. 2010), and it is believed that wild cucumber (*Cucumis sativus* var. *hardwickii* R.) is the progenitor of the present-day cultivated types. The wild forms of cucumbers are widely distributed at southern foothills of Himalayas. Cucumber is a summer season vegetable crop and mainly grown for its edible tender fruits, preferred as salad ingredient, pickles, dessert fruit and cooked vegetable. Even though fresh cucumbers are mostly composed of water, they still pack a lot of nutrition. The flesh of cucumber is a very good source of vitamins such as A, C and folic acid. The hard skin is rich in a variety of minerals including calcium, potassium and magnesium. Cucumber has got a cooling effect; hence, fruits are often used as cooling vegetable. It is ideal for people suffering from jaundice and allied diseases and also very much useful in preventing constipation. Seeds contain oil, which is helpful for brain development and body smoothness. Hence, it is being used in Ayurvedic preparations (Robinson and Decker-Walters 1999). Besides, the whole fruit is used in cosmetic and soap industries.

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Development of improved plant type with higher productivity, tolerance to different biotic and abiotic stresses and climate smart genotypes is the major objective in the modern-day cucumber breeding programme. Conventional breeding methods have contributed immensely in developing improved genotypes and introgression of desirable traits into varied background of cucumber. After evolution from wild types, domestication and suitable agronomic measures have played important role in developing crop species to meet mankind's early food security and human evolution (Harlan 1992; Forster and Shu 2012). The enormous contribution of these two phases was eclipsed by subsequent achievements in plant breeding later on. Plant breeding has remained as the key of scientific innovation in the fields of the genetics, botany, physiology and biotechnology. Plant breeding has achieved success in improving crop plants through recombination, heritability, polyploidy, chromosome engineering, tissue culture, heterosis, genetic linkage mapping, molecular genetics, mutagenesis and transformation. The food and nutritional security of mankind is attributed to the magnificent achievement though application of plant breeding tools (Fehr 1984; Duvick 1986; Borlaug 2002). The total population in 2050 will be around 9.73 billion from the current population of around 7.71 billion (https://www.worldometers.info/world-population). Therefore, the plant breeders face a tremendous job of increasing the food production in a much higher rate to feed the 9.73 billon of population in 2050 under the threat of decreasing cultivable land and uncertain climatic regions. In this context, accelerated plant breeding approaches will be instrumental to meet the future needs of mankind. Development of an end product through conventional breeding selection and other approaches requires more than ten generations to achieve the required homogeneity and their evaluation thereafter under multiple climatic regions. Recent development of genomics tools in the last two decades and their rapid progress in the last 7–8 years enable the plant breeders to achieve the required success in much shorter time period. Besides, various biotechnological tools and in vitro techniques have immense potential in reducing the time cycle and aid the plant breeding programme in a great way. In this chapter, we will specifically discuss the strategies which would be instrumental in rapid breeding of cucumber and achievements till date in the following sections. However, understanding the breeding behaviour and genetic constitution of this crop is necessary before going in details about the different strategies.

10.2 Taxonomy and Genomic Structure

Botanical name: *Cucumis sativus* var. *sativus* L.

Family: *Cucurbitaceae*

Sub-family: *Cucurbitoideae*

Tribe: *Melothrieae*

Sub-tribe: *Cucutmerinae*

Table 10.1 Salient features of the seven chromosomes of *Cucumis sativus* L

Linkage group (LG)	Physical distance (Mb)	Genetic distance (cM)	Remarks
LG1	25–30	90–100	–
LG2	20–25	90–100	Smaller centromeric region with highest rate of recombination
LG3	30–35	110–120	–
LG4	20–25	30–40	–
LG5	25–30	50–60	Largest centromeric region with lowest rate of recombination
LG6	25–30	100–110	Smaller centromeric region with highest rate of recombination
LG7	15–20	60–70	–

Cucumber belongs to the family Cucurbitaceae. Cucumber is a diploid species with basic chromosome number of 7. Salient features of the seven chromosomes of cucumber based on its integrated genetic and physical map (Huang et al. 2009a, b) are presented in Table 10.1. The genetic map was constructed using a recombinant inbred line mapping population from the intersubspecific cross between Gy14 (domestic cucumber) and PI183967 (wild cucumber).

Based on cDNA-EST, homology-based and ab initio gene prediction methods through protein-coding genes, they have predicted 26,682 genes, with a mean coding sequence size of 1046 bp and an average exons per gene of 4.39. Based on pairwise protein sequence similarities, they carried out a gene family clustering analysis on all genes in sequenced plants, using rice as an outgroup. It was found that the cucumber genes consist of 15,669 families. Of these, 4362 are unique families of cucumber, among which 3784 are single-gene families.

At present, genome sequences are available for three different cucumber genotypes: Chinese Long 9930 (GenBank:GCA_000004075.2), a Chinese variety sequenced in Chinaby Huang et al. (2009a, b; new version 2014); Gy14 (http://wenglab.horticulture.wisc.edu/), an American variety sequenced by the USDA-ARS Vegetable Crops Research Unit, Madison, WI (Cavagnaro et al. 2010); and B10 (GenBank: GCA_000224045.1), a northern European Borszczagowski line sequenced by the Polish Consortium of Cucumber Genome Sequencing (Wóycicki et al. 2011) and recently (2017) updated with PacBio reads (GenBank: LKUO00000000.2; https://www.ncbi.nlm.nih.gov/Traces/wgs/?val=LKUO02#contigs). The latter sequence covers 94% of the cucumber genome and is currently the most complete draft version (Osipowski et al. 2016).

C. sativus var. *hardwickii* is believed to be the progenitor of the present-day cultivated genotypes, and it is cross-compatible with the cultivated *C. sativus* var. *sativus*. The fruits of most of the *C. sativus* var. *hardwickii* are extremely bitter in nature because of the presence of cucurbitacins, and the plants have multiple branching nature unlike the cultivated cucumber genotypes. The seedlings of cucumber are

epigean in nature, germinating with the tip of the cotyledons initially inverted and later erect. The photosynthetic cotyledons of cucumber seedlings are oblong in shape and inconspicuous, developing a hypocotyl that lies in between the cotyledons. Cucumber has a very strong tap root system up to 1 m in length, and several secondary roots are found near the soil surface. Sometimes adventitious roots appeared from the node of the stem without having any contact with the soil. The herbaceous and softly woody stems are typically prostrate, trailing or climbing, angled in cross section, centrally hollow, sap filled and branched. Primary and secondary branches can reach 5–7 m in length. The leaves of cucumber are simple in structure without any leaflets and palmately veined and shallowly 3–5 lobed. Cucumber possesses solitary tendrils at its leaf axils and the tendrils are unbranched in nature. Tendrils are coiled quire often to help the plants to cling on trellises and other support. The tendrils of cucumber are mainly considered as modified shoots. The typically unisexual flowers occur in leaf axils, either alone or as inflorescence (Robinson and Decker-Walters 1999).

10.3 Floral Biology and Sex Expression

Cucumber is generally monoecious in nature with the presence of male and female flowers in separate nodes in a single plant. All the primitive forms of cucurbits are hermaphrodites in nature with the presence of male and female reproductive organs within a single flower. Presence of bisexual flowers is still reported in cucumber under natural condition. Based on the position and occurrence of male, female and hermaphrodite flowers, the plants may be classified as monoecious, gynoecious (Fig. 10.1), andromonoecious, hermaphrodite, trimonoecious or androecious. At a very early stage of development, all the flower buds appear similar, and their morphological difference can be detected only when the buds are 3–5 mm in size. At this stage, the developing ovaries of the female flower buds are visible which distinguishes them from the male flowers, whereas the smaller and thickened ovaries of the hermaphrodite flowers can be identified at later stage of development. Besides, the thickness of the pedicels/stalks is also indicative of the type of the flowers, and the male flowers have the thinnest pedicels, whereas the female flowers have the thickest pedicels to support the fruits. The ovaries of the female flowers look like tiny cucumber and smaller in size, whereas the hermaphrodite flowers have shorter and bulgier ovaries. Bai et al. (2004) conducted a precise morphogenetic analysis for the development of floral organs. They divided unisexual flower development into 12 stages ranging from floral meristem initiation to anthesis according to the existence of distinct morphological events. During the first five stages (up to ~0.5 mm), morphological changes are identical in both sex types. In later stages (~0.6–40 mm), increasing differences are evident between female and male flowers.

Fig. 10.1 Tropical cucumber gynoecious line, DC-102, with only female flowers. This line is stable and produces only female flowers even at temperature of 45 °C and being used commercially in the development of F1 hybrids in cucumber

Three major phases of flower differentiation explained by Hao et al. (2003) and Bai et al. (2004) are as follows:

Phase 1: Initial stage (immediately after formation): Flower buds are bisexual with both stamen and pistil primordia

Phase 2: Flower buds of about 0.5–2.0 mm long: Development of unisexual flowers begins with the inhibition of primordial organs' opposite sex form

Phase 3: Flower buds of 3.0–5.0 mm long: Determination of sex form is complete; development of primordial pistils or stamens then continues according to the sexual type of the mature flower.

The process of differentiation of female flowers was explained by Calderon-Urrea and Dellaporta (1999) and Hao et al. (2003). They observed that female flower differentiation takes place after stamen differentiation into filament and anther. The anther, but not the complete stamen, is inhibited in this process. They studied the phenomenon of female flower bud and found that the activity of likely anther-specific DNases and chromatin condensation was observed in primordial stamen anthers, but the mitochondria and nuclei remained unchanged, and

programmed cell death (PCD)-characteristic laddering was detected. Although some PCD-connected phenomena were detected in differentiating buds, the responsible mechanism was not the same as that operating in model crop like maize where PCD is responsible for the inhibition of opposite sex organ. It was found that developmental arrest of the inappropriate stamen occurs mainly in the anther primordium. This inhibition was correlated with DNA damage, detected by the TUNEL assay. Therefore, it was concluded that this is the result of anther-specific DNase activation. It was also found that DNA damage does not lead to cell degeneration, although chromatin condensation was observed in the anther primordia. Female organ inhibition processes in male flower buds have not yet been sufficiently explained. Some researchers have observed DNA laddering in male buds, which may be an evidence of stamen inhibition through PCD (Delorme et al. 2000). However, in cucumber, Yang et al. (2000) showed that primordial pistils remain metabolically active during male flower development. Because treatment of cucumber protoplast cultures with ethephon activates endogenous DNases that damage cellular DNA, ethylene has been proposed to be a possible inhibitor of male organ growth in female flowers via PCD (Wang et al. 2010). To date, the identity of the primary signal triggering the development of floral sex types remains unknown (Table 10.2).

Based on the above studies, the sex expression of different genotypes is as follows:

Genotype	Corresponding sex forms
mmff	Andromonoecious
*mmF_*	Hermaphroditic
*M_F_*	Female
M_ff	Monoecious with mostly male flowers
m*mffaa/M_ffaa*	Completely male

Whereas information on the function of the F and M genes is described, little is known about the functions of A/a, Gy/gy and H/h alleles. In one study, the A/a allele was suggested to be linked to the copper transporter gene CsRAN1 (Terefe 2005), whereas the dominant allele Gy/gy gene can be correlated with the putative serine/threonine kinase gene CsPSTK1 (Pawełkowicz et al. 2012). Because CsPSTK1 expression is related to the recessive gy allele, the associated inhibition of the CsPSTK1 gene may require the presence of the dominant Gy allele. Presumably, CsPSTK1 inhibition by the Gy allele negatively affects ethylene biosynthesis. When gy is present, the inhibition is removed, and the CsPSTK1 gene has a positive effect on ethylene levels (Pawełkowicz et al. 2012). According to recent reports, the recessive female state is conditioned (similar to *Cucumis melo*) by mutational changes in the WIP (CmWIP1-CsWIP1) gene, which possesses a zinc finger domain and controls transcription (Martin et al. 2009; Boualem et al. 2015; Chen et al. 2016). Despite the above findings, the mechanism of sex determination is far more complicated than detailed above, and many other genes are correlated with this process in cucumber.

Table 10.2 Genes involved in floral biology with their function and molecular mechanism

Genes	Function	Molecular mechanism	References
F (female)	Plants with the dominant F allele are female (dominant femininity dF) Expression of this gene is influenced by other sex genes and the environment, a situation called 'soft femininity' Lines homozygous for the dominant F allele are female	The F locus has been cloned and shown to be an additional copy of the CsACS1G gene encoding 1-aminocyclopropane-1-carboxylic acid synthase (ACS)	Trebitsh et al. (1997) and Mibus and Tatlioglu (2004)
m (andromonoecious)	This recessive allele has an influence on the bisexual flower creation	The Mlocus has also been cloned and shown to be the CsACS2 gene encoding another member of the ACS family, while the m allele has a mutation at a conserved site (Gly33Cys). This isoform displays reduced enzymatic activity	Li et al. (2009) and Boualem et al. (2009)
a (androecious)	aa homozygotes are characterized by the intensification of male features. The gene is hypostatic to the F gene	This gene may relate to CsACS11	Boualem et al. (2015)
gy (gynoecious)	This recessive allele is responsible for femininity that is much more highly stable than that conferred by the F gene (recessive femininity rF; hard femininity). The gy gene (also abbreviated as g) is frequently assumed to be the gene indirectly influencing sex	It could be correlated with CsWIP	Pawełkowicz et al. (2012) and Boualem et al. (2015)
h (andromonoecious-2)	This allele produces bisexual flowers with normal ovaries	Unknown	Kubicki (1974)
In-F (intensifier of female sex expression)	It causes intensification of F gene action	Unknown	Malepszy and Niemirowicz-Szczytt (1991)
Tr (trimonoecious)	This regulates the formation of female, male and bisexual flowers, but the ratio of the different types of flowers could be influenced by environmental factors	Unknown	Kubicki (1969b)

10.4 Breeding Behaviour of Cucumber

Cucumber is a cross-pollinated crop and entomophilous in nature. The bee like *Apis florea*, *Apis dorsata* and *Apis mellifera*, *Nomioides* sp. and *Helictine* sp. are the principal pollinating agents of cucumber. Few beetles like *Conpophilus* sp. and moths like *Planidia* sp. and *Pygargonia* sp. are reported to act as pollinators in certain areas. However, the different species of honeybees are the main agents to effect pollination in most of cucumber-growing areas. The cucurbit group of vegetables is significantly different from other classical cross-pollinated vegetable crops like cabbage, onion, carrot, etc. in terms of their breeding behaviour and system. In spite of being cross-pollinated, very low inbreeding depression is recorded in these crops including cucumber. Therefore, it is possible to practice single plant selection even from F_2 population to derive superior genotypes. In heterozygous crop like cucumber with low or insignificant inbreeding depression is explained by homozygous balance. This is mainly because of higher planting distance and growing of small population over time. All the genes showing deleterious effects under homozygous condition are eliminated in the process of evolution. Therefore, selfing in these crops is not affected by inbreeding depression. Therefore, different modified breeding methods performed in both self-pollinated and cross-pollinated crops are practiced in cucumber based on the breeding objectives.

10.5 Genomics-Based Strategies for Accelerated Breeding in Cucumber

10.5.1 Utilization of Wild Species in Broadening the Genetic Base

Access to novel germplasm and creation of genetic diversity are one of the most important objectives to sustain improvement programme of any crop. Gene pool of any crop is important in the creation of genetic diversity and broadening of the genetic base. In cucumber, the primary gene pool consists of the species *Cucumis sativus* var. *sativus* and its wild relative, *C. sativus* var. *hardwickii*. The fruits of *C. sativus* var. *hardwickii* are smaller and round to oblong in shape and are extremely bitter in most of the cases. This wild relative is readily crossable with the cultivated species, and on pollination, they produce fully fertile F_1 hybrids. The secondary gene pool of *C. sativus* includes cross-incompatible (e.g. wild African) or sparingly cross-compatible (e.g. *C. hystrix*) species (Chen et al. 1997; Chung et al. 2006). The tertiary gene pool of cucumber consists of distantly related species from other genera or subgenera (e.g. *Cucumis melo* L. and *Cucurbita* L.), which do not hybridize with cucumber (Chung et al. 2006; Staub et al. 1997a, b). Attempts to exploit resources beyond the secondary cucumber gene pool, e.g. *Cucumis metuliferus* and *C. melo*, have been unsuccessful.

The species, *C. hardwickii*, has several economically important traits like multiple branching, capacity to bear large number of fruits (20–25), resistance to

different viruses (CMV, ToLCNDV) and resistance to different fungal diseases like downy mildew and powdery mildew. However, this species has not been utilized in introgression of different important traits into cultivated genotypes mainly because of its bitter principle which makes the fruits unfit for consumption. Therefore, understanding the genetics of bitterness and identification of closely linked markers with the bitter principle will help in utilizing this wild species for the improvement of the present-day cultivated types.

10.5.1.1 Genetic and Molecular Basis of Bitterness in Cucumber

Bitter fruits in foliage in most of the species of cucumber and its relatives are attributed to the presence of cucurbitacins, which belong to the triterpenoid class of secondary metabolites. These bitter compounds play important role in plant defence against insects and other herbivores (Agrawal et al. 2002; Balkema-Boomstra et al. 2003). Bitterness in cucumber is not only controlled by genetic factor but also influenced by environmental conditions (Kano and Goto 2003; Zhang et al. 2013a, b). However, most of the commercially cultivated cucumber cultivars are non-bitter type with the absence of bitterness in foliage and fruits even under varied climatic condition. It was found that non-bitterness in the foliage of an American cultivar, 'Long Green', is controlled by a single recessive allele, *bi* of the *Bi* gene (Andeweg and DeBruyn 1959). Cucumber plants carrying the *bi* allele exhibit non-bitter foliage and fruits irrespective of environmental conditions. The dominant *Bi* allele that was found responsible for bitterness in cucumber encodes oxidosqualene cyclase, which catalyses the formation of the triterpenoid carbon skeleton in cucurbitacin biosynthesis (Phillips et al. 2006). Recently, the *Bi* gene (Csa6G088690) was mapped on chromosome 6 of cucumber (Huang et al. 2009a, b; Shang et al. 2014). While studying the molecular aspects of the Bi gene, it was found that *Bi* gene has an open reading frame of 2358 bp, encoding a protein of 785 amino acids. Moreover, it was established that the *Bi* is an ortholog of the squash (*Cucurbita pepo*) cucurbitadienol synthase gene CPQ (Shibuya et al. 2004; Huang et al. 2009a, b).

Earlier studies by Qi et al. (2013) and Shang et al. (2014) also demonstrated presence of other genes in relation to bitterness in cucumber. They reported two basic helix-loop-helix (bHLH) transcription factor genes, *Bl* and *Bt*, which regulate the expression of the Bi gene. The *Bl* gene (Csa5G156220) regulates cucurbitacin biosynthesis in cucumber leaves, whereas *Bt* regulates cucurbitacin biosynthesis in fruits.

Cucurbitacin biosynthesis is influenced by different abiotic stresses through modulation of the expression of Bl and/or Bt (Shang et al. 2014). The *Bt* and *Bl* genes are tightly linked on cucumber chromosome 5 (Shang et al. 2014). Presently, few markers linked to bitterness in cucumber are available. An insertion-deletion (InDel) marker, Bt-InDel-1, which is mapped on cucumber chromosome 5, 0.8 cM away from the Bt locus, is tightly linked to cucumber fruit bitterness (Zhang et al. 2011). Although the *Bi* gene is known (Huang et al. 2009a, b), reports of molecular markers predicting the cucumber bitterness trait are few. An amplified fragment length polymorphism (AFLP) marker, TG/GCT_(150), was identified as located 6.43 cM from the Bi locus (Gu et al. 2006; Chi et al. 2007). Li et al. (2010) and

Huang et al. (2009a, b) mapped the *Bi* gene on chromosome 6, and two linked simple sequence repeat (SSR) markers (SSR02309 and SSR00004) were identified with a genetic distances of 1.7 and 2.2 cM, respectively, corresponding to a physical interval of 35 Kb. A recent study involving genome-wide association approach indicated a nonsynonymous mutation (G1178A) in the coding region of the *Bi* gene (Csa6G088690), resulting in the conversion of a cysteine to a tyrosine (C393Y), which leads to conformational changes in the protein. This conformational change results in impaired catalytic activity of cucurbitadienol synthase and is responsible for the variation in bitterness (Shang et al. 2014). Venkatesh et al. (2018) developed reliable and cost-effective high-resolution melting (HRM)- and kompetitive allele-specific PCR (KASP)-based molecular markers (BiHRM1and Bi-KASP). These gene-based SNP markers could enhance the accuracy and effectiveness of marker-assisted selection for bitter-free cucumber lines in cucumber breeding programmes. With the available molecular markers, it is possible to knock out the genes responsible for bitterness in the cucumber while introgressing the different desirable traits from bitter wild relatives into the cultivated types. However, the available molecular markers should be validated in the wild relatives before their application in marker-assisted back-cross breeding.

10.5.2 Rapid Development of Cucumber Genotypes with Resistance to Major Biotic Stresses

Among the different fungal diseases, downy mildew (caused by *Pseudoperonospora cubensis*), powdery mildew (*Sphaerotheca fuliginea*) and leaf spot (caused by *Colletotrichum* spp.) are most important causing significant yield loss in different regions of the world. Availability of draft genome in cucumber has made possible to undertake several studies related to the identification of closely linked and functional markers related to these diseases. However, till now most of the studies are restricted to identification and development of molecular markers, and introgression of these traits into different nuclear background is limited. Now, it is possible to undertake the marker-assisted back-cross breeding programme with the availability of these reliable markers.

10.5.2.1 Downy Mildew

Extensive studies have been conducted for downy mildew resistance in cucumber, and an array of genotypes were identified with effective resistance against this important disease. Most of the genotypes identified as source of resistance against downy mildew are directly or indirectly linked with Indian origin genetic resources. A large number of molecular markers closely linked with R genes were developed. These genetic resources and molecular markers would be extremely useful in rapid introgression of R genes into the desired nuclear background of cucumber. The available genetic information and molecular analysis are therefore summarized in Table 10.3.

Table 10.3 Genetics of downy mildew resistance in cucumber

Sl No.	Genotype	Inheritance	Molecular markers (if any)	Linkage group (if any)	Research groups
	PI 197087	Single recessive gene, *dm-1*	–	–	Barnes and Epps (1954)
1	Aojihai	Three recessive genes (proposed s1, s2 and s3)	–	–	Shimizu et al. (1963)
2	Sadao Rischu	At least three major genes exhibiting partial dominance	–	–	Pershin et al. (1988)
3	Poinsett/PI 197087	A single recessive gene that they named *p/dm-1*	SSRs/SNPs	LG-5	Van Vliet and Meysing (1974), Fanourakis and Simon (1987), Kennard et al. (1994), and Horejsi et al. (2000)
4	Ashley/PI 197087	A single recessive gene, *dm-1*	–	LG-5	Fanourakis and Simon (1987) and Van Vliet and Meysing (1974)
5	Palmetto and Yomaki	An epistatic interaction between a dominant susceptible gene and a recessive resistance gene	–	–	El-Hafaz et al. (1990)
6	PI 197088	Two recessive genes	–	–	Angelov (1994)
7	Poinsett	Single recessive gene			Angelov (1994)
8	–	Resistance was controlled by three recessive genes (dm-1, dm-2 and dm-3), where dm-3 and either dm-1 or dm-2 had to be homozygous recessive for maximum resistance	–	–	Doruchowski and Lakowska-Ryk (1992)
9	J-13/ Wisconsin 2843/PI 197087	One or two incompletely dominant genes	–	–	Petrov et al. (2000)
10	PI 197088	At least three genes for resistance to downy mildew	–	–	Criswell (2008) and Call (2010)
11	Ames 2354 (a selection from SC50)	Number of genes controlling DM resistance and one of them seems to be allelic to dm-1	–	–	Kozik et al. (2013)
12	129, S94, K8	Five QTLs	RAPD/ SSR/SNP	LG-1, 4, 5, 6	Ding et al. (2007), Bai et al. (2008), and Zhang et al. (2013)
13	*C. hystrix* introgression line IL52	Three QTLs	SSR/SNP	LG-6, 5	Pang et al. (2013)

(continued)

Table 10.3 (continued)

Sl No.	Genotype	Inheritance	Molecular markers (if any)	Linkage group (if any)	Research groups
14	PI 197088	Three QTLs	SSR/SNP	LG-2, 4, 5	Caldwell et al. (2011) and Shetty et al. (2014)
15	CS-PMR1	Ten QTLs	SSR/SNP	LG-1, 3, 5, 6, 7	Yoshioka et al. (2014)
16	PI 197085	Three QTLs	SSR/SNP	LG-5	Szczechura et al. (2015)
17	WI7120 (PI 330628)	Five QTLs	SSR/SNP	LG-2, 4, 5. 6	Wang et al. (2016)
18	PI 197088	Ten QTLs	SNP	LG-1, 2, 4, 5	Wang et al. (2018)

10.5.2.2 Powdery Mildew

10.5.2.2.1 Inheritance

Besides downy mildew, powdery mildew is another devastating fungal pathogen causing significant yield loss in cucumber worldwide. Several reports regarding inheritance of powdery mildew resistance are available in cucumber. Most of the reports supports the polygenic nature of powdery mildew resistance in different genotypes. However, few reports of single recessive genes and incomplete dominance nature of the resistance genes are available. The following are the mode of inheritance reported by different workers:

(i) Polygenic/quantitative trait controlled by multiple recessive genes: Smith (1948), Barnes and Epps (1956), Fugieda and Akiya (1962), Kooistra (1968), Kooistm (1971), Zhang et al. (2005), and Sakata et al. (2006a, b)
(ii) Single recessive gene: Shanmugasundarum et al. (1971) for hypocotyl resistance (pm-h) which is essential for overall resistance, He et al. (2013) and Walters et al. (2001) for hypocotyl resistance (pm-h), Zhang et al. (2004) and Liu et al. (2008)
(iii) Pair of incomplete recessive genes and two pairs of epistatic genes: Shanmugasundarum et al. (1971) and Mao et al. (2005)
(iv) A pair of recessive genes and a pair of incompletely dominant genes which are thermo-sensitive: Morishita et al. (2003)

10.5.2.2.2 Genetic Resources and Molecular Characterization of Resistant Genotypes

The genotype PI 197087 consistently exhibited a high level of resistance to powdery mildew for almost 40 years (Clark 1975; Zijlstra and Groot 1992; Block and Reitsma 2005; Nie et al. 2015a). Partial dominance of resistance at the early growing stage in the genotype PI 197088 was reported by El Jack and Munger (1983). Morishita et al. (2003) suggested that the resistance in PI 197088-5, a selection of PI 197088, is due to two genes, one recessive and another incompletely dominant. Sakata et al. (2006a, b) identified four temperature-dependent quantitative trait loci (QTL) for PM resistance on chromosomes 1, 5, 6 and 7 in PI 197088-1. With a detached-leaf assay, Fukino et al. (2013) detected seven QTLs on chromosomes 1–6 inCS-PMR1 (also derived from PI 197088). Berg et al. (2015) and Nie et al. (2015a) identified a candidate gene for the major effect PM resistance QTL pm5.1 (syn. pm-h) and found that the resistance is due to loss of function of the susceptibility gene CsMLO1 (or CsMLO8) on chromosome 5. Nie et al. (2015b) further found that a 1 bp insertion in this candidate gene in PI 197088 resulted in a premature stop codon and PM resistance.

10.5.2.3 Anthracnose

Anthracnose is another foliage disease causing severe yield loss under congenial condition. Barnes and Epps (1952, 1954) were the first to report anthracnose resistance in cucumber long back. They studied the resistance reaction in the genotypes PI 197087 and PI 234517. Later on, Barnes and Epps (1955) found that the resistance in PI 197087 was controlled by several major genes. In the same study, they concluded that inoculation responses on PI 197087 seemed to be typically hypersensitive in nature (Barnes and Epps 1955). Barnes and Epps (1952) also identified five additional PI lines, PI 175111, PI 175120, PI 179676, PI 183308 and PI 183445, with moderate resistance to anthracnose. Robinson et al. (1976) found that the resistance in PI 175111 was controlled by a single dominant gene, *Ar*. Abul-Hayja et al. (1978) found that the resistance to race 1 of anthracnose in the genotype SC19B was controlled by a recessive gene, *cla*. In the USA, two more germplasm lines, Gy3 and Gy14, were also reported to possess high degree of resistance against this disease (Wyszogrodzka et al. 1987). Resistance against race -2 of anthracnose was reported in the line AR79-95 which was derived from PI 197087 (Goode and Browers 1973). Linde et al. (1990) found that at least five genes (both dominant and recessive) for resistance in AR79-95. The anthracnose resistance derived from PI 197087 has been used widely in commercial varieties in the USA for nearly 60 years. In fact, the pickling cucumber inbred line Gy14 possessing anthracnose resistance which originated from PI 197087 is a resistant control being used in cucumber breeding programmes. Anthracnose resistance in the genotype WI2757 was shown to be linked with downy mildew resistance (Fanourakis and Simon 1987). A SCAR (sequence-characterized amplified region) marker was identified that was linked with anthracnose resistance in the cucumber line ‘66’ which was controlled by a single recessive

locus (Wang et al. 2007; Li et al. 2008). No candidate genes for anthracnose resistance have been cloned from these studies. Recently, fine mapping and cloning of a resistance gene to race 1 was reported by Pan et al. (2018) in inbreds, Gy14 and WI 2757. They have found that a single recessive gene, *cla*, was the candidate gene in both the lines. Further study confimerd the presence of the resistance locus, *cla* in 32 kb region in chromosome 5 and three genes were predicted in this region for resistance. It was also concluded that cucumber STAYGREEN (CsSGR) gene is a candidate for the anthracnose resistance based on multiple line evidence.

With the under standing of nature of inheritance and availability of closely linked molecular markers, it is now possible to develop cucumber genotypes with multiple disease resistance very rapidly through marker-assisted back-cross (MABC) breeding programme. However, development of sufficient number of molecular markers for recombinant selection is necessary besides the forward selection for the donor traits in any MABC programme.

10.6 Rapid Development of Inbreds and Creation of Genetic Diversity

Doubled haploidy (DH) is of specific interest to plant breeders as it is the single-step approach to produce homozygous lines. Development of homozygous inbreds in cross-pollinated crops like cucumber requires at least seven generations (4–5 years) of selfing through conventional approach. In contrast, application of techniques for induction of haploids and subsequent diploidization requires 1.5–2 years to develop complete homozygous lines in cucumber (Fig. 10.2). Different techniques used for development of haploids and doubled haploids were reported by different workers as summarized in Table 10.4.

Among the different methods of haploid development, parthenogenic development of haploids through pollination with irradiated pollen was found to be the most successful in cucumber and reported widely by several workers (Table 10.5).

The homozygous materials developed within 2–3 years using DH technology can be readily used as one parent development of F_1 hybrids, or they may be recommended as a commercial variety if performed well under multilocation trial. Therefore, the time required is drastically reduced through development of DH in cucumber. Besides, the DH-based populations are ideal in molecular mapping of complex traits. However, development of RILs (recombinant inbred lines) requires several years (5–8 years) for their use in molecular mapping. Therefore, DH technology offers very attractive option to accelerate the breeding methodologies in cucumber.

Development of doubled haploids from the F_1 hybrids offers a unique opportunity to create novel genotype and more diversity from an existing population. This is mainly because of one cycle of meiosis and recombination when the donor (microspore/megaspore) for the DH development is taken from the F_1 hybrids of

Fig. 10.2 Growing of cucumber under polyhouse during winter season for generation advancement of rapid development of inbreds

Table 10.4 Different methods of haploid development in cucumber

Technique employed	Donor plant parts	Stage of culture	Specific treatment	Success rate	Researchers
Gynogenesis	Unfertilized ovule/ovary	6 h before anthesis	Heat treatment of 35 °C for 4–5 days	18.4%	Ge'mesne-Juha'sz et al. (1997, 2002)
Gynogenesis/ parthenocarpic embryogenesis	Ovaries pollinated with irradiated pollens	Embryo rescue 10–25 days after pollination	Gamma irradiation at 200–500 rad	–	Troung-Andre (1988), Niemirowicz-Szczytt and Dumas de Vaulx (1989), Sauton (1989), Przyborowski and Niemirowicz-Szczytt (1994), Sztangret-Wiśniewska et al. (2006), Lofti et al. (1999, 2003), Claveria et al. (2005), Lofti and Salehi (2008), Ari et al. (2010), and Sari et al. (2010)

Table 10.4 (continued)

Technique employed	Donor plant parts	Stage of culture	Specific treatment	Success rate	Researchers
Androgenesis	Isolated microspores	Late uninucleate stage	Heat shock treatment at 32–35 °C	15–25 embryos/plate	Suprunova and Shmykova (2008), Zhan et al. (2009), and Chen et al. 2012
Androgenesis	Anther culture	Small buds 3–7 days before anthesis	Both cold and hear shock treatment	–	Ashok Kumar et al. (2003, 2004, Ashok Kumar and Murthy (2004), Song et al. (2007), and Abdollahi et al. (2016)

Table 10.5 Comparative analysis of development of a homozygous material through DH technology and conventional pedigree inbreeding method (PIM)

Activity	Progeny	Genetic composition	Time line (year)	
			DH	PIM
Crossing of two desired parents	F_1 hybrids	Heterozygous	–	–
Development of doubled haploids/selfing	DH1/F2	Homozygous/heterogeneous and heterozygous	1.0	1.0
Evaluation of DH plants and selection/selfing	DH2/F3	Homozygous/heterogeneous and heterozygous	2.0	2.0
Multilocation trial after attending homozygosity and homogeneity	DH3/F7	Homozygous/mostly homozygous and homogeneous	3.0	8.0
Development of commercial variety	DH_4/$F_{10–12}$	Homozygous/mostly homozygous and homogeneous	4.0	10.0–13.0

two distantly related genotypes. Moreover, this technology is the only option to develop completely homozygous and stable traits like gynoecism and parthenocarpy without going for marker-assisted back-cross breeding. Moreover, there is a possibility that all the developed DH are diverse from each other while possessing the desirable traits like gynoecy, parthenocarpy and tolerance to different biotic stresses. Lofti et al. (2003) demonstrated that it is possible to develop completely homozygous lines with multiple virus resistance through induction of haploids from F_1 and their subsequent diploidization in *C. melo*. It is further explained in the following figure for its possible application in cucumber (Fig. 10.3).

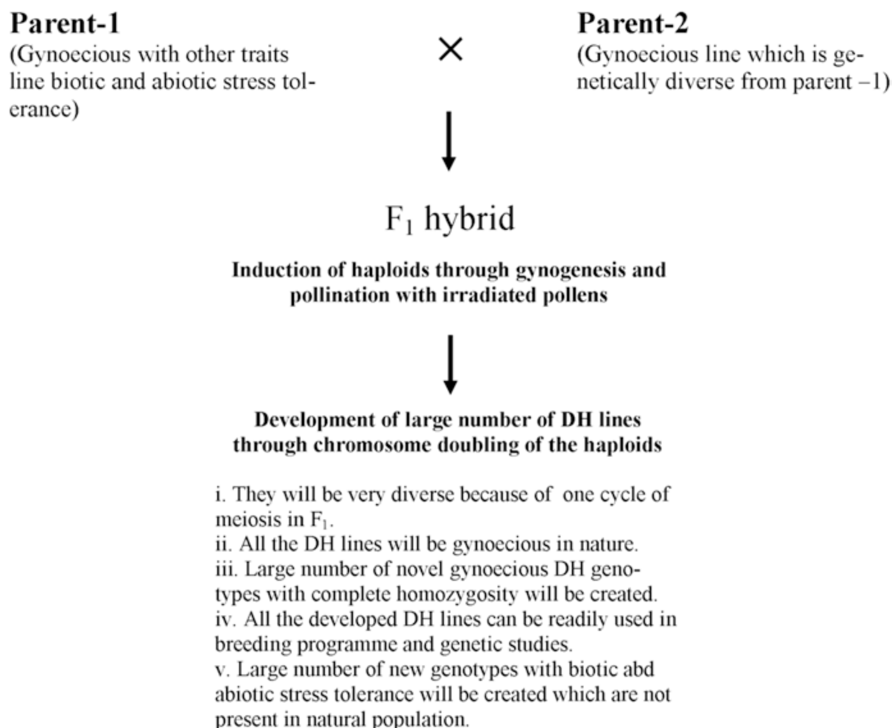

Fig. 10.3 How doubled haploid (DH)-based approaches are useful in germplasm innovation in cucumber?

10.7 Rapid Development of Genotypes with Desirable Agronomic Traits Through Marker-Assisted Back-Crossing

Availability of large number of molecular markers for different traits makes it possible to undertake marker-assisted back-cross introgression of the traits in any desired nuclear background of cucumber. Development and application of high-throughput molecular markers in cucumber are possible after the availability of the draft genome sequence of this important crop. Besides important biotic stresses, a large number of molecular markers were reported for important agronomic traits related to fruit yield and plant architecture (Table 10.6.)

After the availability of cucumber draft genome, QTL mapping for all major yield- and fruit-related traits was reported by different workers throughout the world. This available information can be used in rapid development of improved tailored genotypes through marker-assisted selection strategies.

Table 10.6 Identified traits and corresponding genes reported by different workers in cucumber for important agronomic traits

Gene symbol	Target trait	Reference(s)
a	Androecious. Produces primarily staminate flowers if recessive for F. A from MSU 713-5 and Gy 14; a from An-11 and An314, two selections from 'E-e-szan' of China	Kubicki (1969a)
Ak-2	Adenylate kinase (E.C. # 2.7.4.3). Isozyme variant found segregating in PI 339247 and 271754; 2 alleles observed	Meglic and Staub (1996)
Ak-3	Adenylate kinase (E.C. # 2.7.4.3). Isozyme variant found segregating in PI 113334, 183967 and 285603; 2 alleles observed	Meglic and Staub (1996)
al	Albino cotyledons	Iida and Amano (1990, 1991)
ap	Apetalous. Male-sterile. Anthers become sepal-like	Grimbly (1980)
B	Black or brown spines. Dominant to white spines on fruit	Strong (1931)
B-2	Black spine-2. Interacts with B to produce F2 of 15 black: 1 white spine	Shanmugasundarum et al. (1971)
B-3	Black spine-3. Interacts with B-4 to produce an F2 of nine black: 7 white spine	Cowen and Helsel (1983)
B-4	Black spine-4. Interacts conversely with B3	Cowen and Helsel (1983)
bi	Bitter-free. All plant parts lacking cucurbitacins. Plants with bi less preferred by cucumber beetles	Andeweg and DeBruyn (1959)
bi-2	Bitterfree-2. Leaves lacking cucurbitacins	Wehner et al. (1998)
bl	Blind. Terminal bud lacking after temperature shock	Carlsson (1961)
bla	Blunt leaf. Leaves have obtuse apices and reduced lobing and serration	Robinson (1987)
Bt	Bitter fruit. Fruit with extreme bitter flavour	Barham (1953)
Bu	Bush. Shortened internodes	Pyzenkov and Kosareva (1981)
c	Cream mature fruit colour. Interaction with R is evident in the F2 ratio of 9 red (RC): 3 orange (Rc): 3 yellow (rC): 1 cream (rc)	Hutchins (1940)
chp	Choripetalous. Small first true leaf; choripetalous flowers; glossy ovary; small fruits; few seeds	Kubicki et al. (1984)
cl	Closed flower. Staminate and pistillate flowers do not open; male-sterile	Groff and Odland (1963)
co	Green corolla. Green petals that turn white with age and enlarged reproductive organs	Hutchins (1935)
cor-1	Cordate leaves-1. Leaves are cordate	Gornitskaya (1967)
cp	Compact. Reduced internode length, poorly developed tendrils, small flowers	Kauffman and Lower (1976)
cr	Crinkled leaf. Leaves and seed are crinkled	Odland and Groff (1963)
cs	Carpel splitting. Fruits develop deep longitudinal splits	Carruth (1975)
D	Dull fruit skin. Dull skin of American cultivars, dominant to glossy skin of most European cultivars	Poole (1944)

(continued)

Table 10.6 (continued)

Gene symbol	Target trait	Reference(s)
de	Determinate habit. Short vine with stem terminating in flowers	Denna (1971)
df	Delayed flowering. Flowering delayed by long photoperiod; associated with dormancy	Della Vecchia et al. (1982)
dl	Delayed growth. Reduced growth rate; shortening of hypocotyl and first internodes	Miller and George (1979)
dvl	Divided leaf. True leaves are partly or fully divided	den Nijs and Mackiewicz (1983)
dw	Dwarf. Short internodes. dw from an induced mutant of 'lemon'	Robinson and Mishanec (1965)
dwc-1	Dwarf cotyledons-1. Small cotyledons; late germination; small first true leaf; died after third true leaf	Iida and Amano (1990, 1991)
dwc-2	Dwarf cotyledons-2. Small cotyledons; late germination; small first true leaf	Iida and Amano (1990, 1991)
Es-1	Empty chambers-1. Carpels of fruits separated from each other, leaving a small to large cavity in the seed cell	Kubicki and Korzeniewska (1983)
Es-2	Empty chambers-2. Carpels of fruits separated from each other, leaving a small to large cavity in the seed cell	Kubicki and Korzeniewska (1983)
F	Female. High degree of pistillate sex expression; interacts with a and M; strongly modified by environment and gene background	Galun (1961)
fa	Fasciated. Plants have flat stems, short internodes and rugose leaves	Robinson (1987)
Fba	Flower bud abortion. Preanthesis abortion of floral buds, ranging from 10% to 100%	Miller and Quisenberry (1978)
Fl	Fruit length. Expressed in an additive fashion, fruit length decreases incrementally with each copy of f	Wilson (1968)
g	Golden leaves. Golden colour of lower leaves	Tkachenko (1935)
gb	Gooseberry fruit. Small, oval-shaped fruit	Tkachenko (1935)
gc	Golden cotyledon. Butter-coloured cotyledons; seedlings die after 6–7 days	Whelan (1971)
gi	Ginkgo. Leaves reduced and distorted, resembling leaves of ginkgo; male- and female-sterile	John and Wilson (1952)
gig	Gigantism. First leaf larger than normal	Kubicki et al. (1984)
gl	Glabrous. Foliage lacking trichomes; fruit without spines. Iron deficiency symptoms (chlorosis) induced by high temperature	Inggamer and de Ponti (1980)
glb	Glabrate. Stem and petioles glabrous, laminae slightly pubescent	Whelan (1973)
gn	Green mature fruit. Green mature fruits when rrgngn; cream coloured when rrGnGn; orange when R---	Peterson and Pike (1992)
gy	Gynoecious. Recessive gene for high degree of pistillate sex expression	Kubicki (1969a)

(continued)

Table 10.6 (continued)

Gene symbol	Target trait	Reference(s)
H	Heavy netting of fruit. Dominant to no netting	Hutchins (1940) and Tkachenko (1935)
hl	Heart leaf. Heart-shaped leaves	Vakalounakis (1992)
hn	Hornlike cotyledons. Cotyledons shaped like bull horns; true leaves with round shape rather than normal lobes	Iida and Amano (1990, 1991)
hsl	Heart-shaped leaves. Leaves heart shaped rather than lobed; tendrils branched	Iida and Amano (1990, 1991)
l	Locule number. Many fruit locules and pentamerous androecium; five locules recessive to the normal number of three	Youngner (1952)
lh	Long hypocotyl. As much as a three fold increase in hypocotyl length	Robinson and Shail (1981)
ls	Light sensitive. Pale and smaller cotyledons, lethal at high light intensity	Whelan (1973)
m	Andromonoecious. Plants are andromonoecious if *mm ff*; monoecious if *MM ff*; gynoecious if *MM FF* and hermaphroditic if *mm FF*	Tkachenko (1935)
m-2	Andromonoecious-2. Bisexual flowers with normal ovaries	Kubicki (1969a)
mp	Multi-pistillate. Several pistillate flowers per node, recessive to single pistillate flower per node	Nandgaonkar and Baker (1981)
mpy	Male pygmy. Dwarf plant with only staminate flowers	Pyzhenkov and Kosareva (1981)
ms-1	Male sterile-1. Staminate flowers abort before anthesis; partially female-sterile	Shifriss (1950)
ms-2	Male-sterile-2. Male-sterile; pollen abortion occurs after first mitotic division of the pollen grain nucleus	Whelan (1973)
ns	Numerous spines. Few spines on the fruit are dominant to many	Fanourakis and Simon (1987)
O	Orange-yellow corolla. Orange-yellow dominant to light yellow	Tkachenko (1935)
opp	Opposite leaf arrangement. Opposite leaf arrangement is recessive to alternate and has incomplete penetrance	Robinson (1987)
P	*Prominent tubercles*. Prominent on yellow rind of *Cucumis sativus* var. *tuberculatus*, incompletely dominant to brown rind without tubercles	Tkachenko (1935)
Pc	*Parthenocarpy*. Sets fruit without pollination	Pike and Peterson (1969)
pl	*Pale lethal*. Slightly smaller pale-green cotyledons; lethal after 6–7 days	Whelan (1973)
rc	*Revolute cotyledon*. Cotyledons are short, narrow and cupped downwards	Whelan (1973)
sa	*Salt tolerance*. Tolerance to high salt levels is attributable to a major gene in the homozygous recessive state and may be modified by several minor genes	Jones (1984)
sh	*Short hypocotyl*. Hypocotyl of seedlings 2/3 the length of normal	Soltysiak and Kubicki (1988)

(continued)

Table 10.6 (continued)

Gene symbol	Target trait	Reference(s)
sp	*Short petiole*. Leaf petioles of first nodes 20% the length of normal	den Nijs and de Ponti (1983)
ss	*Small spines*. Large, coarse fruit spines are dominant to small, fine fruit spines	Fanourakis and Simon (1987)
T	*Tall plant*. Tall incompletely dominant to short	Hutchins (1940)
td	*Tendrilless*. Tendrils lacking; associated with misshapen ovaries and brittle leaves	Rowe and Bowers (1965)
tf	*Twin fused fruit*. Two fruits fused into single unit	Klosinska et al. (2006)
Tr	*Trimonoecious*. Producing staminate, perfect and pistillate flowers in this sequence during plant development	Kubicki (1969a)
Tu	*Tuberculate fruit*. Warty fruit characteristic of American cultivars is dominant to smooth, non-warty fruits characteristic of European cultivars	Strong (1931)
u	*Uniform immature fruit colour*	Strong (1931)
v	*Virescent*. Yellow leaves becoming green	Poole (1944)
w	*White immature fruit colour*. White is recessive to green	Cochran (1938)
wy	*Wavy rimed cotyledons*. Wavy rimed cotyledons, with white centres; true leaves normal	Iida and Amano (1990, 1991)
yc-1	*Yellow cotyledons-1*. Cotyledons yellow at first, later turning green	Aalders (1959)
yf	*Yellow flesh*. Interacts with *wf* to produce F2 of 12 white *(WfYf* and *wfYf)*: 3 yellow *(Wfyf)*: 1 orange *(wfyf)*	Kooistra (1971)
yg	*Yellow-green immature fruit colour*	Youngner (1952)
gl2	*Glabrous2, glabrous trait in cucumber, tubercles and spine on cucumber peel*	Mengnan et al. (2015)
qgf5.1	*Major effect QTL for green flesh colour*	Bo et al. (2019)
yf	*Yellow flesh fruit colour*	Lu et al. (2015)

10.8 Rapid Generation Cycling Using Greenhouse and Protected Structures and In Vitro Culture

The production of new generations is a fundamental component of plant breeding as this allows another round of meiosis from which new recombinants can be produced. The time taken to obtain new segregating materials is often a major time constraint. Classic forms of speeding up generation times are single seed descent (SSD) and shuttle breeding. Moreover, off-season flowering and fruiting can also be achieved by using the greenhouse facilities. In vitro-based techniques can also be used for flowering under in vitro condition and generation advancement. However, very limited research works have been conducted in these aspects, and most of the findings are restricted in major grain crops only. There is no such report in cucumber till now.

10.9 Future Strategies

In cucumber, conventional breeding strategies contributed immensely in developing improved cultivars with enhanced productivity, superior quality and resistance to different biotic and abiotic stresses. However, significant progress in cucumber improvement was made after the discovery of the molecular markers. With the availability of the draft genome sequence and next-generation sequencing (NGS) technologies, enormous progress has been made in development of molecular markers, understanding the complex molecular pathways and association of markers with different traits of interest. However, to keep the pace with increasing population and climatic adversaries, more novel efforts are required to meet the future need and development of climate-resilient cultivars. However, very limited work has been undertaken to accelerate the cucumber breeding with the application of induced mutation, in vitro-based techniques for generation advancement, high-throughput phenotyping and generation advancement under greenhouse condition. Therefore, to accelerate the breeding programme of cucumber, a lot of concerted efforts are required in these areas. However, because of the availability of the genomics-based data sets and large number of markers for different traits, it is possible to speed up the improvement programme with the help of different bioinformatics-based tools. Development of haploids through $CeNH_3$-based strategies and genome editing through CRISPR/CAS9 are areas need to be investigated, and proper pipelines need to be developed to exploit these technologies in genomics-based speed breeding of cucumber.

References

Aalders LE (1959) 'Yellow Cotyledon', a new cucumber mutation. Can J Cytol 1:10–12

Abdollahi MR, Najafi S, Sarikhani H, Moosavi SS (2016) Induction and development of anther-derived gametic embryos in cucumber (Cucumis sativus L.) by optimizing the macronutrient and agar concentrations in culture medium. Plant Cell Rep 35:1991–2019

Abul-Hayja Z, Williams PH, Peterson CE (1978) Inheritance of resistance to anthracnose and target leaf spot in cucumbers. Plant Dis Rep 62:43–45

Agrawal AA, Janssen A, Bruin J, Posthumus MA, Sabelis MW (2002) An ecological cost of plant defence: attractiveness of bitter cucumber plants to natural enemies of herbivores. Ecol Lett 5:377–385

Andeweg JM, DeBruyn JW (1959) Breeding of non-bitter cucumbers. Euphytica 8:13–20

Angelov D (1994) Inheritance of resistance to downy mildew, Pseudoperonospora cubensis (Berk. & Curt.) Rostow. Rep. 2nd National Symposium. Plant Immunity (Plovdiv) 3:99–105

Ari E, Ikten H, Gocmen M, Coskun R, Eren A (2010) Comparative evaluation of different embryo rescue techniques on parthenogenetic melon (Cucumis melo L.) fruits induced with irradiated pollen. Afr J Biotechnol 9(33):5347–5356

Ashok Kumar HG, Murthy HN (2004) Effect of sugars and amino acids on androgenesis of Cucumis sativus L. Plant Cell Tissue Organ Cult 78:201–208

Ashok Kumar HG, Murthy HN, Paek KY (2003) Embryogenesis and plant regeneration from anther cultures of Cucumis sativus L. Sci Hortic 98:213–222

Ashok Kumar HG, Ravishankar BV, Murthy HN (2004) The influence of polyamines on androgenesis of Cucumis sativus L. Eur J Hortic Sci 69:201–205

Bai SL, Peng YB, Cui JX, Gu HT, Xu LY, Li YQ, Xu ZH, Bai SN (2004) Developmental analyses reveal early arrests of the spore-bearing parts of reproductive organs in unisexual flowers of cucumber (Cucumis sativus L.). Planta 220:230–240

Bai Z, Yuan X, Cai R, Liu L, He H, Zhou H, Pan J (2008) QTL analysis of downy mildew resistance in cucumber. Prog Nat Sci 18:706–710. (in Chinese)

Balkema-Boomstra AG, Zijlstra S, Verstappen FW, Inggamer H, Mercke PE, Jongsma MA, Bouwmeester HJ (2003) Role of cucurbitacin C in resistance to spider mite (*Tetranychus urticae*) in cucumber (*Cucumis sativus* L.). J Chem Ecol 29:225–235

Barnes WC, Epps WM (1952) Two types of anthracnose resistance in cucumbers. Plant Dis Rep 36:479–480

Barnes WC, Epps WM (1954) A unreported type of resistance to cucumber downy mildew. Plant Dis Rep 38:620

Barnes WC, Epps WM (1955) Progress in breeding cucumbers resistant to anthracnose and downy mildew. Proc Am Soc Hortic Sci 65:409–415

Barnes WC, Epps WM (1956) Powdery mildew resistance in South Carolina cucumbers. Plant Dis Res 40:1093

Berg JA, Appiano M, Martínez MS et al (2015) A transposable element insertion in the susceptibility gene CsaMLO8 results inhypocotyl resistance to powdery mildew in cucumber. BMC Plant Biol 15:243

Block CC, Reitsma KR (2005) Powdery mildew resistance in the US National plant germplasm system cucumber collection. Hortic Sci 40:416–420

Bo K, Wei S, Wang W, Han M, Dong S, Zhang S, Gu X (2019) QTL mapping and genome-wide association study reveal two novel loci associated with green flesh color in cucumber. BMC Plant Biol 19:243. https://doi.org/10.1186/s12870-019-1835-6

Borlaug N (2002) Feeding a world of 10 billion people: the miracle ahead. In vitro Cell Devel Biol Plant 38:221–228

Boualem A, Troadec C, Kovalski I, Sari MA, Perl-Treves R, Bendahmane A (2009) A conserved ethylene biosynthesis enzyme leads to andromonoecy in two Cucumis species. PLoS One 4:e6144

Boualem A, Troadec C, Camps C, Lemhemdi A, Morin H, Sari MA, Fraenkel-Zagouri R, Kovalski I, Dogimont C, Perl-Treves R, Bendahmane A (2015) A cucurbit androecy gene reveals how unisexual flowers develop and dioecy emerges. Science 350:688–691

Calderon-Urrea A, Dellaporta SL (1999) Cell death and cell protection genes determine the fate of pistils in maize. Development 126:435–441

Caldwell D, Chan E, de Vries J et al (2011) Methods and compositions for identifying downy mildew resistant cucumber plants. United States patent US 2011/0126309 A1

Call AD, Wehner TC (2010) Search for resistance to the new race of downy mildew in cucumber. Cucurbitaceae 2010 Proceeding. ASHS Press, Alexandria, pp 112–115

Carlsson G (1961) Studies of blind top shoot and its effect on the yield of greenhouse cucumbers. Acta Agr Scand 11:160–162

Cavagnaro PF, Senalik DA, Yang L et al (2010) Genome-wide characterization of simple sequence repeats in cucumber (Cucumis sativus L.). BMC Genomics 11:569. Published 2010 Oct 15. https://doi.org/10.1186/1471-2164-11-569

Chen JF, Staub JE, Tashiro Y, Isshiki S, Miyazaki S (1997) Successful interspecific hybridization between Cucumis sativus L. and C. hystrix Chakr. Euphytica 96:413–419

Chen YH, Fu XM, Wu H, Zang J (2012) CsACO4, an ACC oxidase gene regulating male differentiation in cucumber. Afr J Biotechnol 11:13069–13074

Chen H, Sun J, Li S, Cui Q, Zhang H, Xin F, Wang H, Lin T, Gao D, Wang S, Li X, Wang D, Zhang Z, Xu Z, Huang S (2016) An ACC oxidase gene essential for cucumber carpel development. Mol Plant 9:1315–1327

Chi X, Gu X, Zhang S, Wang X, Wang Y (2007) Identification of molecular markers linked to foliage non-bitterness gene (bi) in Cucumis sativus L. Acta Hortic Sin 34:1177–1182

Chung SM, Staub JE, Chen JF (2006) Molecular phylogeny of Cucumis species as revealed by consensus chloroplast SSR marker length and sequence variation. Genome 49:219–229

Clark LR (1975) Powdery mildew resistance in plant introductions of cucumber in Iowa. Plant Dis Rep 59:1024–1028
Claveria E, Garcia-Mas J, Dolcet-Sanjuan R (2005) Optimization of cucumber doubled haploid line production using in vitro rescue of in vivo induced parthenogenic embryos. J Am Soc Hortic Sci 130(4):555–560
Cochran FD (1938) Breeding cucumbers for resistance to downy mildew. Proc. Amer. Soc. Hort. Sci. 35:541–543
Criswell AD (2008) Screening for downy mildew resistance in cucumber. M. S. thesis, North Carolina State University, Raleigh
Delorme VG, McCabe PF, Kim DJ, Leaver CJ (2000) A matrix metalloproteinase gene is expressed at the boundary of senescence and programmed cell death in cucumber. Plant Physiol 123:917–927
den Nijs APM, de Ponti OMB (1983) Umbrella leaf: a gene for sensitivity to low humidity in cucumber. Cucurbit Genet. Coop. Rpt. 6:24
Ding G, Qin Z, Zhou X, Fan J (2007) RAPD and SCAR markers linked to downy mildew resistance genes in cucumber. Acta Botan Boreali-Occiden Sin 27:1747–1751. (in Chinese)
Doruchowski RW, Lakowska-Ryk E (1992) Inheritance of resistance to downy mildew (Pseudoperonospora cubensis Berk & Curt) in Cucumis sativus. In: Doruchowski RW, Kozik E, NiemirowiczSzczytt K (eds) Proceedings of 5th EUCARPIA symposium, pp 132138, 2731 July, Warsaw. Published by Research Institute of Vegetables Crops, and Warsaw University of Agriculture, Warsaw
Duvick DN (1986) Plant breeding past achievements and expectations for the future. Econ Bot 40:289–297
El Jack A, Munger H (1983) Two sources conferring partial dominant resistance to powdery mildew (Sphaerotheca fuliginea Poll.) in cucumber. Cucurbit Genet Coop Rep 6:7–8
El-Hafaz A, El-Din B, El-Doweny HH, Awad MMW (1990) Inheritance of downy mildew resistance and its nature of resistance in cucumber. Ann Agric Sci Moshtohor 28(3):1681–1697
Fanourakis NE, Simon PW (1987) Analysis of genetic linkage in the cucumber. J Hered 78:238–242
Fehr WR (1984) Genetic contribution to yield gains in five major crop plants. Crop Sci Soc Am 7:9. isbn 978-0-89118-586-4
Forster BP, Shu Q (2012) Plant mutagenesis in crop improvement: basic terms and applications. In: Shu Q, Forster BP, Nakagawa H (eds) Plant mutation breeding and biotechnology, vol C01. CABI, Wallingford, pp 9–20
Fugieda K, Akiya Y (1962) Genetic study of powdery mildew resistance and spine color on fruit in cucumber. J Jpn Soc Hortic Sci 31:30–32. https://doi.org/10.2503/jjshs.31.30
Fukino N, Yoshioka Y, Sugiyama M et al (2013) Identification and validation of powdery mildew (*Podosphaera xanthii*)-resistant loci in recombinant inbred lines of cucumber (Cucumis sativus L.). Mol Breed 32:267–277
Galun E (1961) Study of the inheritance of sex expression in the cucumber. The interaction of major genes with modifying genetic and non-genetic factors. Genetica 32:134–163
Ge'mes-Juha'sz A, Balogh P, Ferenczy A, Kristo'f Z (2002) Effect of optimal stage of female gametophyte and heat treatment on in vitro gynogenesis induction in cucumber (Cucumis sativus L.). Plant Cell Rep 21(2):105–111
Ge'mesne-Juha'sz A, Venczel G, Balogh P (1997) Haploid plant induction in zucchini (Cucurbita pepo L. convar. Giromontiina Duch) and in cucumber (Cucumis sativus L.) lines through in vitro gynogenesis. Acta Hortic 447:623–625
Goode MJ, Browers JL (1973) Breeding anthracnose resistance in cucumber. Phytopathology 63:442
Gu X, Zhang S, Zhang S (2006) The AFLP markers linked with the bitter fruit gene (Bt) in cucumber. Acta Hortic Sin 33:140–142
Hao YJ, Wang DH, Peng YB, Bai SL, Xu LY, Li YQ, Xu ZH, Bai SN (2003) DNA damage in the early primordial anther is closely correlated with stamen arrest in the female flower of cucumber (Cucumis sativus L.). Planta 217:888–895
Harlan JR (1992) Crops and man. Am Soc Agron Crop Sci 284

He X, Li Y, Pandey S, Yandell BS et al (2013) QTL mapping of powdery mildew resistance in WI 2757 cucumber (Cucumis sativus L.). Theor Appl Genet 126:2149–2161. https://doi.org/10.1007/s00122-013-2125-6

Horejsi T, Staub JE, Thomas C (2000) Linkage of random amplified polymorphic DNA markers to downy mildew resistance in cucumber (Cucumis sativus L.). Euphytica 115:105–113

Huang S, Li R, Zhang Z et al (2009a) The genome of the cucumber, Cucumis sativus L. Nat Genet 41:1275–1281

Huang S, Li R, Zhang Z, Li L, Gu X, Fan W, Lucas WJ, Wang X, Xie B et al (2009b) The genome of the cucumber, Cucumis sativus L. Nat Genet 41:1275–1281

Huang S, Li R, Zhang Z et al (2011) Genomic data for the domestic cucumber (Cucumis sativus var. sativus L.). GigaScience Database. https://doi.org/10.5524/100025

Hutchins AE (1940) Inheritance in the cucumber. J Agr Res 60:117–128

Iida S, Amano E (1990) Pollen irradiation to obtain mutants in monoecious cucumber. In: Gamma field Symp, 29:95–111

Iida S, Amano E (1991) Mutants induced by pollen irradiation in cucumber. Cucurbit Genet Coop Rpt 14:32–33

Inggamer H, de Ponti OMB (1980) The identity of genes for glabrousness in Cucumis sativus L. Cucurbit Genet. Coop. Rpt. 3:14

John CA, Wilson JD (1952) A “ginko leafed” mutation in the cucumber. J Hered 43:47–48

Kano Y, Goto H (2003) Relationship between the occurrence of bitter fruit in cucumber and the contents of total nitrogen, amino acid nitrogen, protein, and HMG-CoA reductase activity. Sci Hortic 98:1–8

Kennard WC, Poetter K, Dijkhuizen A, Meglic V, Staub JE, Havey MJ (1994) Linkages among RFLP, RAPD, isozyme, disease-resistance, and morphological markers in narrow and wide crosses of cucumber. Theor Appl Genet 89:42–48

Klosinska U, Kozik EU, Wehner TC (2006) Inheritance of a new trait - twin fused fruit - in cucumber. HortScience 41:313–314

Kooistm E (1971) Inheritance of flesh and skin colors in powdery mildew resistance cucumber (Cucummis sativas L). Euphytica 20:521–523

Kooistra E (1968) Powdery mildew resistance in cucumber. Euphytica 17:236–244

Kooistra E (1971) Inheritance of flesh and skin colors in powdery mildew resistant cucumbers (Cucumis sativus L.). Euphytica 20:521–523

Kozik EU, Klosin’ska U, Call AD, Wehner TC (2013) Heritability and genetic variance estimates for resistance to downy mildew in cucumber accession Ames 2354. Crop Sci 53:177–182

Kubicki B (1969a) Investigations of sex determination in cucumber (Cucumis sativus L.). IV. Multiple alleles of locus Acr. Genetica Polonica 10:23–68

Kubicki B (1969b) Investigation of sex determination in cucumber (Cucumis sativus L.). VI. Androecism. Genet Pol 10:87–99

Kubicki B (1969c) Investigation of sex determination in cucumber (Cucumis sativus L.). VII Trimonoecism. Genet Pol 10:123–143

Kubicki B (1974) New sex types in cucumber and their uses in breeding work. Proc XIX Intl Hort Congr 3:475–485

Kubicki B, Goszczycka I, Korzeniewska A (1984) Induced mutations in cucumber (Cucumis sativus L.) II. Mutant of gigantism. Genetica Polonica 25:41–52

Li SJ, Wang HZ, Huo ZR, Guan W (2008) Development of SCAR marker linked to cucumber anthracnose resistance-related gene from an AFLP marker. Acta Hortic Sin 35:123–126. (in Chinese)

Li Z, Huang S, Liu S, Pan J, Zhang Z, Tao Q, Shi Q, Jia Z, Zhang W, Chen H, Si L, Zhu L, Cai R (2009) Molecular isolation of the M gene suggests that a conserved-residue conversion induces the formation of bisexual flowers in cucumber plants. Genetics 182:1381–1385

Li M, Gong Y, Miao H, Wu J, Gu X, Zhang S, Wang X (2010) Fine mapping of the foliage bitterness gene (Bi) in Cucumis sativus. Acta Hortic Sin 37:1073–1078

Linde DC, Bridges WC, Rhodes BB (1990) Inheritance of resistance in cucumber to race 2 of Colletotrichum lagenarium. Theor Appl Genet 79:13–16

Liu LZ, He HL, Pan JS (2008) Genetic test of resistance to powdery mildew for cucumber germplasm R17. Resour Utiliz 27:46–48

Lofti M, Salehi S (2008) Detection of cucumber parthenogenic haploid embryos by floating the immature seeds in liquid medium. In: Cucurbitaceae 2008, Pitrat M (ed) Proceedings of IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae, 21–24 May, Avignon, pp 375–380

Lofti M, Kashi A, Onsinejad R (1999) Induction of parthenogenetic embryos by irradiated pollen in cucumber. Acta Hortic 492:323–326

Lofti M, Alan AR, Henning MJ, Jahn MM, Earle ED (2003) Production of haploid and double haploid plants of melon (Cucumis melo L.) for use in breeding for multiple virus resistance. Plant Cell Rep 21(11):1121–1128

Lu HW, Miao H, Tian GL, Wehner TC, Gu XF, Zhang SP (2015) Molecular mapping and candidate gene analysis for yellow fruit flesh in cucumber. Mol Breed 35:64. https://doi.org/10.1007/s11032-015-0263-z

Meglic V, Staub JE (1996) Inheritance and linkage relationships between allozyme and morphological loci in cucumber (Cucumis sativusL.). Theor Appl Genet 92:865–872

Malepszy S, Niemirowicz-Szczytt K (1991) Sex determination in cucumber (Cucumis sativus) as a model system for molecular biology. Plant Sci 80:39–47

Mao AJ, Zhang F, Zhang HY, Zhang LR et al (2005) Inheritance of resistance to powdery mildew in two cucumber varieties. Zhongguo Nongxue Tongbao 21:302–305

Martin A, Troadec C, Boualem A, Rajab M, Fernandez R, Morin H, Pitrat M, Dogimont C, Bendahmane A (2009) A transposon-induced epigenetic change leads to sex determination in melon. Nature 461:1135–1138

Mengnan X, Shenhao W, Shu Z, Quinzhi C, Dongli G, Huiming C, Huang S (2015) A new gene conferring the Glabrous trait in cucumber identified using MutMap. Horticultural Plant Journal 29–34

Mibus H, Tatlioglu ĆT (2004) Molecular characterization and isolation of the F/f gene for femaleness in cucumber (Cucumis sativus L.). Theor Appl Genet 109:1669–1676

Miller JC Jr, Quisenberry JE (1978) Inheritance of flower bud abortion in cucumber. HortScience 13:44–45

Morishita M, Sugiyama K, Saito T, Sakata Y (2003) Powdery mildew resistance in cucumber. JARQ 37(1):7–14. https://doi.org/10.6090/jarq.37.7

Nandgaonkar AK, Baker LR (1981) Inheritance of multi-pistillate flowering habit in gynoecious pickling cucumber. J Amer Soc Hort Sci 106:755–757

Nie J, He H, Peng J et al (2015a) Identification and fine mapping of pm 5.1: a recessive gene for powdery mildew resistance in cucumber (Cucumis sativus L.). Mol Breed 35:7

Nie J, Wang Y, He H et al (2015b) Loss-of-Function mutations in CsMLO1 confer durable powdery mildew resistance in cucumber (Cucumis sativus L.). Front Plant Sci 6:1–14

Niemirowicz-Szczytt K, Dumas de Vaulx R (1989) Preliminary data on haploid cucumber (Cucumis sativus L.) induction. Cucurbit Genet Coop 12:24–25

Osipowski P, Wojcieszek M, Pawełkowicz M, Skarzyńska A, Koren S, Lomsadze A, Wóycicki R, Pląder W, Yagi K, Borodovsky M, Malepszy S, Przybecki Z (2016) Progress in assembling the cucumber (Cucumis sativus) Borszczagowski B10 line genome using long single molecule, real-time reads. In: Cucurbitaceae 2016, XIth Eucarpia Meeting on Cucurbit Genetics & Breeding, July 24–28, 2016, Warsaw, Poland, pp 72–74 ref 17

Pan J, Tan J, Wang Y, Zheng X, Owens K, Li D, Li Y, Weng Y (2018) STAYGREEN (CsSGR) is a candidate for the anthracnose (Colletotrichum orbiculare) resistance locus cla in Gy14 cucumber. Theor Appl Genet 131:1577–1587

Pang X, Zhou X, Wan H, Chen J (2013) QTL mapping of downy mildew resistance in an introgression line derived from interspecific hybridization between cucumber and Cucumis hystrix. J Phytopathol 161:536–543

Pawełkowicz M, Osipowski P, Wojcieszek M et al (2012) Identification and characterization of genes connected with flower morphogenesis in cucumber. Biotechnologia 93:123–134

Pershin AF, Medvedeva NI, Medvedev AV (1988) Quantitative approach to studying the genetics of disease resistance. IV. Interaction of the genetic systems for resistance to powdery and downy mildews in cucumber. Geneticka, USSR. (abstract only) 24(3):484–493

Peterson GC, Pike LM (1992) Inheritance of green mature seed-stage fruit color in Cucumis sativus L. J. Amer. Soc. Hort. Sci. 117:643–645

Petrov L, Boodert K, Sheck L, Baider A, Rubin E, Cohen Y, Katzir N, Paris HS (2000) Resistance to downy mildew, Pseudoperonospora cubensis, in cucumbers. Acta Hortic 510:203–209

Phillips DR, Rasbery JM, Bartel B, Matsuda SP (2006) Biosynthetic diversity in plant triterpene cyclization. Curr Opin Plant Biol 9:305–314

Pike LM, Peterson CE (1969) Inheritance of parthenocarpy in the cucumber (Cucumis sativus L.). Euphytica 18:101–105

Poole CF (1944) Genetics of cultivated cucurbits. J Hered 35:122–128

Pyzenkov VI, Kosareva GA (1981) A spontaneous mutant of the dwarf type. Bul Applied Bot Plant Breeding 69:15–21

Przyborowski J, Niemirowicz-Szczytt K (1994) Main factors affecting cucumber (Cucumis sativus L.) haploid embryo development and haploid plant characteristics. Plant Breed 112:70–75

Qi J, Liu X, Shen D, Miao H, Xie B, Li X, Zeng P, Wang S, Shang Y et al (2013) A genomic variation map provides insights into the genetic basis of cucumber domestication and diversity. Nat Genet 45:1510–1515

Robinson RW (1987) Chlorosis induced in glabrous cucumber by high temperature. Cucurbit Genet. Coop. Rpt. 10:7

Robinson RW, Decker-Walter DS (1999) Cucurbits. CAB International, Wallingford

Robinson RW, Mishanec W (1965) A new dwarf cucumber. Veg Imp Nwsl 7:23

Robinson RW, Munger HM, Whitaker TW, Bohn GW (1976) Genes of the Cucurbitaceae. HortScience 11:564–568

Robinson RW, Shail JW (1981) A cucumber mutant with increased hypocotyl and internode length. Cucurbit Genet. Coop. Rpt. 4:19–20

Rowe JT, Bowers JL (1965) The inheritance and potential of an irradiation induced tendrilless character in cucumbers. Proc. Amer. Soc. Hort. Sci. 86:436–441

Sakata Y, Kubo N, Morishita M et al (2006a) QTL analysis of powdery mildew resistance in cucumber (Cucumis sativus L.). Theor Appl Genet 112:243–250

Sakata Y, Kubo N, Morishita M, Kitadani E et al (2006b) QTL analysis of powdery mildew resistance in cucumber (Cucumis sativus L.). Theor Appl Genet 112:243–250. https://doi.org/10.1007/s00122-005-0121-1

Sari N, Solmaz I, Kasapoglu S, Gursoy I, Szamosi C, Unlu H et al (2010) Effect of different pollination dates with irradiated pollens on fruit set, haploid embryo induction and plant obtention in Turkish (Kirkagac, Yuva and Hasanbey) melons. Acta Hortic 871:639–648

Sauton A (1989) Haploid gynogenesis in Cucumis sativus induced by irradiated pollen. Rep Cucurbit Genet Coop 12:22–23

Sebastian P, Schaefer H, Telford IRH, Renner SS (2010) Cucumber (*Cucumis sativus*) and melon (*C. melo*) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia. Proc Natl Acad Sci U S A 107:14269–14273

Shang Y, Ma Y, Zhou Y, Zhang H, Duan L, Chen H, Zeng J, Zhou Q, Wang S et al (2014) Biosynthesis, regulation, and domesticationof bitterness in cucumber. Science 346:1084–1088

Shanmugasundarum S, Williams PH, Peterson CE (1971) Inheritance of resistance to powdery mildew in cucumber. Phytopathology 61:1218–1221. https://doi.org/10.1094/Phyto-61-1218

Shifriss O (1950) Spontaneous mutations in the American varieties of Cucumis sativus L. Proc Amer Soc Hort Sci 55:351–335

Shetty N, van Kooten H, Sipeyre B et al (2014) Downy Mildew resistant cucumber plants. United States patent US 8859859 B2

Shibuya M, Adachi S, Ebizuka Y (2004) Cucurbitadienol synthase, the first committed enzyme for cucurbitacin biosynthesis, is a distinct enzyme from cycloartenol synthase for phytosterol biosynthesis. Tetrahedron 60:6995–7003

Shimizu S, Kanazawa K, Kato A, Yokota Y, Koyama T (1963) Studies on the breeding of cucumber for the resistance to downy mildew and other fruit characters. Engei Shikenjo ho koku (abstract only) 2:65–81

Smith PG (1948) Powdery mildew resistance in cucumber. Phytopathology 39:1027–1028

Soltysiak U, Kubicki B (1988) Induced mutations in the cucumber (Cucumis sativus L.). VII. Short hypocotyl mutant. Genetica Polonica 29:315–321

Song H, Lou QF, Luo XD, Wolukau J, Diao WP, Qian CT, Chen JF (2007) Regeneration of doubled haploid plants by androgenesis of cucumber (Cucumis sativus L). Plant Cell Tiss Organ Cult 90:245–254

Staub JE, Knerr LD, Holder DJ, May B (1997a) Phylogenetic relationships among several African Cucumis species. Can J Bot 70:509–517

Staub JE, Box J, Meglic V, Horejsi TF, McCreight JD (1997b) Comparison of isozyme and random amplified polymorphic DNA data for determining intraspecific variation in Cucumis. Genet Res Crop Evol 44:257–269

Strong WJ (1931) Breeding experiments with the cucumber (Cucumis sativus L.). Sci Agr 11:333–346

Suprunova T, Shmykova N (2008) In vitro induction of haploid plants in unpollinated ovules, anther and microspore culture of Cucumis sativus. In: Pitrat M (ed) Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae. Avignon, pp 371–374

Szczechura W, Staniaszek M, Klosinska U, Kozik E (2015) Molecular analysis of new sources of resistance to Pseudoperonospora cubensis (Berk. et Curt.) Rostovzev in cucumber. Russ J Genet 51:974–979

Sztangret-Wiśniewska J, Gałecka T, Korzeniewska A, Marzec L, Kołakowska G, Piskurewicz U et al. (2006) Characteristics of double-haploid cucumber (Cucumis sativus L.) lines resistant to downy mildew (Pseudoperonospora cubensis [Berk. et Curt.] Rostovzev). In: Proceedings of Cucurbitaceae 2006. Holmes GJ (ed) Raleigh, pp 515–526

Tkachenko NN (1935) Preliminary results of a genetic investigation of the cucumber, Cucumis sativus L. Bul. Applied Plant Breeding, Ser. 2(9):311–356

Trebitsh T, Staub JE, O'Neill SD (1997) Identification of a 1- aminocyclopropane-1-carboxylic acid synthase gene linked to the Female (F) locus that enhances female sex expression in cucumber. Plant Physiol 113:987–995

Troung-Andre I (1988) In vitro haploid plants derived from pollination by irradiated pollen of cucumber. In: Proceedings of Eucarpia meet cucurbit. Avignon-Monfavet, pp 143–144

Vakalounakis DJ (1992) Heart leaf, a recessive leaf shape marker in cucumber: linkage with disease resistance and other traits. J Hered 83:217–221

Van Vliet GJA, Meysing WD (1974) Inheritance of resistance toPseudoperonospora cubensis Rost. In cucumber (Cucumis sativus L.). Euphytica 23:251–255

Venkatesh J, Song K, Lee JH, Kwon JK, Kang BC (2018) Development of bi gene-based SNP markers for genotyping for bitter-free cucumber lines. Hortic Environ Biotechnol 59:231–238

Walters SA, Shetty NV, Wehner TC (2001) Segregation and linkage of several genes in cucumber. J Am Soc Hortic Sci 126:442–450

Wang HJ, Li SJ, Liu XF, Li P, Huo ZR, Guan W (2007) AFLP markers of cucumber anthracnose resistance-related gene. Acta Hortic Sin 34:213–216. (in Chinese)

Wang DH, Li F, Duan QH, Han T, Xu ZH, Bai SN (2010) Ethylene perception is involved in female cucumber flower development. Plant J 61:862–872

Wang Y, VandenLangenberg KM, Wehner TC et al (2016) QTL mapping for downy mildew resistance in cucumber inbred line WI7120 (PI 330628). Theor Appl Genet 129:1493–1505

Wang Y, VandenLangenberg K, Wen C, Wehner TC, Weng Y (2018) QTL mapping of downy and powdery mildew resistances in PI 197088 cucumber with genotyping-by-sequencing in RIL population. Theor Appl Genet 131:597–611

Whelan EDP (1971) Golden cotyledon: a radiation-induced mutant in cucumber. HortScience 6:343

Whelan EDP (1973) Inheritance and linkage relationship of two radiation-induced seedling mutants of cucumber. Can J Genet Cytol 15:597–603

Wilson JM (1968) The relationship between scab resistance and fruit length in cucumber, Cucumis sativus L.M.S. Thesis. Cornell Univ, Ithaca, NY

Wóycicki R, Witkowicz J, Gawroński P, Dąbrowska J, Lomsadze A, Pawełkowicz M et al (2011) The genome sequence of the north-European cucumber (Cucumis sativus L.) unravels evolutionary adaptation mechanisms in plants. PLoS One 6(7):e22728. https://doi.org/10.1371/journal.pone.0022728

Wyszogrodzka A, Williams P, Peterson C (1987) Multiple-pathogen inoculation of cucumber (Cucumis sativus) seedlings. Plant Dis 71:275

Yang L, Min C, Fuquan L et al (2000) Carpel of cucumber (Cucumis sativus L.) male flowers maintains early primordia characteristics during organ development. Chin Sci Bull 8:729–733

Yoshioka Y, Sakata Y, Sugiyama M, Fukino N (2014) Identification of quantitative trait loci for downy mildew resistance in cucumber (Cucumis sativus L.). Euphytica 198:265–276

Youngner VB (1952) A study of the inheritance of several characters in the cucumber. Ph.D. Diss. Univ. of Minnesota, St. Paul

Zhan Y, Chen JF, Malik AA (2009) Embryoid induction and plant regeneration of cucumber (Cucumis sativus L.) through microspore culture. Acta Hortic Sin 36(2):221–226

Zhang GH, Du SL, Wang M, Ma DH (2004) AFLP markers of cucumber powdery mildew resistance-related gene. Yuan Yi Xue Bao 31:189–192

Zhang SQ, Gu XF, Zhang SP, Zou ZR (2005) The genetic mechanism of resistance to powdery mildew in cucumber. Yuan Yi Xue Bao 32:899–901

Zhang S, Miao H, Cheng Z, Zhang Z, Wu J, Sun R, Gu X (2011) The insertion-deletion (Indel) marker linked to the fruit bitterness gene (bt) in cucumber. J Agric Biotechnol 19:649–653

Zhang S, Liu MM, Miao H et al (2013a) Chromosomal mapping and QTL analysis of resistance to downy mildew in Cucumis sativus. Plant Dis 97:245–251

Zhang S, Miao H, Sun R, Wang X, Huang S, Wehner TC, Gu X (2013b) Localization of a new gene for bitterness in cucumber. J Hered 104:134–139

Zhang X, Zhou Y, Ding L, Wu Z, Liu R, Meyerowitz EM (2013) Transcription repressor HANABA TARANU controls flower development by integrating the actions of multiple hormones, floral organ specification genes, and GATA3 family genes in Arabidopsis. Plant Cell 25:83–101. https://doi.org/10.1105/tpc.112.107854

Zijlstra S, Groot SPC (1992) Search for novel genes for resistance to powdery mildew (Sphaerotheca fuliginea) in cucumber (Cucumis sativus). Euphytica 64:31–37

Chapter 11
Advances in Improvement of Pumpkin and Squashes

Ajmer S. Dhatt, Madhu Sharma, and Barinder Kaur

11.1 Introduction

Pumpkins and squashes grown extensively all over the world were domesticated during the early era of civilization (Smith 1997; Kates et al. 2017). Cross-pollinated nature of this crop generated huge genetic diversity and wider adaptation from temperate to tropical regimes. There are 5 domesticated and 22 wild species with 20 pairs of chromosomes, but all maintain their distinctiveness due to genetic barriers, and all are considered to have domesticated independently in different parts of the world (Whitaker and Bemis 1975; Decker 1988). Among domesticated cucurbits, *Cucurbita pepo* is considered the oldest due to recovery of 9000-year-old seeds from the Valley of Oaxaca and Ocampo Caves in Mexico. The genus *Cucurbita* is considered to have originated from Central and South America (Grubben and Chigumira-Ngwerume 2004; Kiramana and Isutsa 2017) and was introduced to the Old World by European from the America (Ferriol and Pico 2008; Sun et al. 2017). In India, it has been grown as backyard garden crop from ancient times, and its presence has been documented in *Atharvaveda* (800 BC), Buddhist *Jatakas* and Hindu texts (fourth-century AD), *Ayurveda* (ninth century) and in mediaeval text (Levey 1966; Singh and Nigam 2017). The adaptation and diversification attained by this crop in Asian continent have forced scientists to declare China-Japan and India-Myanmar as secondary centre of domestication for pumpkin and squashes (Nee 1990; Ferriol and Pico 2008; Sun et al. 2017).

Cucurbita is one of the most variable genera of plant kingdom and has been studied extensively for growth, yield and quality traits (Aruah et al. 2010; Kiramana and Isutsa 2017). Among vegetables, it holds prominent position due to ease in production practices, high yield, longer storability, extended period of availability,

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rich nutrition and excellent transportability (Hazara et al. 2007a). The average nutritive value of pumpkin (2.68) is higher than brinjal (2.41), tomato (2.09) and cucumber (1.69) mainly due to high stored carbohydrate (mainly glucose) and carotenoids (74% β-carotene) along with a moderate quantity of ascorbic, nicotinic, pantothenic and folic acids and minerals (Gupta and Rai 1990; Hazara et al. 2007b). Though cucurbits are outbreeding in nature, but they do not show inbreeding depression upon selfing. Therefore, breeding approaches like inbreeding, hybridization, recurrent selection, pedigree and backcrossing have been employed for improvement of various traits like earliness, vine growth, fruit shape, size, colour, uniformity, quality, yield and resistance to diseases and insect-pests. Biotechnological tools are also helping to tackle the issues of cross-compatibility, trait-specific selection and fast multiplication of the desirable genotypes. Therefore keeping in view the significance, information on the advancements for improvement of pumpkin and squashes has been complied in this chapter.

11.2 Germplasm Characterization

Germplasm characterization is an important link between the conservation and utilization of plant genetic resources. To design successful breeding programme, availability and determination of genetic variability among different species and their natural habitats are important (Escribano et al. 1998; Muralidhara and Narasegowda 2014). Till date, few morphological and molecular diversity studies have been conducted on *Cucurbita* species to estimate genetic relationships among the species (Ferriol et al. 2004a; Balkaya et al. 2009). The 14 species (Table 11.1) of genus *Cucurbita* ($2n = 40$) have their roots in Americas (Nesom 2011; Kates et al. 2017). Its wild relatives are distributed from the mid-Western United States to Southern Argentina; however, predominance falls in the Mexico (Kates et al. 2017, www.gbif.org). Among all, five wild species are xerophytic perennials found in Mexico and Southwestern United States; seven are mesophytic annuals available in Mexico, Southeastern to Southcentral United States and Central and South America. Five species, namely, *C. moschata* Duchesne, *C. pepo* L., *C. maxima* Duchesne, *C. argyrosperma* Huber and *C. ficifolia* Bouche have been domesticated, and *C. pepo* is the most variable (Paris and Nerson 2003; Hadia et al. 2008). Its cultivars are categorized into eight horticultural groups, viz. pumpkin, vegetable marrow, cocozelle and zucchini (squash), scallop, acorn, crookneck and straightneck. Mostly, pumpkins are referred to the cultivars having round fruits and used upon maturity for baking or feeding livestock whereas squashes to those having edible immature fruits. As per Nee (1990), the cultivation practices and selection pressure over years led to adaptation of these cultivated species to different regions. *C. ficifolia* (figleaf gourd) is adapted to cool temperatures and short-days conditions, *C. moschata* to humid tropics and *C. pepo* to wide range of habitats. Molecular and morphophylogenetic studies have confirmed that C. *andreana* Naud, *C. sororia* Bailey and *C. fraterna* Bailey/*C. texana* (Scheele) Gray are the possible ancestor of *C. maxima*, *C.*

Table 11.1 List of *Cucurbita* species

Species	Subspecies	Crop taxon	Crop types
C. pepo	*C. pepo* ssp. *pepo*	Cultivated	Pumpkin, zucchini, summer squash, spaghetti squash, acorn squash, jack-o-lantern Ornamental gourd
	C. pepo ssp. *ovifera var. ovifera*	Cultivated	
	C. pepo ssp. *ovifera var. texana*	Wild	
	C. pepo ssp. *ovifera var. ozarkana*	Wild	
	C. pepo ssp. *fraternal*	Wild	
C. maxima	*C. maxima* ssp. *maxima*	Cultivated	Giant pumpkin, Hubbard squash, kabocha squash
	C. maxima ssp. *andreana*	Wild	
C. argyrosperma	*C. argyrosperma* ssp. *argyrosperma*	Cultivated	Cushaw pumpkin, Japanese pie pumpkin
	C. argyrosperma ssp. *sororia*	Wild	
C. moschata	-	Cultivated	Butternut squash, Seminole pumpkin, canned pumpkin, crookneck squash
C. ficifolia	-	Cultivated	Figleaf gourd, Malabar melon, Thai melon
C. cordata	-	Wild, xerophytic perennial	-
C. digitata	-	Wild, xerophytic perennial	-
C. ecuadorensis	-	Wild	-
C. foetidissima	-	Wild xerophytic perennial, paraphyletic	-
C. lundelliana	-	Wild polyphyletic	-
C. okeechobeensis	*C. okeechobeensis* ssp. *okeechobeensis*	Wild	-
	C. okeechobeensis ssp. *martinezii*	Wild polyphyletic	-
C. palmata	-	Wild xerophytic perennial and polyphyletic	-
C. pedatifolia	-	Wild xerophytic perennial and polyphyletic	-
C. radicans	-	Wild xerophytic perennial	-

argyrosperma and *C. pepo*, respectively (Decker and Wilson 1987; Decker 1988; Kirkpatrick and Wilson 1988; Decker-Walters et al. 1990, 2002; Nee 1990; Wilson et al. 1992; Jobst et al. 1998; Katzir et al. 2000; Sanjur et al. 2002).

Morphological and molecular characterization of germplasm is essential for breeders to plan crop improvement programme. Morphologically cucurbits have been characterized for size, shape, colour, texture and hardiness of the fruit, vine, peduncle, seeds, leaf and floral traits (Castetter 1925; Whitaker and Bemis 1965, 1975; Paris 1986; Nee 1990; Rios et al. 1997; Chung et al. 1998; Wessel-Beaver 2000; Bisognin 2002; Liu et al. 2013; Hamdi et al. 2017). Morphological genetic diversity for oligogenic and polygenic traits has been conducted in *C. pepo* (Katzir et al. 2000; Ferriol et al. 2003a; Paris et al. 2003), *C. moschata* (Ferriol et al. 2004a; Du et al. 2011; Liu et al. 2013) and *C. maxima* (Ferriol et al. 2004b). Whereas at molecular level, *Cucurbita* species were assessed with RAPD (Stachel et al. 1998; Youn and Chung 1998; Baranek et al. 2000; Gwanama et al. 2000; Ferriol et al. 2001; Decker-Walters et al. 2002; Chung et al. 2003; Chu et al. 2007), AFLP (Katzir et al. 2002; Ferriol et al. 2003a, 2004a, b; Paris et al. 2003; Wu et al. 2011), SRAP (Ferriol et al. 2003a, b, 2004a, b), SCAR (Gong et al. 2012), SSRs (Katzir et al. 2000, 2002; Paris et al. 2003, 2004; Gong et al. 2012; Liu et al. 2013; Hamdi et al. 2017) and ISSRs (Katzir et al. 2000, 2002; Paris et al. 2003) markers.

11.3 Utilization of *Cucurbita* Species in Hybridization

The goal of improvement programmes in pumpkin and squashes is the development of elite line, combinations or recombinants with desirable horticultural traits like earliness, increased productivity, environmental adaptation, resistance or tolerance to diseases and pests, growth habit, fruit colour, size, shape and enhanced nutritional value such as β-carotene (Robinson and Decker-Walters 1997). Most of these traits are available in the wild or cultivated gene pool and need to be introgressed in new cultivar by adopting suitable breeding methodology based upon cross-compatibility among the species (Munger 1993). To congregate relationships numerous attempts have been made between wild and cultivated species of *Cucurbita* (Table 11.2) and extensively reviewed by Whitaker (1951), Whitaker and Davis (1962), Whitaker and Bemis (1965), Whitaker and Robinson (1986), Merrick (1990), Lira et al. (1995) and Lebeda and Kristkova (2007). Consequently, interspecific hybridization between xerophytic species (*C. digitata*, *C. foetidissima*, *C. pedatifolia* and *C. radicans*) and mesophytic species (*C. argyrosperma*, *C. ecuadorensis*, *C. ficifolia*, *C. lundelliana*, *C. maxima*, *C. moschata*, *C. okeechobeensis* and *C. pepo*) was attempted, but none of the cross produced fertile progenies (Lebeda et al. 2006). Among various wild mesophytic taxa, *C. lundelliana*, *C. okeechobeensis* and *C. ecuadorensis* were found promising for hybridization with cultivated, but with inconsistent results. However, *C. lundelliana* is the most crossable with other mesophytic species, being in the tertiary gene pool of *C. ficifolia*, *C. maxima*, *C. moschata* and *C. pepo*.

Among the cultivated, *C. ficifolia* is the least compatible with other cultivated species as well as the remaining mesophytic species of the genus (Whitaker 1951; Whitaker and Davis 1962; Whitaker and Bemis 1965; Robinson and Decker-Walters

Table 11.2 Cross-compatibility behaviour of cultivated *Cucurbita* species (Lebeda et al. 2006)

Species	Primary gene pool	Secondary gene pool	Tertiary gene pool
C. argyrosperma	*C. argyrosperma* ssp. *soraria* *C. argyrosperma* ssp. *argyrosperma*	*C. moschata*	*C. pepo*, *C. maxima* *C. foetidissima*
C. ficifolia	*C. ficifolia*	*C. pedatifolia* *C. foetidissima*	*C. lundelliana*, *C. maxima* *C. pepo*
C. maxima	*C. maxima* ssp. *maxima* *C. maxima* ssp. *andreana*	*C. ecuadorensis*	*C. lundelliana*, *C. argyrosperma*, *C. ficifolia*
C. moschata	*C. moschata*	*C. argyrosperma*	*C. lundelliana*, *C. maxima* *C. pepo*
C. pepo	*C. pepo* ssp. *pepo* *C. pepo* ssp. *ovifera* *C. pepo* ssp. *ovifera* var. *texana* *C. pepo* ssp. *ovifera* var. *ozarkana* *C. pepo* ssp. *fraternal*	*C. argyrosperma* *C. okeechobeensis* *C. moschata* *C. ecuadorensis*	*C. lundelliana*, *C. ficifolia*, *C. maxima*

1997). Earlier efforts of crossing *C. moschata* with *C. argyrosperma* produced the infertile progenies; however, Wessel-Beaver (2000), Wessel-Beaver et al. (2004) and Ortiz-Alamillo et al. (2007) reported generation of fertile hybrids using *C. argyrosperma* as a female parent. It is easiest to achieve interspecific progenies of *C. maxima* with *C. andreana*, and, thus, some authors have classified it as *C. maxima* subspecies (Lira et al. 1995; Lebeda et al. 2006). Various edible and ornamental cultivars as well as populations of the wild taxa ssp. *fraternal* and ssp. *ovifera* var. *texana* and var. *ozarkana* form the primary gene pool of *C. pepo*. These wild taxa were also identified as distinct species (Singh 1990). In Mexico, many *C. pepo* cultivars having particular characteristics together with local landraces constitute an extraordinary genetic stock (Lebeda et al. 2006).

Molecularly phylogenetic studies were also conducted for precise assessments of genetic relationships within the genus *Cucurbita*. Sister lines cladding among mesophytic and xerophytic species were resolved with the polymorphisms of mitochondrial and chloroplast DNA (Sanjur et al. 2002; Zheng et al. 2013; Kistler et al. 2015), which confirmed that *C. foetidissima* and other xerophytic species are at distant and outlying to the mesophytes. Recently, the species level phylogeny has been inferred using 44 loci derived from introns of single-copy nuclear genes (Kates et al. 2017). It contains two major clades of mesophytic species (six *Cucurbita* crop lineages and their close wild relatives), five species clade from North and Central America and two species clade from South America, which were differing from the previous studies. The maximum likelihood tree construction using concatenation method recovered *C. pepo* as sister to *C. lundelliana* + *C. okeechobeensis*, and together these are sister to *C. moschata* + *C. argyrosperma*. A separate pair of clade containing *C. maxima* + *C. ecuadorensis* resolved as sister to the remaining

mesophytic species. To date only two-sister line clades were consistent among the seven mesophytic species, viz. *C. maxima*/*C. ecuadorensis* and *C. lundelliana*/*C. okeechobeensis*, and the position of *C. pepo* fluctuates among all the molecular phylogenetic studies. Therefore, *C. okeechobeensis* is the most central species of the genus, and *C. pepo* is rather outlying. Previous studies postulated *C. ficifolia* as a part of xerophytic species; however, Kates et al. (2017) resolved it, as a sister line clade with the remaining mesophytic species. Mostly, these results were in concordance with the findings of morphology, habitat and interspecific crossability.

11.4 Breeding Objectives

Cultivars of the pumpkin and squashes are breed to use flesh of immature and mature fruits in India. It is also used as ornaments during Halloween and Oktoberfest in America and European countries. Most traits of economic values of genus *Cucurbita* have been derived from *C. pepo* ssp. *pepo* (Ferriol and Pico 2008; Paris 2008). The genes responsible for important traits in different *Cucurbita* species are summarized in Table 11.3. The breeding objectives in pumpkin and squashes are high fruit yield, early fruiting, mottled, green or yellow fruit skin, thick and orange flesh, small cavity, smooth surface, round/oblong or flat-round shape, storage life, high β-carotene, resistance to mosaic viruses, powdery mildew, downy mildew and red pumpkin beetle, tolerant to low temperature and salinity. Genetic diversity available with the breeder is the key for success, and pictures of some advance breeding lines developed at PAU are given in Fig. 11.1a, b.

Table 11.3 Identification and introgression of desirable traits in *Cucurbita* species

Character	Gene symbol	Reference(s)
Fruit flesh colour		
Yellow/orange	*C. maxima:* B^{max} or *B-2* *C. pepo: B* *C. moschata: B*	Paris (1986, 1988), Paris et al. (1985), and Shifriss (1955, 1966, 1981, 1989)
Green/blue	*C. maxima:* Bl	Hutchins (1935)
Fruit shape		
Butternut	*C. moschata: B*n	Mutschler and Pearson (1987)
Crookneck	*C. moschata: b*n	
Fruit skin colour		
External skin colour: Red > green > white > yellow > grey	*C. maxima: Rd*	Lotsy (1920)
Seed characters		
Naked seeds	*C. pepo: h* *C. moschata: n*	Schoniger (1952), Grebenscikov (1954), Zraidi and Lelley (2004), Zraidi et al. (2007) and Gong et al. (2008a)

(continued)

Table 11.3 (continued)

Character	Gene symbol	Reference(s)
Cooking quality		
Milling	*C. pepo:* *sp (spaghetti* flesh) *Wf (white flesh)*	Mazurek and Niemirowicz-Szczytt (1992), Dutta and Nath (1972), Paris (1995) and Sinnott and Durham (1929)
Male sterility		
Male sterility	*C. maxima: ms^{-1}, ms^{-3}* *C. pepo: ms^{-2}*	Scott and Riner (1946), Eisa and Munger (1968) and Korzeniewska (1992)
Growth habit		
Bush habit and short internodes	*C. pepo: Bu or D* *C. maxima: Bu* *C. moschata: Bu*	Shifriss (1947), Grebenscikov (1958), Denna and Munger (1963) and Wu et al. (2007)
Vine habit	*C. maxima: bu* *C. moschata: bu*	
Fused vein	*C. pepo: Fv*	Carle and Loy (1996)
Absence of trichomes (glabrous)	*C. maxima: gl^{-1}or gl* *C. pepo: gl^{-2}*	Korzeniewska (1992) and Xiao and Loy (2007)
Disease resistance		
Cucumber mosaic virus	*C. moschata: CMV* (Nigerian local)	Brown et al. (2003)
Crown rot (*Phytophthora capsici*)	Three complementary genes in *C. lundelliana and C. keechobeensis* subsp. *Okeechobeensis: Crr-1*, *Crr-2*, *Crr-3*	Padley et al. (2009)
Zucchini yellow mosaic virus resistance	*C. ecuadorensis: zym^{ecu}* *C. moschata: Zym-0*, *Zym-1*, *Zym-2* and *Zym-3*, *Zym-4*, *Zym-5* and *Zym-6*	Robinson et al. (1988), Paris and Cohen (2000), Brown et al. (2003), Pachner and Lelley (2004) and Pachner et al. (2011)
Powdery mildew resistance	*C. lundelliana: Pm* *C. okeechobeensis: Pm-0* *C. pepo: Pm-0* *C. moschata: pm-1*, *pm-2*	Rhodes (1964), Contin (1978), Adeniji and Coyne (1983), Jahn et al. (2002) and Cohen et al. (2003)
Silver leaf resistance	*C. moschata: Sl* (Soler) and *sl* (PI 162889) *C. pepo: Sl* (black beauty) and *sl* (Zuc76)	Young and Kabelka (2009)
Squash leaf curl virus resistance	C. moschata: *Slc-1*, *slc-2*	Montes-Garcia et al. (1998) and Vilmorin and Cie (2010)
Watermelon mosaic virus resistance	C. *moschata: Wmv* *C. maxima × ecuadorensis: Wmv^{ecu}*	Weeden et al. (1986), Brown et al. (2003) and Gilbert-Albertini et al. (1993)
Insect resistance		
Cucurbitacin B	*C. pepo*: *Cu > cu*	Sharma and Hall (1971)
Fruit fly resistance	*C. maxima: Fr > fr*	Nath et al. (1976)

Fig. 11.1 (**a**, **b**) Genetic diversity in germplasm

Fig. 11.1 (continued)

11.4.1 Yield

High yield can be obtained by increasing the fruit weight and fruit number. The fruit weight is directly correlated with the size, cavity and thickness of the flesh. The round-shaped genotypes contribute towards thick mesocarp and vigorous vines for the size. However, high flesh recovery can be obtained from butternut-type pumpkins, which have thick flesh, small seed cavity and oblong-cylindrical shape. Earliness can be attained through shorter internodes and bush growth habit. Cultivars with more compact growth habit have increased substantially during the past decades. The bush growth habit of *C. pepo* was introgressed into *C. moschata* and

Fig. 11.2 Hybrids and varieties developed at Punjab Agricultural University. (**a**) PPH-1, (**b**) PPH-2, (**c**) Punjab Samrat, (**d**) Punjab Nawab, (**e**) MBN-6242, (**f**) Hull-less: PAU Magaz Kadoo-1

C. maxima (Munger 1989; Chesney et al. 2004). Bushy habit is controlled by single dominant gene (Bu), and Punjab Agricultural University (PAU) exploited this for release of two hybrids- PPH-1 and PPH-2 of *C. moschata* for cultivation in the state (Fig. 11.2 a, b).

Flowering in these hybrids starts after 40 days, and fruits of 800–900 g weight can be harvested after 65 days of sowing (Dhatt and Sidhu 2017). Multiple branching is an important trait for synchronous flowering and fruit setting, particularly in vine-type pumpkin and winter squash varieties. Production of more than one flower bud per leaf axil is also desirable to increase the yield. This trait is common in *C. pepo* ssp. *texana* and was introgressed into cocozelle and zucchini germplasm (Paris 2010).

11.4.2 Quality

Mottled green skin colour is preferred over smooth yellow in Indian markets. Mottled green skin pattern dominates the yellow and has been used in PPH-1, where female parent is mottled green and male smooth yellow. Colour of young fruits in *C. pepo* is either green or yellow, consisting of two shades of colour, if carrying the gene for striping, l-1BSt, or both green and yellow, if carrying the bicolor gene-B. The variations in fruit colour of *C. pepo* are under control of 13 gene loci, and some are multi-allelic (Paris and Brown 2005). Carotenoids content is very high in zucchini, crookneck and straightneck squash. Zucchini has also higher potassium content than the others (Paris et al. 1985; Schaffer et al. 1986). Parthenocarpy is important in *C. pepo* particularly for cultivation during offseason under greenhouse conditions (Martinez et al. 2014a).

11.4.3 Shelf Life

Storage life is important for distant transportation and retaining the fruits for longer period. Pumpkin fruits harvested after full maturity can be stored for 2–3 months at room temperature, whereas in summer squash high perishability is a major issue, particularly for desiccation and chilling injury (Mencarelli et al. 1982; Sherman et al. 1987; McCollum 1990).

11.4.4 Hull-Less Seed

Pumpkin seeds contain macro- and micro-constituents like triterpenes, phytosterols, polyunsaturated fatty acids, antioxidative phenolic compounds, tocopherol and minerals. Seeds have 35–45% oil, which is fortified with health-enhancing fatty acids like oleic (40.5%), stearic (27.1%), palmitic (17.4%) and linoleic (14.9%). The essential omega (ω)-6 fatty acid is present in the range of 35–65% of the total polyunsaturated fatty acids content (Habib et al. 2015). The oil extracted from oil-seed pumpkins is well-adapted for use on salads and possesses a number of prospective health benefits (Wagner 2000; Lelley et al. 2010). For extraction of oil from the seeds, cultivation of pumpkin started in early eighteenth century in Styria, Austria (Teppner 2004). At onset seeds were manually de-hulled for oil extraction, which gain industrial-scale expansion after the discovery of spontaneous hull-less or 'naked-seeded' mutant in Austria around 1880 (Teppner 2000). Today, the oil-seed pumpkin industry is of considerable economic importance in Austria and elsewhere in Central and Eastern Europe (Artyomenko and Chaban 2000; Cook 2000; Konrad 2000; Loy 2000). The industry is based entirely on cultivars having the recessive hull-less (*h*) seed trait. In Austria alone, approximately 18,000 ha are planted under oil-seed pumpkins, with an average seed yield of 0.61 t/ha (Lelley et al. 2010). Oil-seed pumpkins are being breed intensively in Austria, Serbia and

Hungary (Berenji and Papp 2000; Winkler 2000; Berenji 2011). The first oil-seed pumpkin cultivar in Austria 'GleisdoferOlkurbis' was developed from the hull-less landraces. Others are, namely, *Lady Godiva*, *Baby Bear*, *Eat All*, *Snack Jack*, *Streaker* and *Triple Treat* (Ferriol and Pico 2008). In India, the first variety of hull-seed '*PAU Magaz Kadoo-1*' (Fig. 11.2f) has also been released for cultivation (Dhatt 2019). Round fruits (pumpkins) are preferred because they have more and larger seeds than elongate fruits (Nerson et al. 2000; Paris and Nerson 2003; Nerson 2005). Hull-less-seeded pumpkins are also being bred in the United States, for extraction of oil and nutritious snack food as well (Loy 2004). Breeding for seed production differs from breeding for improved fruit flesh quality, because photosynthate needs to be more efficiently channeled into the growth and maturation of seeds rather than flesh (Loy 1988). Seed embryos usually do not reach their maximum size until 50 or even 60 days postanthesis (Vining and Loy 1998). In a way, this is a return to some of the characteristics of wild gourds, which have thin flesh and are full of seeds.

11.4.5 Abiotic Stress Resistance

Abiotic factors affect the cultivation of pumpkins and squash. Generally, *Cucurbita* plants thrive well under warm temperatures (25–30 °C), but *C. pepo*, *C. maxima*, and *C. ficifolia* are more tolerant of low temperature while *C. moschata* and *C. argyrosperma* to high (Ferriol and Pico 2008). Many *Cucurbita* landraces are of tropical origin and are not adapted to temperate regions like *C. moschata* cultivars. The short-cycle variety 'Xmejem Cum' is commonly grown in typical Mayan maize-bean-squash milpa system (Lira-Saade and Montes-Hernandez 1994; Graephe 2003). Other species with adaptation problems are *C. argyrosperma* and *C. ficifolia* which often fail to flower under long-day length (Ferriol et al. 2005). Early flowering, day-neutral forms of *C. ficifolia* have been selected to use as rootstocks (Robinson and Decker-Walters 1997). The use of grafted seedlings in cucurbits has increased in recent years. Interspecific hybrid between *Cucurbita maxima* and *Cucurbita moschata* has become a preferred rootstock for watermelon, muskmelon and cucumber. The hybrid seed production of *C. maxima* × *C. moschata* mainly depends on genotype compatibility. MA9 × MO8, MA12 × MO2 and MA4 × MO8 hybrid combinations were found most promising candidates for rootstock breeding (Karaagac and Balkaya 2013). Pumpkin rootstock has also been used for salt and cold tolerance in cucumber (Lei et al. 2014; Xu et al. 2017).

11.4.6 Virus Resistance

Zucchini yellow mosaic virus (ZYMV) is the most prevalent and destructive pathogen of cucurbits. Resistance to different strains of ZYMV has been identified in genetic resources of *C. moschata* originating from Nigeria, Portugal and Puerto

Rico (Paris et al. 1985; Munger and Provvidenti 1987; Pachner et al. 2011). Seven gene loci in *C. moschata* have been reported to confer resistance to ZYMV (Pachner et al. 2011). 'Nigerian local' carries two genes for resistance, designated *Zym-0* and *Zym-4* (confers resistance only in complementary interaction with the gene *zym-5*), 'Menina' carries three genes for resistance, with resistance conferred by the complementary interaction of the *Zym-1*gene with either one of two genes, *Zym-2* or *Zym-3*, and the Puerto Rican pumpkin 'Soler' carries a recessive gene for resistance, designated zym-6. This resistance has been introgressed into *C. pepo* from the Nigerian and Portuguese sources (Provvidenti 1997; Paris and Cohen 2000; Pachner et al. 2015). Also, Nigerian local has been used as a source of resistance to *Watermelon mosaic virus* (WMV), *Papaya ringspot virus*-W (PRSV-W) and *Cucumber mosaic virus* (CMV) for *C. moschata* and *C. pepo* (Brown et al. 2003; De oliveira et al. 2003). The resistance to *Squash leaf curl virus* (SqLCV) has been reported in *C. moschata*, transferred from *C. ecuadorensis* and *C. lundelliana* to *C. maxima*. Pumpkin yellow vein mosaic virus (PYVMV) transmitted by whitefly does not make possible to grow *C. moschata* during rainy (June to Oct.) season under northwestern plains of India. PAU has recommended variety resistant to PYVMV, namely, '*Punjab Nawab*' (Fig. 11.2d) for cultivation in the state (Dhatt and Sharma 2019).

11.4.7 Disease Resistance

Powdery mildew, caused by *Podosphaera xanthii* (commonly reported) and *Golovinomyces cichoracearum*, is the most destructive disease of cucurbits. Genetic resistance to powdery mildew has not been identified in *C. pepo* and is found only in few wild accessions of *C. moschata* (Cohen et al. 1993; Jahn et al. 2002; Paris and Cohen 2002; Lebeda and Kristkova 2007; Zhou et al. 2010). Wild *Cucurbita* species with which *C. pepo* and *C. moschata* are sparingly cross-compatible have been used to introgress resistance genes (Robinson and Decker-Walters 1997). The wild *Cucurbita* species *C. lundelliana* contains a dominant resistance gene that was introgressed into *C. pepo* through a bridge cross of *C. moschata.* Cultivars with these introgressions have not been commercialized due to linkage drag associated with the introgression and incomplete resistance (Contin 1978; Jahn et al. 2002). A breakthrough occurred when a resistant gene *Pm-0* from wild species *C. okeechobeensis* ssp. *martinezii* was successfully introgressed into squash and pumpkin at Cornell University. This was achieved first in *C. moschata* with a cross to 'Butternut' and later in *C. pepo* through the interspecific hybrid cross: (*C. pepo* 'Yankee Hybrid' × *C. moschata* 'Butternut') × ('Yankee Hybrid') × (*C. moschata* 'Butternut 23' × *C. okeechobeensis* ssp. *martinezii* F_1) (Cohen et al. 2003; Paris and Brown 2005). Following the initial crosses, the gene was incorporated into the open-pollinated *C. moschata* butternut cultivars 'Bugle' and 'PMT Large Butternut' and into open-pollinated cultivars of multiple morphotypes of both cultivated *C. pepo* subspecies. These included 'Success PM', 'PMR Bush Delicata' and 'Sweet REBA', representing the straightneck, delicata and acorn morphotypes, respectively, in the subspecies

C. pepo ssp. *texana*, and 'Romulus', 'PMR Caserta', 'Improved Costata', and 'PMR Naked Seeded', representing the zucchini, vegetable marrow, cocozelle and pumpkin morphotypes, respectively, in the subspecies *C. pepo* ssp. *pepo* (Paris et al. 2003; Gong et al. 2012). These Cornell cultivars or their progenitors have been used widely by other public and private breeding programmes. At present, the *Pm-0* gene is responsible for resistance in nearly all powdery mildew-resistant (PMR) commercial cultivars of *C. moschata* and *C. pepo* (Holdsworth et al. 2016). Similarly, resistance to *P. capsici* crown rot has been introgressed into *C. moschata* from wild species, *C. lundelliana* and *C. okeechobeensis* (Padley et al. 2009). *Phytophthora capsici* causes seedling death, crown and root rot, fruit rot and foliar blight on squash and pumpkins.

11.4.8 Insect Resistance

Leaf-silvering disorder in *Cucurbita* is a response to the feeding of immature stage of the silver leaf whitefly *Bemisia argentifolii*. The resistance to this whitefly has been reported in *C. moschata*, namely, the Paraguayan landrace PI resistance (Gonzalez-Roman and Wessel-Beaver 2002). Resistance to other pests have been reported in different species, such as fruit fly (*Dacuscucurbitae*) in *C. maxima*, squash vine borer in *C. moschata* and pickle worm in introductions of *C. pepo*, *C. moschata* and *C. maxima*.

11.5 Molecular Markers Development and Their Utilization

Molecular markers are envisaged as powerful tools for marker-assisted selection (MAS), tracing the origin of the species and diversity analysis within and between the species. The prerequisite to MAS is the high-density genetic maps, which facilitates localization of numerous QTLs governing traits of economic importance tightly linked to the molecular marker(s). The mapping of traits fosters breeders to accelerate breeding and identify candidate gene- and map-based cloning. The recent published whole genome sequences of *Cucurbita* species along with advanced genomic resources are assisting in construction of saturated genetic linkage maps. Molecular mapping of genus *Cucurbita* has been summarized in Tables 11.4 and 11.5. Genetic divergence among *C. pepo*, *C. moschata* and *C. maxima* accessions has been assessed with molecular markers like RAPD, AFLP, ISSR (Inter Simple Sequence Repeats), SBAP (sequence-based amplified polymorphism) and SSR (Jeon et al. 1994; Katzir et al. 1998; Stachel et al. 1998; Youn and Chung 1998; Baranek et al. 2000; Gwanama et al. 2000; Decker-Walters et al. 2002; Ferriol et al. 2003a, b, 2004a, b; Paris et al. 2003; Ramos 2007; Heikal et al. 2008; Tsivelikas et al. 2009; Wu et al. 2010; Gong et al. 2012; Inan et al. 2012; Liu et al. 2013; Muralidhara and Narasegowda 2014; Radwan 2014; Murovec 2015; Ntuli et al.

Table 11.4 Genetic map of genus *Cucurbita* using molecular markers

Parents	Number of markers	No. of linkage groups/av. marker distance(c)/genome coverage(cM)	Gene locus	Linked marker/distance (cM)	Marker type	Reference
F_2 *C. maxima* × *C. ecuadorensis*	11 isozyme loci	5/–/–	No morphological trait	–	–	Weeden and Robinson (1990)
F_2 *C. pepo* × *C. moschata*	28 RAPD	5/–/–	No morphological trait	–	–	Lee et al. (1995)
162 BC_1 (*C. pepo*, yellow straightneck inbred, A0449 × *C. moschata*, tropical landrace, Nigerian local) × A0449	148 RAPD	28/12.9/1954	B (precocious yellow fruit), LG5 M gene (mottled leaves), LG6 (rind colour), LG8 (fruit shape) LG10 Leaf indentations-LG5	Fruit shape – B8_900/10.09 Indentations – K11_950/12.59	RAPD	Brown and Myers (2002)
92 F_2 *C. pepo* subsp. *pepo* cross SZG1 (oil pumpkin) × True French resistant (*Zucchini yellow mosaic virus*)	247 RAPD, 82 AFLP, 3 SSR	21 (major) + 3 (minor)/6.4/2140	*n* (hull-less seed trait) – LGpz9	AK11-340, AN10-340, AB14-235, H18-385 and AB07-590/<1.5 AO11-280 and AW11-420/<6.5	RAPD	Zraidi et al. (2007)
92 F_2 *C. pepo* subsp. *pepo* (Lady Godiva, a US oil pumpkin) × *C. pepo* subsp. *ovifera* (Bianco Friulano, an Italian crookneck)	196 RAPD, 125 AFLP	21 (major) + 3 (minor)/6.9/2234	*n* – LGpc9 *Bu* (bush growth habit) – LGpc12	*n* – F09-670, AE07-850, AA12-340 and AB17-980/<7 AW11–420/3	RAPD	Zraidi et al. (2007)
92 F_2 *C. pepo* subsp. *pepo* (Lady Godiva) × *C. pepo* subsp. *ovifera* (Bianco Friulano)	178 SSR, 244 AFLP, 230 RAPD, 5 SCAR	20/2.9/1936	*n* – LGp9 *B* (bush growth habit) – LGp12	*n* – CMTm239, CMTm115, CMTp151 and CMTp58/1.5–3.6 *B* – CMTp131/7.8	SSR	Gong et al. (2008a)
94 F_2 each *C. moschata* crosses Waltham Butternut (WB) × Nigerian local (NL) and ZHOU (hull-less) × WB	205 SSR	27/7/1445.4	*n* – LGm27 *Gr* (green rind of mature fruit) on LGm5	*n* – CMTm166/4.1 *Gr* – CMTmC60/12.7	SSR	Gong et al. (2008b)

(continued)

Table 11.4 (continued)

Parents	Number of markers	No. of linkage groups/av. marker distance(c)/genome coverage(cM)	Gene locus	Linked marker/distance (cM)	Marker type	Reference
201 F_2 *C. maxima* cross '98-2-351' (grey rind) × '06820-1' (orange rind)	57 SSR, 21 AFLP, 3 RAPD	20/12.1/991.5	*Rc* (rind colour) – LG5	*Rc* – PU078072/5.9	SSR	Ge et al. (2015)
177 F_2 *C. okeechobeensis* subsp. *martinezii* PI 532363 × *C. moschata* 'Burpee's Butterbush (genotyping-by-sequencing)	2669 SNP	20/–/2199.2	*Pm-0* (powdery mildew resistance) – LG10	76.4 kb region flanked by SNPs S9_1474683 and S9_1551065 CAPS marker, i.e. NBS_ S9_1495924 (located in the NBS-LRR gene) and S9_1539675 co-segregate with resistance	SNP and CAPS	Holdsworth et al. (2016)
a. True French, TRF (susceptible) × Accession 381e (resistance)-173 F_2 b. San Pasquale, SPQ (susceptible) × Accesssion 381e-88 F_2 SNP-genotyping with Illumina GoldenGate profiling c. (SPQ × 381e F_2–28) × SPQ- 64 BC_1-F_2 d. (SPQ × 381e F_2–47) × SPQ-49 BC_1-F_2 e. (SPQ × 381e F_2–64) × SPQ-60 BC_1-F_2	6 polymorphic SNP among TRF and 381e	–/–/–	ZYMV resistance	90% association between SNP1 and resistance Two nucleotide-binding site leucine-rich repeat (NBS-LRR) protein-encoding genes were located near the SNP1 marker	SNP and CAPS	Capuozzo et al. (2017)
93 F_2 and 30 BC_1 'Zuc76' (a zucchini squash, SSL disorder-resistant line) and 'black beauty'(susceptible)	1152 RAPD and 432 SSR	–/–/–	Squash silver leaf disorder resistant (*sl*)	*sl*- M121/ 3.3	SSR	Kabelka and Young (2010)

Table 11.5 Summary of traits with associated QTL's in genus *Cucurbita*

Population type and parents	Sequencing approach	Number of markers	No of linkage groups/av. marker distance (cM)/ genome coverage (cM)	Trait	QTL/linkage group	R^2 (%)	Reference
146 F_2 *C. pepo* subsp. *pepo* (zucchini, MU-CU-16) × *C. pepo* subsp. *ovifera* (scallop, UPV-196) having contrasting phenotypes	SNP genotyping with Illumina GoldenGate platform	304 SNP, 11 SSR	22 (major) + 1 (minor)/5.56/1740.8	Flowering	6 (*NoMaF*; *NoFeF*, first node with a male and female flower; *DMaF*; *DFeF*, days from sowing to the development of the first male and female flower; *N°MaF*, number of male flowers measured 7 days after the opening of the first female flower; *MaF/FeF*, ratio male to female flowers)/LG3	>25	Esteras et al. (2012)
				Fruit shape	2 (*IFLe*; *MFLe*, fruit length (cm) of immature and mature fruit)/LG6		
				Rind colour of mature fruit	2 (*MLRCo*; *MaRCo*, rind colour, hunter parameter L and a)/LG14		
				Flesh colour of mature fruit	1 (*MbFCo*, flesh colour, hunter parameter b)/ LG 16		
186 F_2 *C. maxima* inbred lines Rimu and SQ026 (bush type)	Genotyping-by-sequencing (GBS) using Illumina HiSeq 2500	458 SNP	20/5.60/2566.8	Dwarf vine	1(*qCmB2*)/LG3, contains *Cma_004516* as a possible candidate gene encodes gibberellin 20-oxidase	21.4	Zhang et al. (2015)

(continued)

Table 11.5 (continued)

Population type and parents	Sequencing approach	Number of markers	No of linkage groups/av. marker distance (cM)/ genome coverage (cM)	Trait	QTL/linkage group	R^2 (%)	Reference
200 F_2 *C. moschata* lines CMO-1 (from Thailand) × CMO-97 (South China 'miben' type germplasm)	Double-digest restriction site-assoc. DNA seq using Illumina HiSeqXten	3470 SNP	20 /0.89/3087.03	Fruit	1 (pericarp colour, pc)/LG8	93	Zhong et al. (2017)
					1 (pericarp strip, ps)/LG8	90	
				Carotenoid	5 (lutein content)/LG8,11,20	15.1–25.6	
					2 (α-carotene content)/LG8,17	12.6–19.1	
					3 (β-carotene content)/LG11,15, 20	10.5–23.2	
					4 (total carotenoid content)/LG8,11, 20	13.2–28.6	
				Sugar	2 (glucose and sucrose cont.)/LG19 and LG10	11.4& 11.3	
					1 (sucrose versus glucose ratio)/LG19	12.4	
				Fruit morphology	2 (fruit tuberculate)/LG8,11	12.3–16.9	
					2 (fruit hollow)/LG8,11	10.3–16.9	
					2 (fruit diameter)/LG8,13	11.1–19.0	
					3 (fruit thickness)/LG8,9	10.3–15.2	
					3 (chamber width)/LG8,13	9.6–11.4	
166 F_2, *C. moschata* inbred lines, i.e. '5-5-6' (chilling stress susceptible) and '8-3-7' (chilling stress tolerant)	QTL mapping	95 SSR	15/8.74/830.7	Chilling index (CI)	3(*qCI*)/LG1 (CMTp163-CMTm140), LG4 (CMTm113–Unigene0027389), LG10 CMTm214 – Unigene0008127	0.30, 1.22 and 20.88	Xu et al. (2017)
120 F_8 RILs *C. pepo cross* zucchini × scallop	Genotyping-by-sequencing using Illumina HiSeq 2000 platform	7718 SNP	21/0.4/2817.6	Leaf	1 (*Li*, Leaf incision)/LG10	50.05	Montero-Pau et al. (2017)
				Fruit	2 (*IFLe*, *MFLe*, immature and mature fruit length)/LG3	31.79 & 38.71	
					2 (*ILRCo*, *MLRCo* immature and mature rind colour, L parameter)/LG4	40.64 & 40.30	
					1 (*IaFCo*, immature flesh colour, a parameter)/ LG10	28.31	
					1 (*MbFCo*, mature flesh colour, b parameter)/ LG19	62.93	

92 F_6 RILs, *C. maxima* inbred lines 802 and 801. 802 from Japanese cultivar 'Uchiki Kuri' (yellow ovary colour with small fruit of orange flesh) and 801 from an Eastern European landrace (light green ovary colour with large fruit and pale orange flesh)	DArTseq genotyping-by-sequencing using a HiSeq2500 system	36 SSR, 1094 SNP and 694 silico-DArTs	20/1.21/2208.3	Ovary colour	At chromosome 14, the closest marker 14-is20584145 linked at a distance of 4.8 cM	–	Kazminska et al. (2018)
				β-carotene content	3 (*qβcar*)/Chr 2,4,14	14.2–25.9	
				α-carotene	3 (*qαcar*)/Chr 2, 4, 14	14.9–30.1	
				Lutein content	2 (*qlut*)/Chr 2, 4	17.1–28.4	
				Violaxanthin	2 (*qviol*)/Chr 2, 4	21.4–27.3	
				Ntheraxanthin	2 (*qant*)/ Chr 2,4	18.8–27.7	
				Zeaxanthin	1 (*qzea*)/Chr 2	18.9–28.2	
				Fruit flesh colour	3 (*qch*)/Chr 1, 4,14	21.6–38.7	

2015; Sim et al. 2015; Miladinovic et al. 2016; Kazminska et al. 2017; Darrudi et al. 2018; Martins et al. 2018; Verdone et al. 2018; Zhao et al. 2018). Twenty-seven SSR markers (Gong et al. 2008a) showed transferability to bottle gourd (Yildiz et al. 2015). Additionally, *C. pepo* (Zucchini) genome is currently available at http://cucurbitgenomics.org/ and contains 263 Mb assembly size with an N50 of 1.8 Mb and 34,240 gene models. The genome is organized in 20 pseudomolecules that represent 81.4% of the assembly. Also, the draft genome of *C. moschata* (Rifu) and *C. maxima* (Rimu) with assembly size 269.9 and 271.4 Mb is available, which represents 72.6% and 70.2% of their estimated genome sizes, respectively. Therefore, the accessibility of these genomic resources will accelerate the breeding programmes in genus *Cucurbita*.

11.6 Development of Doubled Haploids

To speed up breeding programmes, doubled haploid (DH) approach can play significant role in developing cultivars with outstanding characteristics in shortest possible time. DH can be generated through androgenesis (anther culture, microspore culture), gynogenesis (ovule and ovarium culture), parthenogenesis (irradiated pollination, ultrawide hybridization) and centromere histone H3- and (CENH3)-based techniques in a single step (Maluszynski et al. 2003). Through this technique inbreeding depression and deleterious mutations are excluded at the haploid level (Stephenson et al. 2001; Gemes-Juhasz et al. 2002; Cardoso 2004; Datta 2005; Kosmrlj et al. 2013). Haploids have only one version of each gene, and chromosome doubling is needed to get homozygous DH plants. The first haploid in cucurbits was developed in melon using irradiated pollen (Sauton and Dumas De Vaulx 1987). The use of gamma ray for pollen irradiation followed by in vitro culture of immature embryos revealed that season, maternal genotype, irradiation dose, embryo stage and embryo type (necrotic vs. normal) affected the haploid induction in *Cucurbita* species (Kurtar et al. 2002, 2009; Kurtar and Balkaya 2010). X-rays used for parthenogenetic haploid induction in Styrian oil pumpkin gave four ploidy levels (n, 2n, 3n and 4n) having majority of the diploids (Kosmrlj et al. 2013). The haploidization efficiency was poor with irradiated pollen and anther culture techniques (Kurtar et al. 2016). The ovule culture for production of gynogenic haploids in *C. maxima* and *C. moschata* has been demonstrated (Kurtar et al. 2018). However, genotypic differences for di-haploidization were observed by many workers in summer squash, pumpkin and winter squash (Kurtar et al. 2002, 2009, 2016; Kurtar and Balkaya 2010).

11.7 Transcriptome Sequencing for Better Understanding of Genetics and Biology

Transcriptomes are the set of all RNA molecules in one cell or a population of cells. mRNA serves as a transient intermediary molecule in the information network, while noncoding RNAs perform additional diverse functions. Its sequencing gives

better understanding of genetics and biology genes. In pumpkin and squashes, several transcriptomes have been generated, and the first was in *C. pepo* using 454 GS-FLX Titanium technology (Blanca et al. 2011). About 512,751 expressed sequence tags (ESTs) were assembled into 49,610 *Cucurbita* unigenes, and about 34% were identified to have known orthologs in *Arabidopsis* or melon. Some genes annotated in this transcriptome have been functionally characterized for fruit set, sex determination and parthenocarpy in squashes (Martinez et al. 2013, 2014b). The expression analysis of 13 genes regulating ethylene biosynthesis and signaling (i.e. *CpACS2*, *CpACS4* to *CpACS6*, *ACS* (6), *ACO*, *CpACS7* and *CpACO1*) deduced that low ethylene biosynthesis concomitant with fruit set, early fruit development, parthenocarpy and partial andromonoecy. Further transcriptomic studies explained that how ethylene affects the sex expression (Wang et al. 2019). Because the key candidate genes such as *CmaACS7*, *CmaACO1*, *CmaETR1* and *CmaEIN3* involved in ethylene synthesis was positively correlated with the number of female flowers. Also, functional annotation of DEGs (differentially expressed genes) regulating parthenocarpy in zucchini revealed the coordination between auxin, ethylene and gibberellin (Pomares-Viciana et al. 2019). In addition, the high-throughput reverse genetic tools have been developed for functional characterization such as TILLING (Targeting Induced Local Lesions IN Genomes) in zucchini (Vicente-Dolera et al. 2014). Being a monoecious plant, squash is an excellent model for studying nectar biology, due to its large nectaries (Solhaug et al. 2019). The transcriptome revealed a metabolic progression in nectaries leading from starch synthesis to starch degradation and to sucrose biosynthesis.

The first transcriptome of *C. moschata* was developed using IlluminaHiSeq 2000 (Wu et al. 2014). Almost 50 million reads were assembled into 62,480 unigenes. Also, this transcriptome revealed higher expression levels of genes, namely, *Cm-LWD1*, *Cm-FIO1*, *Cm-PIE1*, *Cm-TIC* and *Cm-ELF6* in photoperiod insensitive pumpkin plants indicating their role in light insensitiveness and late flowering. Furthermore, a fruit and seed transcriptomes of acorn squash cultivar 'Sweet Reba' and oilseed pumpkin 'Lady Godiva' were generated over five developmental stages, which possessed the key candidate genes concomitant with carotenoid and carbohydrate metabolism (Wyatt et al. 2015, 2016). Fruit metabolite in squashes was studied at five development stages by Huang et al. (2019a), to identify numerous transcripts enriched in starch, sugar, carotenoid, plant hormone signal transduction and pectin pathways contributing towards quality formation. Another transcriptome profiling was developed in relation to fruit size in pumpkin cultivars, namely, Big Moose (large round fruits) and Munchkin (small round fruits). This transcriptome revealed the upregulation of microtubules genes such as *KINESIN1* and *BETA-TUBULIN* in the large-fruit cultivar and *HEXOKINASE1* and *CNR/FW2.2* gene in small-fruit cultivar (Xanthopoulou et al. 2016, 2017).

Some transcriptomic assemblies were analysed for biotic and abiotic stresses. The key candidate genes imparting powdery mildew resistance in pumpkin were *bHLH87*, *ERF014*, *WRKY2*, *HSF*, *MLO3* and *SGT1* (Guo et al. 2018), while the transcription factors, i.e. *MYB76-like*, *ZAT10-like*, *DELLA* protein *GAIP* and *AP2/ERF* domain regulated chilling tolerance (Carvajal et al. 2018) in zucchini. Huang et al. (2019b) explained the elusive details that how pumpkin is tolerant to salt stress. It might be due to its superior ability for K^+ uptake and higher H_2O_2

accumulation in the root apex. DEGs encode *NADPH oxidase* which increased the plasma membrane activity and mediate the uptake of potassium transporter (*HAK5*) in pumpkin under salinity stress.

These transcriptomes represent repertoire of genes expressed throughout plant, flower and fruit development in pumpkin and squashes. The draft proteome of zucchini cultivar True French was developed (Andolfo et al. 2017), and a total of 64 R-proteins were detected. Additionally, 40 transcriptomes of 12 species of the genus were assembled and used as the foundation for comparative genomic studies (Montero-Pau et al. 2018). Apart from cDNA sequencing, high-throughput sequencing of small RNAs (such as miRNAs), involved in post-transcriptional regulation of gene expression, has been conducted in pumpkin and squashes (Jagadeeswaran et al. 2012). Some miRNAs are differentially expressed in different tissues in response to salt stress (Xie et al. 2015), grafting (Li et al. 2014; Ren et al. 2018) and drought stress (Chaohan et al. 2016).

11.8 Genetic Modification and Transformation

Pumpkins and squashes are infested by wide range of pathogens (fungi, bacteria, viruses, nematodes) and insects. Though resistant sources are available, but cross-compatibility barriers and linkage drag limit the utilization. In pumpkins, serious implications on fruit quality have been noticed because of abiotic and biotic stresses especially viral diseases (Provvidenti 1993; Clough and Hamm 1995). Under natural conditions, all the three key viruses (ZYMV, C MV, WMV) occur as a mixed infection (Quemada 1998; Schultheis and Walters 1998; Walters et al. 2003; Manamohan et al. 2011), and thus resistance against a particular viral disease is broken due to synergistic interactions among multiple infections (Wang et al. 2002, 2004). Therefore, multiple disease resistance is needed but is difficult through conventional approach due to governance by multiple recessive or complementary dominant genes (Kooistra 1969; Danin-Polleg et al. 1997). Therefore to overcome it, genetic transformation or gene-editing approaches can be followed. Protocols standardized for genetic transformation and development of transgenic lines against viral synergism in pumpkin have been summarized in Table 11.6.

11.9 Future Breeding Tools to Accelerate Improvement Programmes

So far, conventional breeding contributed significantly for improvement of pumpkin and squashes. Though yield is important, but focus on specialty traits like β-carotene in fruits and omega-3 fatty acids in seed demands special attention for improvement in pumpkin and squashes. Changing climate is posing new threats for emergence of more aggressive pests and pathogens, particularly the complex of viruses in

Table 11.6 Genetic transformation studies in pumpkin and squashes

Crop	Gene	Type of explant/ method	Targeted trait(s)	Reference
C. pepo	–	Embryo	Regeneration	Jelaska (1972), Chee (1992)
C. pepo	–	Apices, cotyledons, leaves	*Agrobacterium*-mediated transformation	Chee (1997) and Tricoli et al. (2002)
C. pepo	–	Cotyledonary petioles, shoot tips	Maintenance on high levels of cytokinin	Zhong et al. (2002)
Zucchini and cocozelle squash	–	–	Regeneration by organogenesis	Ananthakrishnan et al. (2003)
C. pepo	Coat proteins (CP) of CMV, ZYMV and WMV	*Agrobacterium tumefaciens* mediated	Multiple resistance to CMV, ZYMV	Tricoli et al. (1995)
Yellow crookneck squash	ZYMV and WMV2 coat protein (CP) genes	*Agrobacterium tumefaciens* mediated	ZW-20B, a transgenic line having resistance to ZYMV and WMV	Fuchs and Gonsalves (1995)
C. pepo	WMV-CP, ZYMV-CP	Interfering RNA mechanism (RNAi) through 35S promoter of the *Cauliflower mosaic virus* (CaMV)	ZW20: Resistant to WMV and ZYMV	USDA (1994)
C. pepo	CMV-CP, WMV-CP, ZYMV-CP, NPTII		CZW3: Resistant to CMV, WMV and ZYMV	USDA (1994)
All cucurbits	SqMV-CP	*Agrobacterium tumefaciens* mediated	Resistance against *Squash mosaic comovirus* (SqMV)	Pang et al. (2000)
Squash	–	–	Standardized multiplex PCR protocol to identify the transgenic line ZW-20 with CP genes from WMV II and ZYMV	Wall et al. (2004)
Squash	CP genes of WMV and ZYMV	*Agrobacterium tumefaciens* mediated	Freedom II: First commercially available transgenic from Asgrow Seed Co.	Tricoli et al. (2008)
C. moschata	–	Cotyledonary node	Optimized *Agrobacterium* transformation by the use of 1% (w/v) with aluminium borate	Nanasato et al. (2011)

cucurbits. The introgression of favourable alleles through speed and shuttle breeding is useful to accelerate the improvement programmes. Integration of data generated from genomics, transcriptomics, proteomics, metabolomics and phenomics studies will help in understanding the evolution, lineages and pathways for

designing the desirable plant types of pumpkin and squashes. Genome editing which allows incorporation of desired mutations into elite cultivars can be the target in future breeding programmes. The establishment of crop databases/consortium and incorporation of functional annotation of genome on the basis of high-throughput phenotyping and genotyping has emerged as a great facility to identify gene(s) governing novel traits and their direct utilization. Thus, selection efficiency can be increased several fold, and cultivars possessing specialty trait, resistance to diseases and stresses with high yield can be developed in lesser time than traditional breeding programmes in pumpkin and squashes.

11.10 Conclusion

Cucurbita is highly diverse genera of plant kingdom. With the advancement of human civilization, demand for uniformity, earliness and better fruit size, colour, shape and quality lead to develop improved varieties and hybrids. Interspecific hybridization has the potential for introgression of bush growth habit, fruit colour, naked seed, quality and insect-pest resistance traits in cultivated *Cucurbita* species. Biotechnological tools like embryo rescue, marker-assisted selection, doubled haploids and genetic transformations not only provide the opportunity to overcome cross-compatibility barriers but accelerate the breeding programme also. The advent of next-generation sequencing (NGS) and whole genome sequencing (WGS) of pumpkin and squashes has triggered the genomics and transcriptomic studies. The combination of breeding techniques with genomic and bioinformatics tools have provided the possibility to edit genome at base level, pyramiding or stacking of desirable genes in minimum possible time. In future, new omics analysis tools, extensive genome analysis and editing studies will help in addressing issues of climate change, biotic and abiotic stresses and biofortification in pumpkin and squashes.

References

Adeniji AA, Coyne DP (1983) Genetics and nature of resistance to powdery mildew in crosses of butternut with calabaza squash and 'Seminole Pumpkin'. J Am Soc Hortic Sci 108:360–368

Ananthakrishnan G, Xia X, Elman C, Singer S, Paris HS, Gal-On S, Gaba V (2003) Shoot production in squash (*Cucurbita pepo*) by in vitro organogenesis. Plant Cell Rep 21:739–746

Andolfo G, Donato AD, Darrudi R, Errico A, Cigliano RA, Ercolano MR (2017) Draft of zucchini (*Cucurbita pepo* L.) proteome: a resource for genetic and genomic studies. Front Genet 8:181

Artyomenko SB, Chaban LN (2000) Production of cucurbit seed oil by cold pressing process in the "Farmaol" company. Cucurbit Genet Coop Rep 23:120–121

Aruah CB, Uguru MI, Oyiga BC (2010) Variations among some Nigerian Cucurbita landraces. African J Plant Sci 4(10):374–386

Balkaya A, Yanmaz R, Ozbakır M (2009) Evaluation of variation in seed characters in Turkish winter squash (Cucurbita maxima Duch.) populations. N Z J Crop Hortic Sci 37(3):167–178

Baranek M, Stift G, Vollmann J, Lelly T (2000) Genetic diversity within and between the species *Cucurbita pepo*, *C. moschata* and *C. maxima* as revealed by RAPD markers. Cucurbit Genet Coop Rep 23:73–77

Berenji J (2011) Uljana tikva, *Cucurbita pepo* L. Institut za Ratarstvo Povrtarstvo, Novi Sad

Berenji J, Papp D (2000) Inter-relationships among fruit and seed characteristics of oil pumpkin. In: Katzir N, Paris HS (eds) Proceedings of cucurbitaceae 2000. Acta horticulturae, vol 510, pp 101–104

Bisognin DA (2002) Origin and evolution of cultivated cucurbits. Cienc Rural 32:715–723

Blanca J, Canizares J, Roig C, Ziarsolo P, Nuez F, Pico B (2011) Transcriptome characterization and high throughput SSRs and SNPs discovery in *Cucurbita pepo (Cucurbitaceae)*. BMC Genomics 12:104

Brown RN, Myers JR (2002) A genetic map of squash (*Cucurbita* ssp.) with randomly amplified polymorphic DNA markers and morphological markers. J Am Soc Hortic Sci 127:568–575

Brown RN, Bolanos-Herrera A, Myers JR, Jahn MM (2003) Inheritance of resistance to four cucurbit viruses in *Cucurbita moschata*. Euphytica 129:253–258

Capuozzo C, Formisano G, Iovieno P, Andolfo G, Tomassoli L, Barbella MM, Pico B, Paris HS, Ercolano MR (2017) Inheritance analysis and identification of SNP markers associated with ZYMV resistance in *Cucurbita pepo*. Mol Breed 37:99

Cardoso AII (2004) Depression by inbreeding after four successive self-pollination squash generations. Sci Agric 61:224–227

Carle RB, Loy JB (1996) Genetic analysis of the fused vein trait in *Cucurbita pepo* L. J Am Soc Hortic Sci 121:13–17

Carvajal F, Rosales R, Palma F, Manzano S, Canzares J, Jamilena M, Garrido D (2018) Transcriptomic changes in Cucurbita pepo fruit after cold storage: differential response between two cultivars contrasting in chilling sensitivity. BMC Genomics 19:125

Castetter EF (1925) Horticultural groups of cucurbits. Proc Am Soc Hortic Sci 22:338–340

Chaohan LI, Yansu LI, Longqiang BAI, Chaoxing HE, Xianchang YU (2016) Dynamic expression of miRNAs and their targets in the response to drought stress of grafted cucumber seedlings. Hortic Plant J 2:41–49

Chee PP (1992) Initiation and maturation of somatic embryos of squash (Cucurbita pepo). HortScience 27:59–60

Chee PP (1997) Somatic embryogenesis and transformation of squash. US patent 5,677,157

Chesney P, Wessel-Beaver L, Maynard DN (2004) Both traditional and semi-bush tropical pumpkin can be intercropped with beans or cowpeas. HortScience 39:525–528

Chu PP, Xiang CP, Zhang CX, Liu CP (2007) Genetic diversity of Cucurbita moschata genotypes revealed by RAPD markers and agronomic traits. J Nucl Agric Sci 21:441–444

Chung HD, Youn SJ, Choi YJ (1998) Ecological and morpho-edible logical characteristics of the Korean native squash (Cucurbita moschata). J Korean Soc Hortic Sci 39:377–384

Chung S-M, Decker-Walters D-S, Staub JE (2003) Genetic relationships within the Cucurbitaceae as assessed by consensus chloroplast simple sequence repeats (ccSSR) marker and sequence analyses. Canadian Journal of Botany 81(8):814–832

Clough GH, Hamm PB (1995) Coat protein transgenic resistance to watermelon mosaic and zucchini yellow mosaic virus in squash and cantaloupe. Plant Dis 79:1107–1109

Cohen R, Leibovich G, Shtienberg D, Paris HS (1993) Variability in the reaction of squash (*Cucurbita pepo*) to inoculation with *Sphaerotheca fuliginea* and methodology of breeding for resistance. Plant Pathol 42:510–516

Cohen R, Hanan A, Paris HS (2003) Single-gene resistance to powdery mildew in zucchini squash (*Cucurbita pepo*). Euphytica 130:433–441

Contin M (1978) Interspecific transfer of powdery mildew resistance in the genus Cucurbita. PhD Thesis, Cornell University, Ithaca

Cook JP (2000) Styrian pumpkin oil: the marketing perspective. Cucurbit Genet Coop Rep 23:128

Danin-Polleg Y, Paris HS, Cohen S, Rabinowitch HD, Karchi Z (1997) Oligogenic inheritance of resistance to zucchini yellow mosaic virus in melons. Euphytica 93:331–337

Darrudi R, Nazeri V, Soltani F, Shokrpour M, Ercolano MR (2018) Genetic diversity of *Cucurbita pepo* L. and *Cucurbita moschata* Duchesne accessions using fruit and seed quantitative traits. J Appl Res Med Aromat Plants 8:60–66

Datta SK (2005) Androgenic haploids: factors controlling development and its application in crop improvement. Curr Sci 89:1870–1878

De Oliveira ACB, Maluf WR, Pinto JEBP, Azevedo SM (2003) Resistance to papaya ringspot virus in summer squash *Cucurbita pepo* L. introgressed from an interspecific *C. pepo* × *C. moschata* cross. Euphytica 132:211–215

Decker DS (1988) Origin(s), evolution, and systematics of Cucurbita pepo (Cucurbitaceae). Econ Bot 42:4–15

Decker DS, Wilson HD (1987) Allozyme variation in the Cucurbita pepo complex – Cucurbita-pepo var. ovifera vs Cucurbita texana. Syst Bot 12(2):263–273

Decker-Walters DS, Walters TW, Poslusny U, Kevan PG (1990) Genealogy and gene flow among annual domesticated species of *Cucurbita*. Can J Bot 68:782–789

Decker-Walters DS, Staub JE, Chung SM, Nakata E, Quemada HD (2002) Diversity in free-living populations of *Cucurbita pepo (Cucurbitaceae)* as assessed by random amplified polymorphic DNA. Syst Bot 27:19–28

Denna DW, Munger HM (1963) Morphology of the bush and vine habits and the allelism of the bush genes in *Cucurbita maxima* and *C. pepo* squash. Proc Am Soc Hortic Sci 82:370–377

Dhatt AS (2019) *PAU Magaz Kadoo-1*: First variety of hull-less seeded pumpkin in India. Progressive Farming 55(1):15

Dhatt AS, Sharma M (2019) *Punjab Nawab*: a new variety of pumpkin. Progressive Farming 55(8):16

Dhatt AS, Sidhu MK (2017) PPH-1 and PPH-2: Extra early hybrids of pumpkin. Progressive Farming 53(3):15–16

Du XH, Sun YD, Li XZ, Zhou JG, Li XM (2011) Genetic divergence among inbred lines in Cucurbita moschata from China. Sci Hortic 127:207–213

Dutta LP, Nath P (1972) Inheritance of flower and fruit characters in squash, *Cucurbita pepo* L. In: 3rd international symposium on subtropical and tropical horticulture, pp 69–74

Eisa HM, Munger HM (1968) Male sterility in *Cucurbita pepo*. Proc Am Soc Hortic Sci 92:473–479

Escribano MR, Santalla M, Casquero PA (1998) Patterns of genetic diversity in landraces of common bean (Phaseolus vulgaris L.) from Galicia. Plant Breed 117(1):49–56

Esteras C, Gomez P, Monforte AJ, Blanca J, Vicente-Dolera N, Roig C, Nuez F, Pico B (2012) High-throughput SNP genotyping in *Cucurbita pepo* for map construction and quantitative trait loci mapping. BMC Genomics 13:80

Ferriol M, Pico B (2008) Pumpkin and winter squash. In: Prohens J, Nuez F (eds) Handbook of plant breeding, Vegetables I. Springer, New York, pp 317–349

Ferriol M, Picó B, Nuez F (2001) Genetic variability in pumpkin (Cucurbita maxima) using RAPD markers. Cucurbit Genet Coop Rep 24:94–96

Ferriol M, Picó B, Nuez F (2003a) Genetic diversity of a germplasm collection of Cucurbita pepo using SRAP and AFLP markers. Theor Appl Genet 107:271–282

Ferriol M, Pico B, Nuez F (2003b) Genetic diversity of some accessions of Cucurbita maxima from Spain using RAPD and SRAP markers. Genet Resour Crop Evol 50:227–238

Ferriol M, Pico B, Córdova PF, Nuez F (2004a) Molecular diversity of a germplasm collection of squash (*Cucurbita moschata*) determined by SRAP and AFLP markers. Crop Sci 44:653–664

Ferriol M, Pico B, Nuez F (2004b) Morphological and molecular diversity of a collection of *Cucurbita maxima* landraces. J Am Soc Hortic Sci 129:60–69

Ferriol M, Pico B, Nuez F (2005) Genetic diversity of *Cucurbita* sp. in the Canary Islands: a bridge between America and Europe. In: Bullita S (ed) Plant Genetic Resources of Geographical and other "Islands" (Conservation, Evaluation and Use for Plant Breeding), Book of Abstracts of the XVII EUCARPIA Genetic Resources Section, 30 Mar–2 Apr. CNR, Castelsardo, p 9

Fuchs M, Gonsalves D (1995) Resistance of transgenic hybrid squash ZW-20 expressing the coat protein genes of zucchini yellow mosaic virus and watermelon mosaic virus 2 to mixed infections by both potyviruses. Biotech 13:1466–1473

Ge Y, Li X, Yang XX, Cui CS, Qu SP (2015) Genetic linkage map of *Cucurbita maxima* with molecular and morphological markers. Genet Mol Res 14:5480–5484

Gemes-Juhasz A, Balogh P, Ferenczy A, Kristof Z (2002) Effect of optimal stage of female gametophyte and heat treatment on in vitro gynogenesis induction in cucumber (Cucumis *sativus* L.). Plant Cell Rep 21:105–111

Gilbert-Albertini F, Lecoq H, Pitrat M, Nicolet JL (1993) Resistance of *Cucurbita moschata* to watermelon mosaic virus type 2 and its genetic relation to resistance to zucchini yellow mosaic virus. Euphytica 69:231–237

Gong L, Stift G, Kofler R, Pachner M, Lelley T (2008a) Microsatellites for the genus *Cucurbita* and an SSR-based genetic linkage map of *Cucurbita pepo* L. Theor Appl Genet 117:37–48

Gong L, Pachner M, Kalai K, Lelley T (2008b) SSR-based genetic linkage map of *Cucurbita moschata* and its synteny with *Cucurbita pepo*. Genome 51:878–887

Gong L, Paris HS, Nee MH, Stift G, Pachner M, Vollmann J (2012) Genetic relationships and evolution in *Cucurbita pepo* (pumpkin, squash, gourd) as revealed by simple sequence repeat polymorphisms. Theor Appl Genet 124:875–891

Gonzalez-Roman M, Wessel-Beaver L (2002) Resistance to silverleaf disorder is controlled by a single recessive gene in *Cucurbita moschata* Duchesne. Cucurbit Genet Coop Rep 25:49–50

Graephe S (2003) Crop and soil variability in traditional and modern mayan maize cultivation of Yucatan, Mexico. J Agric Rural Dev Trop Subtrop 75:1–72

Grebenscikov I (1954) Zur Vererbung der Dünnschaligkeit bei *Cucurbita pepo* L. Zuchter 24:162–166

Grebenscikov I (1958) Notulae cucurbitologicae III. Kulturpflanze 6:38–60

Grubben GJH, Chigumira-Ngwerume F (2004) *Cucurbita moschata* Duch. In: Grubben GJH, Denton OA (eds) PROTA 2: vegetables/legumes. [CD-Rom]. PROTA, Wageningen

Guo WL, Chen BH, Chen XJ, Guo YY, Yang HL, Li XZ, Wang GY (2018) Transcriptome profiling of pumpkin (*Cucurbita moschata* Duch.) leaves infected with powdery mildew. PLoS One 13:e0190175

Gupta PN, Rai M (1990) Genetic diversity in cucurbits-pumpkin. Indian Hort 36:1

Gwanama C, Labuschagne MT, Botha AM (2000) Analysis of genetic variation of *Cucurbita moschata* by random amplified polymorphic DNA (RAPD) markers. Euphytica 113:19–24

Habib A, Biswas S, Siddique AH, Manirujjaman M, Uddin B, Hasan S, Khan MMH, Uddin M, Islam M, Hasan M, Rahman M, Asaduzzaman M, Rahman M, Khatun M, Islam MA, Rahman M (2015) Nutritional and lipid composition analysis of pumpkin seed (*Cucurbita maxima* L.). J Nutr Food Sci 5:374

Hadia HA, Abdel-Razzak HS, Hafez EE (2008) Assessment of genetic relationships among and within *Cucurbita* species using RAPD and ISSR markers. J Appl Sci Res 4:515–525

Hamdi K, Ben-amor J, Mokrani K, Mezghanni N, Tarchoun N (2017) Assessment of the genetic diversity of some local squash (*Cucurbita maxima* Duchesne) populations revealed by agromorphological and chemical traits. J New Sci Agriculture Biotechnol 42(5):2306–2317

Hazara P, Mandal AK, Dutta AK, Ram HH (2007a) Breeding pumpkin (*Cucurbita moschata* Duch. ex Poir.) for fruit yield and other characters. Int J Plant Breed *1*(*1*):*51–64*

Hazara P, Mandal AK, Dutta AK, Sikadar D, Pandit MK (2007b) Breeding pumpkin (*Cucurbita moschata* duch. ex poir.) for high yield and carotene content. Acta Hortic 752:431–435

Heikal AH, Abdel-Razzak HS, Hafez EE (2008) Assessment of genetic relationships among and within *Cucurbita* species using RAPD and ISSR markers. J Appl Sci Res 4:515–525

Holdsworth WL, La Plant KE, Bell DC, Jahn MM, Mazourek M (2016) Cultivar-based introgression mapping reveals wild species-derived *pm-0*, the major powdery mildew resistance locus in squash. PLoS One 11:e0167715

Huang H-X, Yu T, Li JX, Qu SP, Wang MM, Wu TQ, Zhong YJ (2019a) Characterization of Cucurbita maxima fruit metabolite profiling and transcriptome to reveal fruit quality and ripening gene expression patterns. J Plant Biol 62:203–216

Huang Y, Cao H, Yang L, Chen C, Shabala L, Xiong M, Niu M, Liu J, Zheng Z, Zhou L, Peng Z, Bie Z, Shabala S (2019b) Tissue-specific respiratory burst oxidase homolog-dependent H_2O_2

signaling to the plasma membrane H⁺-ATPase confers potassium uptake and salinity tolerance in Cucurbitaceae. J Exp Bot. https://doi.org/10.1093/jxb/erz328

Hutchins AE (1935) The interaction of blue and green color factors in hubbard squash. Proc Am Soc Hortic Sci 33:514

Inan N, Yildiz M, Sensoy S, Kafkas S, Abak K (2012) Efficacy of ISSR and SRAP techniques for molecular characterization of some *Cucurbita* genotypes including naked (hull-less) seed pumpkin. J Anim Plant Sci 22:126–136

Jagadeeswaran G, Nimmakayala P, Zheng Y, Gowdu K, Reddy UK, Sunkar R (2012) Characterization of the small RNA component of leaves and fruits from four different cucurbit species. BMC Genomics 13:329

Jahn M, Munger HM, McCreight JD (2002) Breeding cucurbit crops for powdery mildew resistance. In: Bélanger RR, Bushnell WR, Dik AJ, Carver TLW (eds) The powdery mildews, a comprehensive treatise. American Phytopathological Society, St. Paul, pp 239–248

Jelaska S (1972) Embryoid formation by fragment of cotyledons and hypocotyls in *Cucurbita pepo*. Planta 103:278–280

Jeon HJ, Been CG, Hong KH, Om YH, Kim BD (1994) Identification of *Cucurbitaceae* cultivars by using RAPD markers. J Korean Soc Hortic Sci 35:449–456

Jobst J, King K, Hemleben V (1998) Molecular evolution of the internal transcribed spacers (ITS I and ITS2) and phylogenetic relationships among species of the family Cucurbitaceae. Malec Phylogenet Evol 9:204–219

Kabelka EA, Young K (2010) Identification of molecular markers associated with resistance to squash silver leaf disorder in summer squash (*Cucurbita pepo*). Euphytica 173:49–54

Karaagac O, Balkaya A (2013) Interspecific hybridization and hybrid seed yield of winter squash (*Cucurbita maxima* Duch.) and pumpkin (*Cucurbita moschata* Duch.) lines for rootstock breeding. Sci Hortic 149:9–12

Kates HR, Soltis PS, Soltis DE (2017) Evolutionary and domestication history of Cucurbita (pumpkin and squash) species inferred from 44 nuclear loci. Mol Phylogenet Evol 111:98–109

Katzir N, Leshzeshen E, Tzuri G, Reis N, Danin-Poleg Y, Paris HS (1998) Relationships among accessions of *Cucurbita pepo* based on ISSR analysis. In: McCreight JD (ed) Proceedings of Cucurbitaceae'98. ASHS, Alexandria, pp 331–335

Katzir N, Tadmor Y, Tzuri G, Leshzeshen E, Mozes-Daube N, Danin-Poleg Y, Paris HS (2000) Further ISSR and preliminary SSR analysis of relationships among accessions of *Cucurbita pepo*. Acta Hortic 510:433–439

Katzir N, Portnoy V, Yonash N, Mozes-Daube N, Tzuri G, Paris HS (2002) Use of AFLP, ISSR, and SSR marker systems to assess genetic diversity in Cucurbita pepo. In: Plant, animal and microbe genomes conference, San Diego, vol 10, p 121

Kazminska K, Sobieszek K, Targonska-Karasek M, Korzeniewska A, Niemirowicz-Szczytt K, Bartoszewski G (2017) Genetic diversity assessment of a winter squash and pumpkin (*Cucurbita maxima* Duchesne) germplasm collection based on genomic *Cucurbita*-conserved SSR markers. Sci Hortic 219:37–44

Kazminska K, Hallmann E, Rusaczonek A, Korzeniewska A, Sobczak M, Filipczak J, Kuczerski KS, Steciuk J, Sitarek-Andrzejczyk M, Gajewski M, Niemirowicz-Szczytt K, Bartoszewski G (2018) Genetic mapping of ovary colour and quantitative trait loci for carotenoid content in the fruit of *Cucurbita maxima* Duchesne. Mol Breed 38:114. https://doi.org/10.1007/s11032-018-0869-z

Kiramana JK, Isutsa DK (2017) First detailed morphological characterisation of qualitative traits of extensive naturalized pumpkin germplasm in Kenya. Int J Dev Sustain 6(7):500–525

Kirkpatrick KJ, Wilson HD (1988) Interspecific geneflow in *Cucurbita: C. texana* vs. *C. pepo*. Am J Bot 75:519–527

Kistler L, Newsom LA, Ryan TM, Clarke AC, Smith BD, Perry GH (2015) Gourds and squashes (*Cucurbita spp*.) adapted to megafaunal extinction and ecological anachronism through domestication. Proc Natl Acad Sci U S A 112:15107–15112

Konrad C (2000) Styrian pumpkin-seed oil. A-over one million control numbers have been assigned. Cucurbit Genet Coop Rep 23:124–125

Kooistra E (1969) The inheritance of resistance to *Cucumis* virus 1 in cucumber (Cucumis sativus L.). Euphytica 18:326–332

Korzeniewska A (1992) New genes in *Cucurbita maxima* Duch. In: Doruchowski RW, Kozik E, Niemirowicz-Szczytt K (eds) Proceedings of Cucurbitaceae'92: the 5th Eucarpia meeting on cucurbit genetics and breeding, pp 75–78

Kosmrlj K, Murovec J, Bohanec B (2013) Haploid induction in hulless seed pumpkin through parthenogenesis induced by X-ray irradiated pollen. J Am Soc Hortic Sci 138:310–316

Kurtar ES, Balkaya A (2010) Production of in vitro haploid plants from in situ induced haploid embryos in winter squash (*Cucurbita maxima* Duchesne ex Lam.) via irradiated pollen. Plant Cell Tissue Organ Cult 102:267–277

Kurtar ES, Sari N, Abak K (2002) Obtention of haploid embryos and plants through irradiated pollen technique in squash (*Cucurbita pepo* L.). Euphytica 127:335–344

Kurtar ES, Balkaya A, Ozbakir M, Ofluoglu T (2009) Induction of haploid embryo and plant regeneration via irradiated pollen technique in pumpkin (*Cucurbita moschata* Duchesne ex. Poir). Afr J Biotechnol 8:5944–5951

Kurtar ES, Balkaya A, Kandemir D (2016) Evaluation of haploidization efficiency in winter squash (*Cucurbita maxima* Duch.) and pumpkin (*Cucurbita moschata* Duch.) through anther culture. Plant Cell Tissue Org Cult 127:497–411

Kurtar ES, Balkaya A, Ozbakir Ozer M (2018) Production of callus mediated gynogenic haploids in winter squash (*Cucurbita maxima* Duch.) and pumpkin (*Cucurbita moschata* Duch.). Czech J Genet Plant Breed 54:9–16

Lebeda A, Kristkova AE (2007) Genotypic variation in field resistance of *Cucurbita pepo* cultivars to powdery mildew (*Erysiphe cichoracearum*). Genet Resour Crop Evol 43:79–84

Lebeda A, Widrlechner MP, Staub J, Ezura H, Zalapa J, Kristkova E (2006) Cucurbits (Cucurbitaceae; *Cucumis* spp., *Cucurbita* spp., *Citrullus* spp.). In: Singh RJ (ed) Genetic resources, chromosome engineering, and crop improvement, vol 3. CRC Press, Boca Raton, pp 271–376

Lee YH, Jeon HJ, Hong KH, Kim BD (1995) Use of random amplified polymorphic DNA for linkage group analysis in an interspecific cross hybrid F_2 generation of *Cucurbita*. J Kor Soc Hortic Sci 36:323–330

Lei B, Huang Y, Xie JJ, Liu ZX, Zhen A, Fan ML, Bie ZL (2014) Increased cucumber salt tolerance by grafting on pumpkin rootstock and after application of calcium. Biol Plant 58:179–184

Lelley T, Loy JB, Murkovic M (2010) Breeding oil seed pumpkins. In: Vollmann J, Rajcan I (eds) Handbook of plant breeding, Oil crops, vol 4. Springer, New York, pp 469–492

Levey M (1966) The medical formulary of Aqrabadhin of Al-Kindi, Translated with a study of its materia medica. University of Wisconsin Press, Milwaukee

Li C, Li Y, Bai L, Zhang T, He C, Yan Y, Yu X (2014) Grafting-responsive miRNAs in cucumber and pumpkin seedlings identified by high-throughput sequencing at whole genome level. Physiol Plant 151:406–422

Lira R, Andres TC, Nee M (1995) Cucurbita L. In: Lira R (ed) Estudios Taxonómicos y Ecogeográficos de lasCucurbitaceae Latinoamericanas de Importancia Económica: Cucurbita, Sechium, Sicana y Cyclanthera. Systematic and ecogeographic studies on crop genepools, vol 9. International Plant Genetic Resources Institute, Rome, pp 1–115

Lira-Saade R, Montes-Hernandez S (1994) Cucurbits (*Cucurbita* spp.). In: Bermejo JEH, Leon J (eds) Neglected Crops: 1492 from a different perspective. Plant production and protection series, vol 26. FAO, Rome, pp 63–77

Liu C, Ge Y, Wang DJ, Li X, Yang XX, Cui C-S, Qu SP (2013) Morphological and molecular diversity in a germplasm collection of seed pumpkin. Sci Hortic 154:8–16

Lotsy JP (1920) *Cucurbita* Strijdvragen. II. Eigen Onderzoekingen. Genetica 2:1–21

Loy JB (1988) Improving seed yield in hull-less strains of *Cucurbita pepo*. Cucurbit Genet Coop Rep 11:72–73

Loy JB (2000) Seed development in Cucurbita pepo: an overview with emphasis on hull-less seeded genotypes of pumpkin. Cucurbit Genet Coop Rep 23:89–95

Loy JB (2004) Morpho-physiological aspects of productivity and quality in squash and pumpkins (*Cucurbita* spp.). Crit Rev Plant Sci 23:337–363

Maluszynski M, Kasha KJ, Szarejko I (2003) Doubled haploid protocols in plant species. In: Maluszynski M, Kasha KJ, Forster BP, Szarejko I (eds) Doubled haploid production in crop plants: a manual. Kluwer Academic Publishers, Dordrecht, pp 309–335

Manamohan M, Prakash N, Chandra GS (2011) Cucurbits. In: Advances in horticulture biotechnology – gene cloning & transgenics, Westville Publishing House, New Delhi, vol 5, pp 227–253

Martinez C, Manzano S, Megias Z, Garrido D, Pico B, Jamilena M (2013) Involvement of ethylene biosynthesis and signaling in fruit set and early fruit development in zucchini squash (*Cucurbita pepo* L.). BMC Plant Biol 13:139

Martinez C, Manzano S, Megias Z, Garrido D, Pico B, Jamilena M (2014a) Sources of parthenocarpy or zucchini breeding. Euphytica 200:349–362

Martinez C, Manzano S, Megias Z, Barrera A, Boualem A, Garrido D, Bendahmane A, Jamilena M (2014b) Molecular and functional characterization of CpACS27A gene reveals its involvement in monoecy instability and other associated traits in squash (*Cucurbita pepo* L.). Planta 239:1201–1215

Martins LHP, Lopes MTG, Noda H, Neto P, De QC, Martins ALU, De Almeida FV, Demosthenes LCR (2018) Molecular study of the genetic variability of pumpkins landraces from Brazilian amazon. Agric Sci 9:511–523

Mazurek Z, Niemirowicz-Szczytt K (1992) Inheritance of spaghetti traits in *Cucurbita pepo*. In: Mookann M 2015. *Direct organogenesis from cotyledonary node explants of Cucurbita pepo (L.)-an important zucchini type vegetable crop*. Am J Plant Sci 6:157–162

McCollum TG (1990) Gene B influences susceptibility to chilling injury in *Cucurbita pepo*. J Am Soc HortScience 115:618–622

Mencarelli F, Anelli G, Tesi R (1982) Idoneità alla conservazione di alcune cultivars di carciofo e di zucca da zucchini. Frutticoltura 44:47–50

Merrick LC (1990) Systematics and evolution of a domesticated squash, Cucurbita argyrosperma, and its wild and weedy relatives. In: Bates DM, Robinson RW, Jeffrey C (eds) Biology and utilization of the Cucurbitaceae. Cornell University Press, Ithaca, pp 77–95

Miladinovic D, Dimitrijevic A, Brdar-Jokanovic M, Imerovski I, Sikora V, Marjanovic JA, Jocic S (2016) Molecular analysis of NS *Cucurbita moschata* collection. In: Proceedings of III international congress "food technology, quality and safety", Novi Sad, 25–27 Oct, pp 6–9

Montero-Pau J, Blanca J, Esteras C, Martinez-Perez EM, Gomez P, Monforte AJ, Canizares J, Pico B (2017) An SNP-based saturated genetic map and QTL analysis of fruit-related traits in Zucchini using Genotyping-by-sequencing. BMC Genomics 18:94

Montero-Pau J, Blanca J, Bombarely A, Ziarsolo P, Esteras C, Marti-Gomez C, Ferriol M, Gomez P, Jamilena M, Mueller L, Pico B, Canizares J (2018) De novo assembly of the zucchini genome reveals whole-genome duplication associated with the origin of the *Cucurbita* genus. Plant Biotechnol J 16:1161–1171

Montes-Garcia CE, Garza-Ortega S, Brown JK (1998) Inheritance of the resistance to squash leaf curl virus in *Cucurbita pepo* L. In: McCreight JD (ed) Cucurbitaceae'98: evaluation and enhancement of cucurbit germplasm. A S H S, Alexandria, pp 328–330

Munger HM (1989) Availability and use of interspecific populations involving *Cucurbita moschata* and *C. pepo*. Cucurbit Genet Coop Rep 13:49

Munger HM (1993) Breeding for viral disease resistance in cucurbits. In: Kyle MM (ed) Resistance to viral diseases of vegetables: genetics and breeding, Timber Press, Portland, USA, pp 44–60

Munger HM, Provvidenti R (1987) Inheritance of resistance to zucchini yellow mosaic virus in *Cucurbita moschata*. Cucurbit Genet Coop Rep 10:80–81

Muralidhara MS, Narasegowda NC (2014) Genetic diversity analysis of pumpkin genotypes (*Cucurbita moschata* Duch ex. Poir) using morphological and RAPD markers. Asian J Bio Sci 9:188–194

Murovec J (2015) Phenotypic and genetic diversity in pumpkin accessions with mutated seed coats. HortScience 50:211–217

Mutschler MA, Pearson OH (1987) The origin, inheritance, and instability of butternut squash (*Cucurbita moschata* Duchesne). HortScience 22:535–539

Nanasato Y, Konagaya KI, Okuzaki A, Tsuda M, Tabei Y (2011) Development of agrobacterium-mediated transformation of pumpkin (Cucurbita moschata Duch) by wounding with aluminum borate whisker. In: Plant & animal genomes XIX conference, San Diego, 15–19 Jan

Nath P, Dutta OP, Velayudhan S, Swamy KRM (1976) Inheritance of resistance to fruit fly in pumpkin. SABRAO J 8:117–119

Nee M (1990) The domestication of Cucurbita (Cucurbitaceae). Econ Bot 44(3):56–68

Nerson H (2005) Effects of fruit shape and plant density on seed yield and quality of squash. Sci Hortic 105:293–204

Nerson H, Paris HS, Paris EP (2000) Fruit shape, size and seed yield in *Cucurbita pepo*. In: Katzir N, Paris HS (eds) Proceedings of Cucurbitaceae 2000. Acta horticulturae, vol 10, pp 227–230

Nesom GL (2011) New state records for *Citrullus, Cucumis*, and *Cucurbita* (*Cucurbitaceae*) outside of cultivation in the USA. Phytoneuron 1:1–7

Ntuli NR, Tongoonab PB, Zobolo AM (2015) Genetic diversity in *Cucurbita pepo* landraces revealed by RAPD and SSR markers. Sci Hortic 189:192–200

Ortiz-Alamillo O, Garza-Ortega S, Sanchez-Estrada A, Troncoso-Rojas R (2007) Yield and quality of the interspecific cross *Cucurbita argyrosperma* x *C. moschata*. Cucurbit Genet Coop Rep 30:56–59

Pachner M, Lelley T (2004) Different genes for resistance to zucchini yellow mosaic virus (ZYMV) in *Cucurbita moschata*. In: Lebeda A, Paris HS (eds) Proceedings of Cucurbitaceae Palacky University, Olomouc, Czech Republic, pp 237–243

Pachner M, Paris HS, Lelley T (2011) Genes for resistance to zucchini yellow mosaic in tropical pumpkin. J Hered 102:330–335

Pachner M, Paris HS, Winkler J, Lelley T (2015) Phenotypic and marker-assisted pyramiding of genes for resistance to zucchini yellow mosaic virus in oilseed pumpkin (*Cucurbita pepo*). Plant Breed 134:121–128

Padley LD, Kabelka EA Jr, Roberts PD (2009) Inheritance of resistance to crown rot caused by *Phytophthora capsici* in *Cucurbita*. HortScience 44:211–213

Pang SZ, Jan FJ, Tricoli DM, Russell PF, Carne KJ, Hu JS, Fuchs M, Quemada HD, Gonsalves D (2000) Resistance to squash mosaic comovirus in transgenic squash plants expressing its coat protein genes. Mol Breed 6:87–93

Paris HS (1986) A proposed subspecific classification for Cucurbita pepo. Phytologia 61:133–138

Paris HS (1988) Complementary genes for orange fruit flesh color in *Cucurbita pepo*. HortScience 23:601–603

Paris HS (1995) The dominant *Wf* (*white flesh*) allele is necessary for expression of “white” mature fruit color in *Cucurbita pepo*. In: Lester G, Dunlap J (eds) Cucurbitaceae’94. Gateway, Edinburg, pp 219–220

Paris HS (2008) Summer squash. In: Prohens J, Nuez F (eds) Handbook of plant breeding, vegetables I. Springer, New York, pp 351–379

Paris HS (2010) Multiple flowering as an adaptation of summer squash for growing in protected culture. In: Thies JA, Kousik S, Levi A (eds) Proc Cucurbitaceae 2010. ASHS Press, Alexandria, pp 88–90

Paris HS, Brown RN (2005) The genes of pumpkin and squash. HortScience 40:1620–1630

Paris HS, Cohen S (2000) Oligogenic inheritance for resistance to zucchini yellow mosaic virus in *Cucurbita pepo*. Ann Appl Biol 136:209–214

Paris HS, Cohen R (2002) Powdery mildew-resistant summer squash hybrids having higher yields than their susceptible, commercial counterparts. Euphytica 124:121–128

Paris HS, Nerson H (2003) Seed dimensions in the subspecies and cultivar-groups of *Cucurbita pepo*. Genet Resour Crop Evol 50:615–625

Paris HS, Nerson H, Burger Y (1985) Precocious PI 165561 and Precocious PI 165561R pumpkin breeding lines. HortScience 20:778–779

Paris HS, Yonash N, Portnoy V, Mozes-Daube N, Tzuri G, Katzir N (2003) Assessment of genetic relationships in *Cucurbita pepo* (*Cucurbitaceae*) using DNA markers. Theor Appl Genet 106:971–978

Paris HS, Portnoy V, Mozes-Daube N, Tzuri G, Katzir N, Yonash N (2004) AFLP, ISSR, and SSR polymorphisms are in accordance with botanical and cultivated plant taxonomies of the highly polymorphic Cucurbita pepo. In: Davidson CG, Trehane P (eds) Proceedings of 26th international horticultural congress. Acta horticulturae, vol 634, pp 167–173

Pomares-Viciana T, Rio-Celestino MD, Roman B, Die J, Pico B, Gomez P (2019) First RNA-seq approach to study fruit set and parthenocarpy in zucchini (*Cucurbita pepo* L.). BMC Plant Biol 19:61

Provvidenti R (1993) Resistance to viral diseases of cucurbits In: Kyle MM (ed) Resistance to viral diseases of vegetables, Timber Press, Portland, USA, pp 8–43

Provvidenti R (1997) New American summer squash cultivars possessing a high level of resistance to a strain of zucchini yellow mosaic virus from China. Cucurbit Genet Coop Rep 20:57–58

Quemada HD (1998) The use of coat protein technology to develop virus-resistant cucurbits. In: Ives CL, Bedford BM (eds) Agricultural biotechnology in international development, CAB International, Wallingford, UK, pp 147–160

Radwan SA (2014) Molecular discrimination and genetic relationships between some cultivars of *Cucurbita pepo* ssp. *pepo* using random amplification of polymorphic DNA (RAPD) analysis. Afr J Biotechnol 13:1202–1209

Ramos SR (2007) Genetic diversity based on AFLP molecular markers and indicators for the establishment of a core collection for pumpkin (*Cucurbita moschata*) for north-east Brazil. Plant Genet Resour Newsl Thesis Abstr 145:66–67

Ren Y, Xu Q, Wang L, Guo S, Shu S, Lub N, Sun J (2018) Involvement of metabolic, physiological and hormonal responses in the graft-compatible process of cucumber/pumpkin combinations was revealed through the integrative analysis of mRNA and miRNA expression. Plant Physiol Biochem 129:368–380

Rhodes AM (1964) Inheritance of powdery mildew resistance in the genus *Cucurbita*. Plant Dis Rptr 48:54–55

Rios H, Fernandez A, Batista O (1997) Cuban pumpkin genetic variability under low input conditions. Cucurbit Genet Coop Rep 20:48–49

Robinson RW, Decker-Walters DS (1997) Cucurbits. Crop production science in horticulture, vol 6. CAB International, Cambridge, 226pp

Robinson RW, Weeden NF, Provvidenti R (1988) Inheritance of resistance to zucchini yellow mosaic virus in the interspecific cross *Cucurbita maxima* × *C. ecuadorensis*. Cucurbit Genet Coop Rep 11:74–75

Sanjur OI, Piperno DR, Andres TC, Wessel-Beaver L (2002) Phylogenetic relationships among domesticated and wild species of *Cucurbita* (*Cucurbitaceae*) inferred from a mitochondrial gene: Implications for crop plant evolution and areas of origin. Proc Natl Acad Sci U S A 99:535–540

Sauton A, Dumas De Vaulx R (1987) Obtention de plantes haploides chez le melon (Cucumis melo L.) par par gynogenese induite par pollen irradie. Agronomie 7:141–148

Schaffer AA, Paris HS, Ascarelli IM (1986) Carotenoid and starch content of near-isogenic B+B+ and BB genotypes of *Cucurbita*. J Am Soc Hortic Sci 111:780–783

Schoniger G (1952) Vorläufige Mitteilung über das Verhalten der Testa- und Farbgene bei verschiedenen Kreuzungen innerhalb der Kürbisart *Cucurbita pepo* L. Züchter 22:316–337

Schultheis JR, Walters SA (1998) Yield and virus resistance of summer squash cultivars and breeding lines in North Carolina. Hortic Tech 8:31–39

Scott DH, Riner ME (1946) Inheritance of male sterility in winter squash. Proc Am Soc Hortic Sci 47:375–377

Sharma GC, Hall CV (1971) Cucurbitacin B and total sugar inheritance in *Cucurbita pepo* related to spotted cucumber beetle feeding. J Am Soc Hortic Sci 96:750–754

Sherman M, Paris HS, Allen JJ (1987) Storability of summer squash as affected by gene B and genetic background. HortScience 22:920–922

Shifriss O (1947) Developmental reversal of dominance in *Cucurbita pepo*. Proc Am Soc Hortic Sci 50:330–346

Shifriss O (1955) Genetics and origin of the bicolor gourds. J Hered 46:213–222

Shifriss O (1966) Behavior of gene *B* in *Cucurbita*. Veg Improv Newsl 8:7–8

Shifriss O (1981) Origin, expression, and significance of gene *B* in *Cucurbita pepo* L. J Am Soc Hortic Sci 106:220–232
Shifriss O (1989) Relationship between the *B* genes of two *Cucurbita* species, II. Cucurbit Genet Coop Rep 12:75–78
Sim SC, Hong JH, Kwon YS (2015) DNA profiling of commercial pumpkin cultivars using simple sequence repeat polymorphisms. Hortic Environ Biotechnol 56(6):811–820
Singh AK (1990) Cytogenetics and evolution in the Cucurbitaceae. In: Bates DM, Robinson RW, Jeffrey C (eds) Biology and utilization of the Cucurbitaceae. Cornell University Press, Ithaca, pp 10–28
Singh AK, Nigam SN (2017) Ancient alien crop introductions integral to Indian agriculture: an overview. Proc Indian Natn Sci Acad 83(3):549–568
Sinnott EW, Durham GB (1929) Development history of the fruit in lines of *Cucurbita pepo* differing in fruit shape. Bot Gaz 87:411–421
Smith BD (1997) The initial domestication of *Cucurbita pepo* in the Americas 10,000 years ago. Science 276:932–934
Solhaug EM, Roy R, Chatt EC, Klinkenberg PM, Mohd-Fadzil NA, Hampton M, Nikolau BJ, Carter CJ (2019) An integrated transcriptomics and metabolomics analysis of the *Cucurbita pepo* nectary implicates key modules of primary metabolism involved in nectar synthesis and secretion. Plant Direct 3(2):e00120. Published 2019 Feb 28. doi:10.1002/pld3.120
Stachel M, Csanadi G, Vollmann J, Lelly T (1998) Genetic diversity in pumpkin (*Cucurbita pepo* L.) as revealed in inbred lines using RAPD markers. Cucurbit Genet Coop Rep 21:48–50
Stephenson AG, Hayes CN, Johannsson MH, Winsor JA (2001) The performance of microgametophytes is affected by inbreeding depression and hybrid vigor in the sporophytic generation. Sex Plant Reprod 14:77–83
Sun H, Wu S, Zhang G, Jiao C, Guo S, Ren Y, Zhang J, Zhang H, Gong G, Jia Z, Zhang F, Tian J, Lucas WJ, Doyle JJ, Li H, Fei Z, Xu Y (2017) Karyotype stability and unbiased fractionation in the paleo-allotetraploid *cucurbita* genomes. Mol Plant 10:1293–1206
Teppner H (2000) *Cucurbita pepo* (*Cucurbitaceae*)- history, seed coat types, thin coated seeds and their genetics. Phyton 40:1–42
Teppner H (2004) Notes on *Lagenaria* and *Cucurbita* (*Cucurbitaceae*)-review and new contributions. Phyton 44:245–308
Tricoli DM, Carney KJ, Russell PF, McMaster JR, Groff DW, Hadden KC, Himmel PT, Hubbard JP, Boeshore ML, Quemada HD (1995) Field evaluation of transgenic squash containing single and multiple protein gene constructs for resistance to cucumber mosaic virus, watermelon mosaic virus 2, and zucchini yellow mosaic virus. Biotechnology 13:1458–1465
Tricoli DM, Carney J, Russell PF, Quemada HD, McMaster JR, Reynolds JF, Deng RZ (2002) Transgenic plants expression DNA constructs containing a plurality of genes to impart virus resistance. US patent 6,337,431
Tricoli DM, Staub JE, Kabelka EA, Yi-Hong W, Quemada HD (2008) Cucurbits. In: Kole C, Hall TC (eds) Compendium of transgenic crop plants: transgenic vegetable crops, Blackwell Publishing Ltd. Oxford, UK, pp 145–184
Tsivelikas AL, Koutita O, Anastasiadou A, Skaracis GN, Traka-Mavrona E, Koutsika-Sotiriou M (2009) Description and analysis of genetic diversity among squash accessions. Braz Arch Biol Technol 52:271–283
US Department of Agriculture (1994) Availability of determination of nonregulated status for virus resistant squash. Fed Regist 59:64187–64189
Verdone M, Rao R, Coppola M, Corrado G (2018) Identification of zucchini varieties in commercial food products by DNA typing. Food Control 84:197–204
Vicente-Dolera N, Troadec C, Moya M, Rio-Celestino MD, Pomares-Viciana T, Bendahmane A, Pico B, Roman B, Gomez P (2014) First TILLING platform in *Cucurbita pepo*: new mutant resource for gene function and crop improvement. PLoS One 9:e112743
Vilmorin and Cie (2010) Squash leaf curl virus (SLCV) resistance in cucurbits. EP 2592924 A1
Vining KJ, Loy JB (1998) Seed development and seed fill in hull-less seeded cultigens of pumpkin (*Cucurbita pepo* L.). In: McCreight JD (ed) Cucurbitaceae'98. ASHS Press, Alexandria, pp 64–69

Wagner FS (2000) The health value of Styrian pumpkin-seed oil-science and fiction. Cucurbit Genet Coop Rep 23:122–123

Wall EM, Lawrence TS, Green MJ, Rott ME (2004) Detection and identification of transgenic virus resistant papaya and squash by multiplex PCR. Eur Food Res Technol 219:90–96

Walters SA, Kindhart JD, Hobbs HA, Eastbourne DA (2003) Viruses associated with cucurbit production in southern Illinois. HortScience 38:65–66

Wang YZ, Gaba V, Yang J, Palukaitis P, Gal-On A (2002) Characterization of synergy between cucumber mosaic virus and potyviruses in cucurbit hosts. Phytopathology 92:51–58

Wang YZ, Lee KC, Gaba V, Wong SM, Palukaitis Pand Gal-On A (2004) Breakage of resistance to cucumber mosaic virus by co-infection with zucchini yellow mosaic virus: enhancement of CMV accumulation independent of symptom expression. Arch Virol 249:379–396

Wang Y, Yan C, Zou B, Wang C, Xu W, Cui C, Qu S (2019) Morphological, transcriptomic and hormonal characterization of trimonoecious and subandroecious pumpkin (*Cucurbita maxima*) suggests important roles of ethylene in sex expression. Int J Mol Sci 20:3185

Weeden NF, Robinson RW (1990) Isozyme studies in *Cucurbita*. In: Bates DM, Robinson RW, Jeffrey C (eds) Biology and utilization of the Cucurbitaceae. Cornell University Press, Ithaca, pp 51–59

Weeden NF, Robinson RW, Ignart F (1986) Linkage between an isozyme locus and one of the genes controlling resistance to watermelon mosaic virus in *Cucurbita ecuadorensis*. Cucurbit Genet Coop Rep 7:86–87

Wessel-Beaver L (2000) *Cucurbita argyrosperma* sets fruits in fields where *C. moschata* is the only pollen source. Cucurbit Genet Coop Rep 23:62–63

Wessel-Beaver L, Cuevas HE, Andres TC, Piperno DR (2004) Genetic compatibility between *Cucurbita moschata* and *C. argyrosperma*. In: Lebeda A, Paris HS (eds) Proceedings of Cucurbitaceae 2004. Palacky University, Olomouc, pp 393–400

Whitaker TW (1951) A species cross in *Cucurbita*. J Hered 43:65–69

Whitaker TW, Bemis WP (1965) Evolution in the genus *Cucurbita*. Evolution 18:553–559

Whitaker TW, Bemis WP (1975) Origin and evolution of the cultivated cucurbita. Bull Torrey Bot Club 102:362–368

Whitaker TW, Davis GN (1962) Cucurbits: botany, cultivation, and utilization. Interscience Publishers, New York, 250pp

Whitaker TW, Robinson RW (1986) *Squash breeding*. In: Bassett MJ (ed) Breeding vegetable crops. AVI Publishing, Westport, pp 209–242

Wilson HD, Doebley J, Duvall M (1992) Chloroplast DNA diversity among wild and cultivated members of *Cucurbita* (Cucurbitaceae). Theor Appl Genet 84:859–865

Winkler J (2000) Breeding of hull-less seeded pumpkins (*Cucurbita pepo*) for the use of the oil. In: Katzir N, Paris HS (eds) Proceedings of Cucurbitaceae 2000. Acta horticulturae, vol 510, pp 123–128

Wu T, Zhou J, Zhang Y, Cao J (2007) Characterization and inheritance of a bush-type in tropical pumpkin (*Cucurbita moschata* Duchesne). Sci Hortic 114:1–4

Wu J, Chang Z, Wu Q, Zhan H, Xie S (2010) Molecular diversity of Chinese *Cucurbita moschata* germplasm collections detected by AFLP markers. Sci Hortic 128:7–13

Wu JX, Chang ZJ, Wu QS, Zhan HX, Xie SL (2011) Molecular diversity of Chinese Cucurbita moschata germplasm collections detected by AFLP markers. Sci Hortic 128:7–13

Wu T, Luo S, Wang R, Zhong Y, Xu X, Lin Y, He X, Sun B, Huang H (2014) The first Illumina-based de novo transcriptome sequencing and analysis of pumpkin (*Cucurbita moschata* Duch.) and SSR marker development. Mol Breed 34:1437–1447

Wyatt LE, Strickler SR, Mueller LA, Mazourek M (2015) An acorn squash (*Cucurbita pepo* ssp. *ovifera*) fruit and seed transcriptome as a resource for the study of fruit traits in *Cucurbita*. Hort Res 2:14070

Wyatt LE, Strickler SR, Mueller LA, Mazourek M (2016) Comparative analysis of Cucurbita pepo metabolism throughout fruit development in acorn squash and oilseed pumpkin. Hortic Res 3:16045

Xanthopoulou A, Psomopoulos F, Ganopoulos I, Manioudaki M, Tsaftaris A, Nianiou-Obeidat I, Madesis P (2016) De novo transcriptome assembly of two contrasting pumpkin cultivars. Genomics Data 7:200–201

Xanthopoulou A, Ganopoulos I, Psomopoulos F, Manioudaki M, Moysiadis T, Kapazoglou A, Osathanunkul M, Michailidou S, Kalivas A, Tsaftaris A, Nianiou-Obeidat I, Madesis P (2017) De novo comparative transcriptome analysis of genes involved in fruit morphology of pumpkin cultivars with extreme size difference and development of EST-SSR markers. Gene 622:50–66

Xiao Q, Loy JB (2007) Inheritance and characterization of a glabrous trait in summer squash. J Am Soc Hortic Sci 132:327–333

Xie J, Lei B, Niu M, Huang Y, Kong Q, Bie Z (2015) High throughput sequencing of small RNAs in the two cucurbita germplasm with different sodium accumulation patterns identifies novel microRNAs involved in salt stress response. PLoS One 10:e0127412

Xu Y, Guo S, Shu S, Ren Y, Sun J (2017) Construction of a genetic linkage map of rootstock-used pumpkin using SSR markers and QTL analysis for cold tolerance. Sci Hortic 220:107–113

Yildiz M, Cuevas HE, Sensoy S, Erdinc C, Baloch FS (2015) Transferability of *Cucurbita* SSR markers for genetic diversity assessment of Turkish bottle gourd (*Lagenaria siceraria*) genetic resources. Biochem Syst Ecol 59:45–53

Youn SJ, Chung HD (1998) Genetic relationship among the local varieties of the Korean native squashes (*Cucurbita moschata*) using RAPD technique. J Korean Soc Hortic Sci 35:429–437

Young K, Kabelka EA (2009) Characterization of resistance to squash silver leaf disorder in summer squash. HortScience 44:1213–1214

Zhang G, Ren Y, Sun H, Guo S, Zhang F, Zhang J, Zhang H, Jia Z, Fei Z, Yong X, Haizhen L (2015) A high-density genetic map for anchoring genome sequences and identifying QTLs associated with dwarf vine in pumpkin (*Cucurbita maxima* Duch). BMC Genomics 16:1101

Zhao Q, Wu J, Zhang L, Xu L, Yan C, Gong Z (2018) Identification and characteristics of *Stagonosporopsis cucurbitacearum* pathogenic factors influencing pumpkin seeding survival in north-east China. J Phytopathol 167:41–55

Zheng YH, Alverson AJ, Wang QF, Palmer JD (2013) Chloroplast phylogeny of *Cucurbita*: evolution of the domesticated and wild species. J Syst Evol 51:326–334

Zhong H, Boudreau E, Rouse S, Dunder E, Gu W, Chang YF (2002) Methods for stable transformation of plants. US patent application no. 20020073445

Zhong YJ, Xhou YY, Li JX, Yu T, Wu TQ, Luo JN, Luo SB, Huang HX (2017) A high density linkage map and QTL mapping of fruit-related traits in pumpkin (*Cucurbita moschata* Duch.). Sci Rep 7:12785

Zhou J, Hu H, Li X, Zhou R, Zhang H (2010) Identification of a resource of powdery mildew resistance in *Cucurbita moschata*. In: Proceedings of 4th international symposium on CUCURBITS. Acta horticulturae, vol 87, pp 141–146

Zraidi A, Lelley T (2004) Genetic map for pumpkin *Cucurbita pepo* using random amplified polymorphic DNA markers. In: Lebeda A, Paris HS (eds) Proceedings of Cucurbitaceae 2004. Palacky University, Olomouc, pp 507–514

Zraidi A, Stift G, Pachner M, Shojaeiyan A, Gong L, Lelley T (2007) A consensus map for *Cucurbita pepo*. Mol Breed 20:375–388

Chapter 12
Accelerated Breeding in Okra

S. K. Dhankhar and A. V. V. Koundinya

12.1 Introduction

The population in the country and in the world has been increasing annually. By 2040, the global human population is expected to reach 9.1 billion (Randers 2012). But, the land under cultivation is decreasing rapidly putting a pressure on increasing crop productivity and high-yielding varieties to feed sufficiently the world's growing population. At the same time, agriculture is one of the most vulnerable sectors to the climate change. Under changing climatic situations, crop failures, reduction in yields and quality and increasing in pest and disease problems are common (Koundinya et al. 2014). Consequently, there is a need to develop quickened or speed breeding approaches to accelerate the creation of new varieties which takes a long time through conventional strategies (Comeau et al. 2001; Forster et al. 2014; Watson et al. 2018).

Since the discovery of Mendel's genetic principles, the methods of the crop breeding are evolving continuously. Plant breeding continues to be a dynamic process and based more and more firmly on interdisciplinary science (Forster et al. 2014). Currently, in order to develop new varieties quickly, speed breeding or accelerated breeding is gaining importance among the breeders. Speed breeding in a fully enclosed, controlled-environment growth chambers can accelerate plant development for research purposes, including phenotyping of adult plant traits, mutant studies and transformation (Watson et al. 2018).

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Variation is the first and foremost prerequisite for effective selection to occur. Creation of variation for desirable traits is possible through physical or chemical mutagens and is a solution when there is no sufficient variation for a particular trait among the available germplasm and in cases where sources are available but could not use through the traditional methods due to hybridization barriers. Tissue culture methods like development of haploid plants through anther/ovule culture followed by doubled haploids creation by doubling of the chromosomes are the fastest way of developing homozygous genotypes. With the advent of molecular biology techniques, the selection of superior genotypes based on genotypic data has become possible. Marker-assisted selection helps in identifying and selection of genotypes with desirable alleles in the early stages of plant growth in segregating generations thereby saving time in handling the large populations. Gene transformation technologies opened new paths by making possible the transfer of genes from unrelated organisms into crop plants. High-throughput phenotyping in phenomics facilities supports the study of plant responses for multiple environments/factors and facilitates the selection of desirable genotypes that perform well in variety of environmental conditions.

Okra is an important vegetable crop of the world which has been growing in many parts of the world. India is the largest producer of okra with an average production and productivity of 6.1 mt and 11.6 t/ha, respectively (Horticultural Statistics at A Glance, 2017). More than 99% of okra cultivation is done exclusively in the developing countries of Asia and Africa with very poor productivity, especially in African countries (2.25 t/ha) compared with any other region (Mishra et al. 2017). Okra is facing several production problems like less seed germination during summer season, and flower drop in okra is recorded at high temperatures above 42 °C (Dhankhar and Mishra 2004). The diseases like yellow vein mosaic disease and enation leaf curl disease, infestations of fruit borer and root-knot nematode cause severe yield losses in okra. Despite wide variation available among the wild relatives of okra, significant improvement has not been made to overcome these problems due to hybridization barriers.

12.2 Speeding Up of Selection Generations in Conventional Breeding

The most commonly followed method of breeding for improvement in okra is pedigree method, which generally takes eight to ten generations for developing a homozygous genotype with desirable traits. At this speed of improvement, it is difficult to achieve global demand within a short period of time. When compared to cereal and oilseed crops like rice, wheat, pearl millet and mustard crops, speed breeding has not been taken much promote in vegetable crops especially in okra. The possibilities in speeding up the normal breeding procedure are described below.

12.2.1 Flowering Manipulation

The number of generations that can be grown in a year is dependent on the duration of crop growth. Each generation begins with the sowing of seeds collected from previous generation to collection of seeds for proceeding to the next generation. If one can reduce this duration by getting early flowering and seed set, more number of generations can be grown in a year. For instance, speed breeding can be used to achieve up to six generations per year for spring wheat (*Triticum aestivum*), durum wheat (*T. durum*), barley (*Hordeum vulgare*), chickpea (*Cicer arietinum*) and pea (*Pisum sativum*) and four generations for canola (*Brassica napus*), instead of two to three under normal glasshouse conditions by manipulating the photoperiod so that early flowering takes place than the normal conditions (Watson et al. 2018). Studies related to earlier flowering induction through photoperiod manipulation have not been done in okra. Since okra is a day-neutral plant, earlier flowering through photoperiod manipulation may be difficult to achieve, but hormonal sprays are fruitful in this regard. Significant difference in the number of days to 50% flowering was recorded due to seed soaking in 100 ppm cycocel followed by foliar spray of same regulators, which took 38 days to 50% flowering, whereas control and water soaking of seeds delayed it (48 days) (Arora and Dhankar 1992). Earlier flowering took place on the third node by triacontanol spraying on Parbhani Kranti and Arka Anamika varieties, and 50% plants obtained flowering in 39 days in Parbhani Kranti and 42 days in Arka Anamika by triacontanol spraying (Acharya 2004). Spraying of NAA @ 50 ppm at 20 DAS caused earlier flowering in 31 days in the okra cultivar Utkal Gaurav (Sanodiya et al. 2017). The details of flowering manipulation in okra by using growth regulators are mentioned in Table 12.1.

From this previous literature, it is understood that growth regulators cause earlier flowering in okra, thus enabling the breeders to make crosses and collecting the seeds early. Growing of parents in controlled conditions coupled with early manifestation of flowering and seed set will facilitate in increasing the number of generations per year in okra.

Table 12.1 Flowering manipulation in okra by using growth regulators

S. no.	Findings	Reference
1	A significant difference in the number of days to 50% flowering was recorded due to seed soaking in 100 ppm cycocel followed by foliar spray of same regulators, which took 38 days to 50% flowering, whereas control and water soaking of seeds delayed it (48 days)	Arora and Dhankar (1992)
2	Earlier flowering took place on the third node by triacontanol spraying on Parbhani Kranti and Arka Anamika varieties, and 50% plants obtained flowering in 39 days in Parbhani Kranti and 42 days in Arka Anamika by triacontanol spraying	Acharya (2004)
3	Spraying of NAA @ 50 ppm at 20 DAS caused earlier flowering in 31 days in the okra cultivar Utkal Gaurav	Sanodiya et al. (2017)

12.2.2 Doubled Haploids

It is well-known that conventional breeding is time-consuming especially the methods based on selection in segregating generations such as pedigree and bulk method of breeding. These methods take seven generations to get 99.22% homozygous lines. Moreover, it is laborious and requires manual self-pollination every year. But, development of haploid plants through pollen/ovule culture followed by the doubling of chromosomes will facilitate the 100% homozygous plants in lesser time.

Haploid plants are of extraordinary enthusiasm to geneticists and plant breeders as they offer the chance to inspect qualities in the hemizygous condition and facilitate identification of new mutations. Haploids are the source of homozygosity as they carry only one set of alleles at each locus; creation of homozygous and homogeneous lines is possible by doubling the chromosomes. This method is highly helpful for studying the effect of recessive genes. This method is greater timesaver than conventional method.

The availability and the introduction of in vitro techniques, especially the anther culture for the induction of androgenesis in the early 1980s, had increased the interest in the production of haploids for crop improvement (Bajaj 1983; Hu 1985). Ibrahim (2016) studied the haploid development in okra. He observed that MS medium was the most responsive medium with an average of 95% callus induction. Incubation in a dark place for 28 days gave highest percentage (92.5%) of callus and root induction. He failed to obtain shoot development in okra despite several treatments and further subculturing. Hence, there is a need for further much efforts to generate an effective androgenesis/gynogenesis protocol in okra in order to reap the benefits of double haploids for the sake of accelerated breeding.

12.2.3 Early Multilocation Trials

In traditional breeding multilocation trials will be taken up after attaining homozygosity, which is obtained after seven to eight generations of selfing. In conventional multilocation testing, the reproducibility of cultivar performance over years and sites is disappointing (Comeau et al. 2001). Because the varieties bred in one particular location are mostly adaptable to that location and when these lines are taken for multilocation trials, their performance is often miserable. This is mainly due to the differences in the environmental factors, differences in pest and disease problems, and differences in soil physical, chemical and microbial factors across various locations. Cumulative effect of all these factors reflects in the reduction of yield further affecting the varietal adaptability over many locations.

The goal of the early multilocation trials was to stabilize yield, without necessarily increasing the yield potential. Yet, the cultivars derived from this process combined high yield with yield stability (Comeau et al. 2001). Generally multilocation trials will be taken up after developing the putative desired homozygous lines expected to be released for general cultivation. This costs additional 3–4 years-time between the development of homozygous lines with desired attributes and their release as a variety.

This method suggests the growing of segregating generations in multilocations enabling the breeders to select stable genotypes without necessitating further multilocation trials after 7–8 years of selection in a single location thereby saving the time.

12.2.4 Marker-Assisted Selection

Molecular markers facilitate the confirmation of presence or absence of a particular locus without waiting for the phenotypic expression of a particular trait thereby facilitating the early selection of genotypes. But, unfortunately the utilization of molecular markers in okra breeding is often limited due to few molecular markers; no genetic map or other molecular tools are available. No linkage map has been constructed so far in okra due to large number of chromosomes (polyploidy) and larger genome size, i.e. about 1600 mb (Sastry and Zitter 2014; Mishra et al. 2017; Jatav et al. 2018), and reports on marker development are very scanty and mostly hovering around cultivar characterization (Mishra et al. 2017). In case of genomic/transcriptomic studies, correct de novo assembly of short sequence reads is complex due to the larger size of the transcriptome arising due to polyploidy (Gruenheit et al. 2012). Moreover, majority of the molecular studies in okra are used in genetic diversity-related studies.

Even there is no proper protocol available for DNA isolation and purification in okra due to the presence of large amounts of mucilaginous acidic polysaccharides, having polygalacturonic acid as its main component in the tissue (Ahmed et al. 2013). During cell lysis, the nucleic acids react with these polysaccharides, and the oxidized form of polyphenols binds to the proteins and nucleic acids, thereby forming a brown gelatinous material, resulting in the poor yield and purity of the extracted DNA (Aljanabi et al. 1999). Moreover, the DNA dissolved in the presence of polysaccharides limits several biotechnological activities including restriction digestion, PCR or in vitro labeling (Sahu et al. 2012).

As mentioned earlier, the marker-assisted selection is not much progressed so far in okra. But, Patil et al. (2018) succeeded in identifying the YVMV-resistant genotypes by amplifying the virus DNA present in the plants. They screened 18 okra genotypes with two primers that amplify the OYMV DNA in plants to confirm the infection by virus, and they were able to differentiate the susceptible and resistant genotypes. This study revealed that these primers can be used to identify the resistant genotypes in the early stages of plant growth.

12.2.5 Heterosis Breeding

The hybrid vigour is seen in the first generation (F_1) obtained from a cross between two genetically diverse parents. Since the hybrids are selected in the F_1 generatin itself, it saves a lot of time when compared to pedigree breeding. Moreover, the hybrids are superior interms of performance of various economic traits. A wide range of heterosis has been reported by several workers for various characters in okra.

Fig. 12.1 Hybrid HBH-142, resistant to YVMV disease

Hybrid vigour has also been exploited for developing hybrids with resistance to yellow vein mosaic virus. But practical utilization of heterosis is highly restricted in okra. The significance of heterosis will be only when it indicates improvement over existing best commercial varieties. It has been pointed out by Hamon and Charrier (2001) that in Indian varieties, genetic study of many characteristics shows additivity, sometimes with slight dominance for flowering time, fruits per plant, plant height and branches per plant. The general combining ability is predominant in most cases. However, some specific combinations could prove worth considering for fruit-bearing branches, seeds per fruit, fruit length and fruit diameter. Several hybrids with resistance to YVMV disease were also developed by both private and public sectors, but only few could show consistency in their performance, viz. CO. 2 and CO. 3 developed by Tamil Nadu Agricultural University, Coimbatore and hybrid HBH-142 (Fig. 12.1) resistant to YVMV disease developed by CCS Haryana Agricultural University, Hisar for cultivation in Punjab, Uttarakhand, Utter Pradesh and Karnataka.

12.3 Nonconventional Breeding Methods

As mentioned earlier the conventional breeding methods are time-consuming and laborious. The success of conventional breeding depends upon the amount of existing variability and sources of variability, breeding systems and compatibility within and between the species. Despite okra is having huge genetic base due to many wild relatives, its utilization is often difficult due to polyploidy and hybridization barriers.

For instance, yellow vein mosaic virus (YVMV) disease is most serious disease of okra spread by whitefly in the region of subtropics and tropics. In the beginning, the resistant strain IC1542 collected from West Bengal was utilized for development of a variety Pusa Sawani, conferring resistance by two recessive alleles located at two loci and was symptomless carrier of yellow vein mosaic virus disease (Singh et al. 1962). Later three YVMV-resistant species like *Abelmoschus manihot* (L.) Medik introduced from Japan, *Abelmoschus manihot* (L.) Medic ssp. *manihot* from Ghana and *Abelmoschus tetraphyllus* Wall were involved in crossing programme with cultivated species, *Abelmoschus esculentus*. Several YVMV-resistant cultivars were developed, namely, Punjab-7 and Punjab Padmini by crossing *A. esculentus* Pusa Sawani and Pusa Reshmi varieties, respectively, with *Abelmoschus manihot* ssp. *manihot*; Parbhani Kranti by using *Abelmoschus manihot* (L.) Medik followed by backcross with Puas Sawani; and Arka Anamika and Arka Abhay involving *Abelmoschus tetraphyllus* with IIHR-20-31 followed by backcross and selection in

Fig. 12.2 An okra variety 'Hisar Naveen' developed through inter-varietal hybridization

subsequent generations. Later on other varieties carrying resistance gene were developed through inter-varietal hybridization, namely, Varsha Uphar, Hisar Unnat, Hisar Naveen (Fig. 12.2), A4, Azad Kranti and VRO-5. These wild species are having pseudo resistance by masking the symptoms, which eventually lead to the breaking of resistance by the virus in those varieties (Nariani and Seth 1958; Dhankhar and Mishra 2004). *Abelmoschus pungens* was reported to be having the true resistance against yellow vein mosaic virus, but it produces sterile hybrids with *Abelmoschus esculentus* (Pal et al. 1952). Resistance to YVMV disease in *Abelmoschus manihot* ssp. *manihot* is conditioned by two complimentary genes (Thakur 1976) and by two dominant complimentary genes (Dhankhar et al. 2005). Sharma et al. (1987) indicated the possibility of polygenic control of resistance in this species. However, Jambhale and Nerkar (1981) reported that single dominant gene is responsible for resistance in *Abelmoschus manihot*.

The resistance to YVMV is not stable in the cultivated species, and frequent breakdowns of resistance have been observed in developed varieties so that there is an urgent need to adopt nonconventional methods of breeding combining biotechnological tools for development of pre-breeding lines resistant to biotic stresses (Singh et al. 2007; Shetty et al. 2013).

Enation leaf curl is another virus disease reported in 1984. This disease causes damage to okra crop mainly in southern states. Source of resistance against enation leaf curl virus (ELCV) has not been reported yet among the available cultivated

germplasm. Some of the wild species (*Abelmoschus crinitus*, *A. ficulneus*, *A. angulosus* and *A. manihot*) of okra have stable and reliable sources of resistance to ELCV (Singh et al. 2007, 2009). However, the transfer of resistance from wild relatives has been hampered by sterility problems, and it is difficult to produce subsequent generations or even carry out backcrosses (Yadav et al. 2018). No fruit set was found in crosses where *A. ficulneus* was used either as male or female parent, and the crosses with *A. moschatus* exhibited hybrid inviability (Rajamony et al. 2006). In such cases nonconventional breeding methods like mutation breeding and recombinant DNA technology are useful, which are even faster than conventional breeding methods.

12.3.1 Mutation Breeding

Mutation is a sudden heritable change in the genetic constitution of an organism apart from segregation and recombination. The most commonly used mutagens are gamma rays and ethyl methane sulfonate (EMS). Mutation breeding is useful where there is no variation available for the desired trait in the existed germplasm stock, or it cannot be utilized due to incompatible barriers. In such cases applied mutagenesis is helpful to create variation for the desirable traits. Moreover, the selection starts from first mutagenic generation (M_1) itself, whereas in pedigree breeding, it starts in third year (F_2), thus speeding up of the breeding procedure. In terms of population variability, the second generation (M_2) in mutation breeding can be compared with the third generation (F_2) in pedigree scheme. As per Forster et al. (2014), the early release of varieties through mutation breeding is possible as it takes an already favoured/adopted cultivar and improves it through slightly changing the elite genetic background. So far, 3000 mutants were released as varieties officially over 200 crops as per the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

In okra, efforts have been attempted to create more variability through mutation techniques (Table 12.2). But so far, only a few mutants of economic value have been isolated for cultivation in India, viz. Punjab-8 (EMS-8), an induced mutant of Pusa Sawani through EMS treatment of 1% ethyl methane sulfonate (EMS). This mutant showed a yield increase of 107%, a fruit number increase of 16% and YVMV disease decrease of 99%. The mutant also carries a good amount of resistance to the fruit borer: infestation decreased by 46%. The mutant is the best among the tested varieties for canning, is suitable for dehydration and can be stored pre-packed at room temperature for 6 days (Sharma and Arora 1990); MDU-1, an induced mutant of Pusa Sawani developed by Tamil Nadu Agricultural University, Coimbotore in 1978; and Prabhani Tillu, also an induced mutant suitable for processing (Kulkarni and Nerkar 1992). In another experiment, 12 mutant resistant lines were obtained from okra variety by screening for YVMV resistance under greenhouse and field conditions. A single YVMV-resistant mutant plant, B-21, was identified in the M_4 generation from 400 Gy gamma irradiation (Phadvibulya et al. 2004). Few capable lines in the X_{10} generation were isolated by Kuwada (1970). Three short intermodal-length mutants (<4.0 cm) and two genic male-sterile mutants were isolated in okra

Table 12.2 The results of the previous mutation experiments in okra for various characters

S. no.	Findings	References
1	Mutagenic treatments were very effective in inducing macromutations for various desirable traits which is a consonance of the earlier finding in okra	Shanna and Arora (1991) and Kulkarni and Nerkar (1992)
2	The highest genotypic coefficient of variation and phenotypic coefficient of variation were observed for number of fruits per plant, yield per plant and plant height in a gamma radiation and EMS-induced okra population	Singh et al. (1998)
3	Among all the doses of mutagens 45, 60 kR gamma rays and 0.75% and 1% EMS gave resistance to yellow vein mosaic virus in okra in M_2 and M_3 generations	Singh and Singh (2000)
4	High EMS at 0.2%, 0.35% and 0.5% and diethyl sulphate (DES) at 0.025%, 0.07% and 0.125% induced genetic variation for average fruit length, fruits per plant and fruit weight was observed, and high heritability for average fruit length, fruits per plant and fruit weight were observed in an M_2 generation in okra	Suryakumari (2002)
5	In M_2 and M_3 generations, the mean for various growth characters of okra increased at lower doses of mutagens. A number of mutants for one or more traits, viz. plant height, maturity, branching, pigmentation, fruit size and yield, were isolated in these generations by gamma rays and EMS application in okra	Singh and Singh (2002)
6	High genotypic and phenotypic coefficients of variation were found for number of fruits and yield per plant in EMS-treated M_2 generation	Singh and Singh (2004)
7	When treated with 30 kR, the mean fruit number/plant, fruit length and fruit yield/plant were increased as compared to control in okra	Mishra et al. (2007)
8	Increased number of flowers and fruits per plant as well as weight of fruits, fruit and seed yield (q/ha) was found when okra seeds were irradiated with gamma rays dose of 2000 rad	Dubey et al. (2007)
9	Increased number of okra fruit per plant and fruit length as a result of gamma irradiation	Dubey et al. (2007), Mishra et al. (2007), and Sharma and Mishra (2007)
10	Progenies treated with 400 gamma rays showed high resistance to yellow vein mosaic virus diseases in okra	Phadvibulya et al. (2009)
11	Plants with maximum height for 400 Gy exposure were obtained while using 300, 400 and 500 Gy gamma ray exposures in okra	Hegazi and Hamideldin (2010)
12	Days for first flower appearance, 50% flowering and days required for first fruit set was increased with increase in mutagenic doses	Dalve et al. (2010)
13	Sodium azide and gamma rays mutagens caused to increase in plant height and number of pods in musk okra (*Abelmoschus moschatus*) as compared to control	Warghat et al. (2011)
14	Higher mutagenic doses showed resistance with less disease intensity. The treatments with higher mutagenic doses (40 kR gamma rays + 0.1% EMS and 30 kR gamma rays + 0.1% EMS) had shown resistance against yellow vein mosaic virus	Dalve et al. (2012)

(continued)

Table 12.2 (continued)

S. no.	Findings	References
15	Growth and yield parameters of okra increase with lower doses of gamma rays application, but days to first flower increased with increase in dose of gamma rays	Jagajanantham et al. (2012)
16	In M2 generation, a number of morphological and economical mutants like chlorophyll, tall plant, dwarf plant, fruit size, early and late maturity were isolated in okra by gamma rays and EMS application	Jadhav et al. (2013)
17	Application of EMS mutation leads to increased plant height, pod number/plant, pod length, pod diameter, stem thickness, seed number/pod, number of locules, seed yield/plant and decrease of seed weight in okra	Baghery and Kazemitabar (2014)
18	Increase in pod length was found with EMS treatment (0.01% and 0.15%) than gamma radiation in Parbhani Kranti variety of okra in M_2 generation, but gamma rays (5 kR and 20 kR) induced longer fruit length in case of Arka Anamika	Kashid and Khulte (2014)
19	Very low gamma radiation and EMS-induced GCV values were observed for all the morphological and yield parameters such as days to flowering, plant height, number of branches per plant, number of nodes on the main stem, internodal length, fruit length, fruit diameter, fruit weight, number of seeds per fruit, number of fruits per plant and fruit yield per plant in M_2 generation in GO-2 and GJO-3 varieties of okra	Reddy and Dhaduk (2014)
20	Mutation breeding increased number of fruits per plant, seeds per fruit and seed weight in okra	Kashid (2016)
21	The gamma irradiation doses are 300, 400 and 500 kR; EMS doses 0.3%, 0.4% and 0.5% induced more variation in two okra cultivars BCO-1 and Japanese Jhar Bhindi. Both gamma irradiation and soaking in EMS caused sufficiently high GCV and PCV for effective selection to carry out in the characters as the first node to fruit, the number of flowers per plant, the number of fruits per plant and the number of seeds per pod and seed index. High heritability coupled with high GAM was observed for days to first flower set, first node to flower, first node to fruit, number of flowers per plant, number of fruits per plant, number of seeds per pod, seed index and fruit yield per plant in both the populations; for number of primary branches/plant when treated with gamma radiation; for fruit length when soaked in EMS suggesting that these characters were under strong influence of additive gene action	Ashadevi et al. (2017)

when commercial variety Hisar Unnat was irradiated with 70 krad gamma rays (Dhankhar, 2019, Unpublished data) (Fig. 12.3).

In okra, most effective mutation rates in M_2 generation were obtained by 60–80 kRad gamma ray seed treatment (Abraham and Bhatia 1984). Among the viable mutants obtained, most of them had altered leaf characteristics. Chlorophyll mutant, chlorine, in the variety Vaishali Vadhu, which is governed by single gene, was identified by Jambhale and Nerkar (1979). Supernumerary inflorescences were observed in many plants of a North Nigerian ecotype (Fatokun et al. 1979).

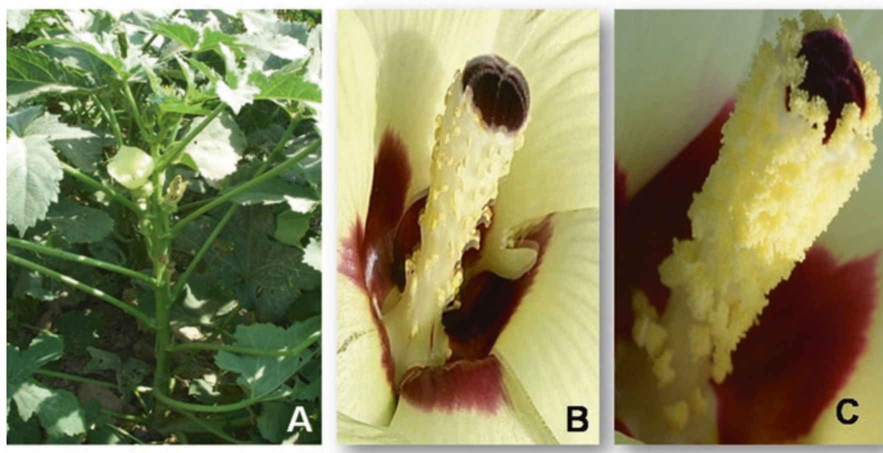

Fig. 12.3 Okra mutants. (**a**) Male sterile plant. (**b**) Male-sterile flower. (**c**) Male-fertile flower

12.3.2 Tissue Culture and Genetic Transformation

The commonly known conventional plant breeding technique for the transfer of single or few genes into a genetic background is backcrossing. Backcrossing is generally followed for transfer of genes from wild relatives. A combination of back-cross and pedigree method was followed for transfer of YMV resistance to cultivated okra from wild relatives. Backcross has certain limitations as these depend on sexual compatibility and often take 10–15 years to release a new variety due to extensive backcrossing (Pauls 1995). In general, seven backcrossings are required to recover 99.22% of recurrent parent genome. These limitations have stimulated the development of more advanced technologies like genetic transformation of plants. A remarkable progress has been made in the development of gene transfer technologies which ultimately have resulted in production of a large number of genetically engineered plants. Potential benefits from these transgenic plants include higher yields, enhanced nutritional values, reduction in pesticides and fertilizers use and improved control of soil and water pollutants (Rekha Rani 2007).

For effective transformation efficient callus induction and regeneration protocols are required. But, the absence of an efficient transformation system has hampered the progress in okra genetic engineering research. Regarding in vitro culture of okra, only limited numbers of protocols were reported for shoot organogenesis (Mishra et al. 2017). Many researchers used various explants so far including cotyledon, cotyledonary node, cotyledonary axis, hypocotyl (Mangat and Roy 1986; Haider et al. 1993; Ganesan et al. 2007; Kabir et al. 2008), shoot tip (Dhande et al. 2012) and plumule from the zygotic embryo (Narendran et al. 2013) of okra for callus induction and regeneration by using various growth regulators. Some important regeneration protocols developed in okra are presented in Table 12.3.

Table 12.3 Regeneration protocols developed in okra

S. no	Findings	References
1	Successfully regenerated the whole plantlets of okra through hypocotyls callus cultures by using combined treatment of benzylaminopurine (BAP) and NAA	Haider et al. (1993)
2	A simple and reliable protocol for the regeneration of okra through somatic embryogenesis from suspension cultures	Ganesan et al. (2007)
3	Embryogenic callus was obtained from hypocotyl explants cultured on media with Murashige and Skoog (MS) salts, Gamborg (B5) vitamins, 2.0 mg dm^{-3}, 2,4-dichlorophenoxyacetic acid (2,4-D), 1.0 mg dm^{-3} naphthaleneacetic acid (NAA), 25 mg dm^{-3} polyvinylpyrrolidone and 30 g dm^{-3} sucrose	Anisuzzaman et al. (2008)
4	More number and high frequency of healthy embryoids appeared individually in suspension culture containing MS salts, B5 vitamins, 2.0 mg dm^{-3} 2,4-D and 1.0 mg dm^{-3} kinetin	
5	The best conversion of the embryo into plantlets (67.3%) was recorded on media with half-strength MS salts, B5 vitamins, 0.2 mg dm^{-3} benzylaminopurine and 0.2 mg dm^{-3} gibberellic acid	
6	Callus induction with high frequency was observed in MS medium with 2.0 mg/L NAA and 0.5 mg/L TDZ by using hypocotyls	
7	The highest per cent shoot regeneration and the high mean number of shoots per callus were observed 2.0 mg/L BAP and 0.1 mg/L IBA. Root formation was observed from callus induced in medium containing 1.5 mg/L	
8	Okra tissue culture regeneration protocol using zygotic embryo explants	Narendran et al. (2013)

Agrobacterium-mediated gene transfer is the most commonly used transformation method. Rekha Rani (2007) standardized *Agrobacterium*-mediated gene transformation protocol in okra. Genetic transformation with *Agrobacterium* carrying the plasmid pBI121 with a selectable marker gene npt-II that confers resistance against the antibiotic kanamycin was carried out in okra. Transformed cells were cultured on kanamycin (50 mg/L) and cefotaxime (300 mg/L). Proliferation of callus was achieved in this selection medium, and *Agrobacterium* was suppressed completely. About 50–60% calli showed GUS expression confirming the transformation.

Fruit borer is a menace in okra whose management is difficult due to the feeding of the larva in the interior of pod. Narendran et al. (2013) have developed an okra tissue culture regeneration protocol using zygotic embryo explants and its integration with *Agrobacterium*-mediated transformation. They developed seven transgenic lines with *cry1Ac* gene in order to achieve resistance against at fruit borer, and the feeding on the transformed fruits from these lines caused 100% larval mortality and therefore confirmed the presence and expression of transgene in transgenic Bt plants.

Recently, Manickavasagam et al. (2015) have established a tissue culture-independent in planta genetic transformation system for okra using seed as an explant. In planta transformation is a tissue culture-independent process which is

Table 12.4 Gene transformation experiments for the introgression of various traits in okra

S. no	Traits	Details	Reference
1	*gus* gene expression	Genetic transformation with *Agrobacterium* carrying the plasmid pBI121 with a selectable marker gene npt-II that confers resistance against the antibiotic kanamycin was carried out in okra. Transformed cells were cultured on kanamycin (50 mg/L) and cefotaxime (300 mg/L). The proliferation of callus was achieved in this selection medium, and *Agrobacterium* was suppressed completely. About 50–60% calli showed GUS expression confirming the transformation	Rekha Rani (2007)
2	Fruit borer resistance	They developed seven transgenic lines with cry1Ac gene through *Agrobacterium*-mediated gene transfer in order to achieve resistance against at fruit borer, and the feeding on the transformed fruits from these lines caused 100% larval mortality thereby confirming the presence and expression of the transgene in transgenic Bt plants	Narendran et al. (2013)
3	*hpt II*, *bar* and *gus A* genes	In planta transformation through *Agrobacterium tumefaciens* EHA 105 harbouring the binary vector pCAMBIA 1301-bar was done, and 18.3% transformation efficiency was recorded. The GUS histochemical analysis confirmed the gus A gene integration and expression, whereas polymerase chain reaction (PCR) and southern blot hybridization confirmed the bar gene integration and copy number in the transformed okra genome	Manickavasagam et al. (2015)

simple, less time-consuming, does not require skilled labours, and importantly, somaclonal variation would not occur (Manickavasagam et al. 2015). Some of the gene transformation experiments for the introgression of various traits are illustrated below (Table 12.4).

Agrobacterium tumefaciens harbouring the binary vector pCAMBIA 1301-bar was used for the transformation, and 18.3% transformation efficiency was recorded. Similarly, there seems an urgent need to shift the research focus to develop more transgenics using coat protein (CP) genes, imparting resistance to the various viral diseases like YVMV and OELCV (Mishra et al. 2017).

12.4 Conclusion

Okra is an important vegetable of high value. There is a necessity to develop high-yielding, pest- and disease-resistant, abiotic stress-tolerant, nutritionally rich varieties at a quicker rate in order to face the future needs of the ever-increasing human population. To achieve this goal, speed or accelerated breeding of okra needs to be taken up. In this chapter, the possibilities for accelerating the breeding of okra are discussed. The methods mentioned will be helpful to the breeders in speeding up of the okra breeding programme.

12.5 Future Prospects

The main focus is to be given for speeding up of the conventional breeding through techniques such as flowering manipulation, double haploids, early multilocation testing and marker-assisted selection to reduce the selection generations. The possibility of applying nonconventional breeding methods that involve biotechnology, radiation biology, etc. is to be exploited. Varietal notification and release take a lot of time before the release of a variety. Early multilocation trials are to be incorporated in varietal release programmes to avoid this delay and faster release of the varieties, seed production, certification and distribution.

References

Abraham V, Bhatia CR (1984) Induced mutation in okra (A. esculentus). Mutation breed. Newsletter 24:1–3

Acharya UK (2004) Thesis submitted to the Tribhuvan University, Institute of Agriculture and Animal Science Rampur, Chitwan, Nepal for the degree of master of science in agriculture (horticulture)

Ahmed N, Nawaz S, Iqbal A, Mubin M, Butt A, Lightfoot DA et al (2013) Extraction of high-quality intact DNA from okra leaves despite their high content of mucilaginous acidic polysaccharides. BIOSCI Methods 4:19–22

Aljanabi SM, Forget L, Dookun A (1999) An improved and rapid protocol for the isolation of polysaccharide-and polyphenol-free sugarcane DNA. Plant Molecular Biology Reports 17:281

Anisuzzaman M, Jarin S, Naher K, Akhtar MM, Alam MJ, Khalekuzzaman M, Alam I, Alam MF (2008) Callus induced organogenesis in okra (Abelmoschus esculents L. Moench.). Asian J Plant Sci 7:677–681

Arora SK, Dhankar BS (1992) Effect of seed soaking and foliar spray of cycocel on germination, growth, flowering, fruit set and yield of okra (*Abelmoschus esculentus* L. Moench). Veg Sci 19(1):79–85

Ashadevi R, Koundinya AVV, Chattopadhyay SB (2017) An estimation of effective mutagen doses, induced genetic variation and characters association in M2 generation of okra. Int J Curr Microbiol App Sci 6(5):2209–2219

Baghery MA, Kazemitabar SK (2014) Effect of EMS induction on some morphological traits of okra (Abelmoschus esculentus (L.) Moench). International Journal of Biosciences 5(9):340–345

Bajaj YPS (1983) *In vitro* production of haploids. In: Evans DA, Sharp WR, Ammirato PV, Yamada Y (eds) Handbook of plant cell culture, vol 1. Macmillan, New York, pp 228–287

Comeau A, Caetand VR, St-Pierre CA, Haber S (2001) Accelerated breeding: dream or reality? In: Bedii Z, Lang L (eds) Wheat in a global environment. Kluwer Academic Publishers, pp 671–679

Dalve PD, Musmade AM, Mane SV, Nimbalkar RR (2010) Response of okra to gamma rays and EMS in M1 generation. The Asian Journal of Horticulture 4(2):398–400

Dalve PD, Musmade AM, Patil RS, Bhalekar MN, Kute NS (2012) Selection for resistance to yellow vein mosaic virus disease of okra by induced mutation. Bioinfolet 9(4B):822–823

Dhande GA, Patil VM, Raut RV, Rajput JC, Ingle AG (2012) Regeneration of okra (*Abelmoschus esculentus*) via apical shoot culture system. Afr J Biotechnol 11:15226–15230

Dhankhar BS, Mishra JP (2004) Objectives of okra breeding. In: Singh PS, Dasgupta SK, Tripathi SK (eds) Hybrid vegetable development. Food Products Press, New York, USA. pp 195–209

Dhankhar SK, Dhankhar BS, Yadav RK (2005) Inheritance of resistance to yellow vein mosaic virus in an interspecific cross of okra (Abelmoschus esculentus). Indian J Agric Sci 75(2):87–89

Dubey AK, Yadav JR, Singh B (2007) Studies on induced mutations by gamma irradiation in okra. [Abelmoschus esculentus (L.) Moench]. Progress Agric 7(1/2):46–48

Fatokun CA, Aken Orea ME, Chheda HR (1979) Supernumerary inflorescence-a mutation of agronomic significance in okra. J Hered 70:270–271

Forster BP, Till BJ, Ghanim AMA, Huynh HOA, Burstmayr H, Caligari PDS (2014) Accelerated plant breeding. CAB Rev 9:1–16

Ganesan M, Chandrasekar R, Ranjitha Kumari BD, Jayabalan N (2007) Somatic embryogenesis and plant regeneration of *Abelmoschus esculentus* through suspension culture. Biol Plant 51(3):414–420

Gruenheit N, Deusch O, Esser C, Becker M, Voelckel C, Lockhart PJ (2012) Cutoffs and k-mers: implications from a transcriptome study in allopolyploid plants. BMC Genomics 13:92

Haider SA, Islam R, Kamal AHM, Rahman SM, Joarder OI (1993) Direct and indirect organogenesis in cultured hypocotyl explants of *Abelmoschus esculentus* (L.) Moench. Plant Tissue Cult 3:85–89

Hamon S, Charrier A (2001) Okra. In: Charrier A, Jacquot M, Hamon S, Nicolas D (eds) Tropical plant breeding. Oxford and IBH pvt. Ltd., Calcutta, pp 361–380

Hegazi AZ, Hamideldin N (2010) The effect of gamma irradiation on enhancement of growth and seed yield of okra [Abelmoschus esculentus (L.) Monech] and associated molecular changes. Journal of Horticulture and Forestry 2(3):038–051

Hu H (1985) Use of haploids in crop improvement. In: Biotechnology in international agricultural research. In: Proceedings of the inter-center seminar on international agricultural centers (IARCs) and biotechnology, 23–27 Apr 1984. IRRI, Manila, pp 75–84

Ibrahim AM (2016) Haploid induction of kenaf (Hibiscus cannabinus L.), okra (Abelmoschus esculentus L.) And spring onion (Allium fistulosum L.) using anther, ovary and ovule cultures faculty of agro based industry. Thesis submitted to the Universiti Malaysia Kelantan for the degree of Doctor of Philosophy

Jadhav PA, Kalpande HV, Arbad SK, Mali AR (2013) Induced mutagenesis in okra [Abelmoschus esculentus (L.) Moench] by gamma rays and ethyl methane sulphonate. Vegetable Science 40(2):223–224

Jagajanantham N, Dhanavel D, Pavadai P, Chidambaram AA (2012) Growth and yield parameters using gamma rays in bhendi [Abelmoschus esculentus (L) Moench] var. Arka Anamika. International Journal of Research in Plant Science 2(4):56–58

Jambhale ND, Nerkar YS (1979) Inheritance of chlorine and induced chlorophyll mutant in okra (Abelmoschus esculentus (L.) Moench). J Maharashtra Agric Univ 4(3):316

Jambhale ND, Nerkar YS (1981) Inheritance of resistance to okra yellow vein mosaic disease in interspecific crosses of Abelmoschus. Theor Appl Genet 60:313–316

Jatav PK, Singh K, Mathapati G, Gowda R, Karoriya S, Nalla MK (2018) Breeding for virus resistance in okra. Int J Curr Microbiol App Sci 7(8):3053–3065

Kabir AH, Sarker KK, Sharmin SA, Islam MS, Alam MF (2008) Callus induction and plantlet regeneration in *Abelmoschus esculentus* (L.) Moench. J Agric Technol 4:193–204

Kashid NG (2016) Mutagenesis in hundred seed weight of okra (Abelmoschus esculentus L. Moench). Asian Journal of Multidisciplinary Studies 4(3):106–109

Kashid NG, Khulte MP (2014) Effect of physical and chemical mutagens on pod length in okra (Abelmoschus esculentus L. Moench). Science Research and Report 4(2):151–154

Koundinya A, Sidhya P, Pandit MK (2014) Impact of climate change on vegetable cultivation-a review. International Journal of Agriculture, Environment & Biotechnology 7(1):145–155

Kulkarni UB, Nerkar YS (1992) 'Parbhani Tillu' an induced bhendi mutant suitable for fruit processing. Journal of Maharastra Agricultural University 17(3):496–497

Kuwada H (1970) X ray induced mutation in okra. Technical bulletin of the faculty of agriculture. Kagawa University 21:2–8

Mangat BS, Roy MK (1986) Tissue culture and plant regeneration of okra (*Abelmoschus esculentus*). Plant Sci 47:57–61

Manickavasagam M, Subramanyam K, Ishwarya I, Elayaraja D, Ganapathi A (2015) Assessment of factors influencing the tissue culture independent Agrobacterium-mediated in planta genetic transformation of okra (*Abelmoschus esculentus* (L.) Moench). Plant Cell Tissue Organ Cult 123:309–320. https://doi.org/10.1007/s11240-015-0836-x

Mishra GP, Singh B, Seth T, Singh AK, Halder J, Krishnan N, Tiwari SK, Singh PM (2017) Biotechnological advancements and Begomovirus Management in Okra (Abelmoschus esculentus): status and perspectives. Front Plant Sci 8:360

Mishra MN, Qadri H, Mishra S (2007) Macro and micro mutations in okra [Abelmoschus esculentus (L.) Moench] by gamma rays and ethyl methane Sulphonate. Vegetable Science 29(1):30–33

Narendran M, Shirale D, Parimi S, Deole SG, Nanote A, Char BR et al (2013) Efficient genetic transformation of okra (Abelmoschus esculentus (L.) Moench) and generation of insect-resistant transgenic plants expressing the cry1Ac gene. Plant Cell Rep 32:1191–1198. https://doi.org/10.1007/s00299-0131415-4

Nariani TK, Seth ML (1958) Reaction of Abelmoschus and Hibiscus species to yellow vein mosaic virus. Indian Phytopathology 11:137–140

Pal BP, Singh HB, Swarup V (1952) Taxonomic relationships and breeding possibilities of species of *Abelmoschus* related to okra. Bot Gaz 113:455–464

Patil S, Bagde E, Tatte R, Mane SS, Naik RM, Gawande PP, Jadhav PV (2018) Characterization of okra genotypes by molecular markers against yellow vein mosaic virus. Int J Chem Stud 6(5):2809–2812

Pauls KP (1995) Plant biotechnology for crop improvement. Biotechnol Adv 13:673–693

Phadvibulya V, Boonsirichai K, Adthalungrong A, Srithongchai W (2009) Selection for resistance to yellow vein mosaic virus disease of okra by induced mutation. In: Shu QY (ed) Induced plant mutations in the genomics era. Food and agriculture organization of the United Nations, Rome, pp 349–351

Phadvibulya V, Puripanyavanich V, Adthalungrong A, Kittipakorn K, Lavapaurya T (2004) Induced mutation breeding for resistance to yellow vein mosaic virus in okra. In: Proceedings of a final research coordination meeting organized by the joint FAO/IAEA division of nuclear techniques in food and agriculture, Pretoria, 19–23 May 2003, pp 155–175

Rajamony L, Chandran M, Rajmohan K (2006) In vitro embryo rescue of interspecific crosses for transferring virus resistance in okra (*Abelmoschus esculentus* (L.) Moench). In: Fári MG, Holb I, Bisztray GYD (eds) Proceedings of Vth IS on in vitro culture and horticultural breeding. Acta horticulturae, p 725

Randers JA (2012) Global forecast for the next forty years 2052. Chelsea Green Publishing, White River Junction, pp 62–159

Reddy PS, Dhaduk LK (2014) Induction of genetic variability in okra [Abelmoschus esculentus (L.) Moench] by gamma and EMS. Electronic Journal of Plant Breeding 5(3):588–593

Rekha Rani M (2007) Standardization of protocol for regeneration and genetic transformation of okra (Abelmoschus esculentus [L.] Moench). Thesis submitted to the Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad for the degree of Master of Science (Agricultural Biotechnology)

Sahu SK, Thangaraj M, Kathiresan K (2012) DNA extraction protocol for plants with high levels of secondary metabolites and polysaccharides without using liquid nitrogen and phenol. Mol Biol 1:1–1: 6

Sanodiya K, Pandey G, Kacholli P, Dubey AK (2017) Effect of growth regulator on growth, yield and seed quality parameters of okra (*Abelmoschus esculentus* L.): cv. Utkal Gaurav. Int J Curr Microbiol App Sci 6(10):3551–3556

Sastry KS, Zitter TA (2014) Management of virus and viroid diseases of crops in the tropics. In: Plant virus and viroid diseases in the tropics Vol. 2: epidemiology and management. Springer, Netherlands, pp 149–480

Shanna DR, Arora SK (1991) Mutation breeding in okra. In: Goldcn Jubilee symposium on genetic research and education: current trends and next 50 years. February 12–15, 1991 New Delhi

Sharma BR, Sharma OP, Bansal RD (1987) Influence of temperature on incidence of yellow vein mosaic virus in okra. Vegetable Science 14:65–69
Sharma BK, Mishra MN (2007) Micro-mutations for fruit number, fruit length and fruit yield characters in gamma-irradiated generation of ANKUR-40 variety of okra. [Abelmoschus esculentus (L.) Monech]. International Journal of Plant Science 2:208–211
Sharma BR, Arora SK (1990) Advances in breeding of okra [Abelmoschus esculentus (L) Moench] in India. Mutation breeding newsletter No 35, International Atomic Energy Agency. Pp 44
Shetty AA, Singh JP, Singh D (2013) Resistance to yellow vein mosaic virus in okra: a review. Biol Agric Hortic Int J Sustain Prod Syst 29(3):159–164. https://doi.org/10.1080/01448765.2013.793165
Singh AK, Singh KP (2000) Seedling injury, reduced pollen and ovule fertility and chlorophyll mutations induced by gamma rays and EMS in okra. Vegetable Science 27:42–44
Singh AK, Singh KP (2002) Induced mutation in okra (Abelmoschus esculentus (L). Moench) by gamma rays and ethyl methane sulphonate. Vegetable Science 29(1):30–33
Singh AK, Singh KP (2004) Induced quantitative variation for yield and its components in okra. [Abelmoschus esculentus (L.) Moench]. Indian Journal of Horticulture 61(3):240–244
Singh AK, Singh KP, Singh VP (1998) Genetic analysis of induced mutants of okra. [Abelmoschus esculentus (L) moench]. Vegetable Science 25(2):174–177
Singh HB, Joshi BS, Khanna PP, Gupta PS (1962) Breeding for field resistance to yellow vein mosaic in bhindi. Indian Journal of Genetics and Plant Breeding 22:137–144
Singh B, Rai M, Kalloo G, Satpathy S, Pandey KK (2007) Wild taxa of okra (*Abelmoschus species*): reservoir of genes for resistance to biotic stresses. Acta Hortic 752:323–328
Singh DR, Singh PK, Syamal MM, Gautam SS (2009) Studies on combining ability in okra. Indian J Hortic 66(2):277–280
Suryakumari S (2002) Induced polygenic mutations in okra [Abelmoschus esculentus (L.) Moench]. South Indian Horticulture 50:533–537
Thakur MR (1976) Inheritance of yellow vein mosaic in a cross of okra species, Abelmoschus esculentus and A. manihot ssp. manihot. SABRAO Journal of Breeding and Genetics 8:69–73
Warghat AR, Rampure NH, Wagh P (2011) Effect of sodium azide and gamma rays treatments on percentage germination, survival, morphological variation and chlorophyll mutation in musk okra (Abelmoschus moschatus L.). Int J Pharm Pharm Sci 3(5):483–486
Watson A, Ghosh S, Williams MJ (2018) Speed breeding is a powerful tool to accelerate crop research and breeding. Nature Plants 4(1):23–29
Yadav Y, Maurya PK, Devi AP, Jamir I, Bhattacharjee T, Banerjee S, Dutta S, Debnath D, Mandal AK, Dutta S, Chattopadhyay A (2018) Enation leaf curl virus (ELCV): a real threat in major okra production belts of India: a review. J Pharmacogn Phytochem 7(2):3795–3802

Chapter 13
New Initiatives in Quick Bitter Gourd Breeding

T. K. Behera, Suman Lata, and S. S. Dey

13.1 Introduction

Bitter gourd belongs to genus *Momordica*. The genus *Momordica* derives its name from the Latin word "mordeo" (momordi to bite) which might be due to the bitter appearance of its seeds. The highest species diversity for this genus is found in Africa and Southeast Asian countries. *Momordica* belongs to the subtribe Thalidianthinae Pax, tribe Joliffieae Schrad., subfamily Cucurbitoideae of Cucurbitaceae (Jeffrey 1980; de Wilde and Duyfjes 2002). Generic and species descriptions (along with keys in some cases) are found in various monographic and floristic treatises (Wight and Arnott 1841; Thwaites 1864; Hooker 1871; Clarke 1879; Keraudren 1975; Jeffrey 1980). The similarity for the common characters which is taken as key to distinguish between dioecious taxa of *Momordica* has led to widely conflicting taxonomic description of this genus in South and Southeast Asia. An understanding of the taxonomy of the target taxa and their distribution is the basic pre-requisite for undertaking a viable improvement and conservation programme. It is essential to designate taxon's correct scientific name if a specimen is to be linked to the wealth of information that may be known about the taxon to which it belongs.

The taxonomic treatment of the genus *Momordica* is very vast. The most reliable classification of *Momordica* is provided by Chakravarty (1982) in his Fascicles of Cucurbitaceae which is widely adopted in India. Seven species from India including *M. denudata* from Kerala and *M. macrophylla* from the Assam-Manipur belt bordering Myanmar were documented in his classification. Gamble and Fischer (1919) also mentioned occurrence of *M. denudata* in Kerala from "low country Quilon", which might have prompted Chakravarty (1982) to mention its distribution in

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Kerala. He has also described a new variety, namely, *M. charantia* var. *muricata*, based on Rheede's plate in *Hortus Malabaricus* as type. Jeffrey (1980) rules out *M. subangulata* from India for the absence of ridged or longitudinally alate fruits and hence treats this component under *M. dioica*. Kumar and Pandey (2002) also reported the taxonomy and available divergence of the genus *Momordica* in India. However, his work indicates the findings with very close resemblance with the classification of Chakravarty (1982). Recently, the genus was revised in India by Joseph and Antony (2010). Similarly, a detailed technical description and key of the species was postulated by Trimen (1893–1900) in Sri Lanka. Detailed floristic account of the species of *Momordica* was given by several workers (Backer and Bakhuizen van den Brink 1963; Henderson 1974; Keraudren 1975) in different Southeast Asian countries. Again, a detailed taxonomic treatment to the genus was given by De Wilde and Duyfjes (2002) in South and Southeast Asia. As per the findings of their study, *M. cochinchinensis* and *M. subangulata* do not occur in South India, and they have also thoroughly revised the species distribution under the genus *Momordica*. They have proposed a new sub-specific rank in *M. subangulata* which also includes material treated under *M. dioica* of Northeast India. A considerable part of the taxa hitherto treated under *M. cochinchinensis* has been taken out and placed under *M. denticulata*. In Africa, detailed description of the species and keys for their identification was proposed by Oliver (1979).

This genus *Momordica* comprises 45 species which are domesticated in regions of Asia and Africa (Robinson and Decker-Walters 1997). However, only six valid species of *Momordica* have been reported from India. The most widely cultivated species, *M. charantia*, was classified into two botanical varieties based on fruit size, shape, colour and surface texture. They are (a) *M. charantia* var. *charantia* with large fusiform fruits with absence of tapering at both ends and characterized by presence of numerous triangular tubercles giving the appearance of a "crocodile's back" and (b) *M. charantia* var. *muricata* (wild type) with small and round fruits with tubercles, tapering to varying extent at both ends (Chakravarty 1990). These two types are widely cultivated throughout tropical and subtropical regions of India.

Bitter gourd was again classified into three horticultural groups or types by Yang and Walters (1992). They are (a) small-fruited type where fruits are 10–20 cm long, 100–300 g in weight, usually dark green and very bitter; (b) long-fruited type (most commonly grown commercially in China) where fruits are 30–60 cm long, 200–600 g in weight, light green in colour with medium-size protuberances and only slightly bitter; and (c) triangular-type cone-shape fruits which are 9–12 cm long, 300–600 g in weight, light to dark green with prominent tubercles and moderately to strongly bitter. Based on the fruit diameter, Reyes et al. (1994) reclassified Indian and Southeast Asian *M. charantia* botanical varieties into two groups (*M. charantia* var. *minima* Williams & Ng <5 cm and *M. charantia* var. *maxima* Williams & Ng >5 cm).

Among the six species distributed in India, two are monoecious (*M. charantia* and *M. balsamina*), and the rest of the four species (*M. dioica*, *M. sahyadrica*, *M. cochinchinensis* and *M. subangulata*) are dioecious in nature (De Wilde and

Fig. 13.1 Bitter gourd growing under protected condition

Duyfjes 2002; Joseph 2005; Joseph and Antony 2007). However, the most widely cultivated species is *M. charantia* (Fig. 13.1), which is considered as one of the major vegetables in different parts of the world (India, Sri Lanka, the Philippines, Thailand, Malaysia, China, Japan, Australia, Tropical Africa, South America and the Caribbean).

13.2 Morphology of *Momordica charantia* L.

Joseph (2005) described the morphological traits and diversity in *M. charantia* in detail. The plants of *M. charantia* are annual and viny in nature. The vines may attain a length of 2–4 m depending on the genotypes and growing condition. Different genotypes may be scarcely to densely pubescent (tender parts woolly) and predominantly monoecious in behaviour. The leaf blades of *M. charantia* are usually palmately 5–9 lobed, reniform to orbicular or sub-orbicular in outline, 2.5–10 cm, cordate at base, acute or acuminate at apex, lobes ovate or obovate, narrowed at base, margins sinuate to undulate, mucronate, petioles 1.5–5 cm long.

13.3 Reproductive Biology

The primary sex form in *Momordica* is monoecious, and it is found in wild-type genotypes and accessions of bitter gourd. Therefore, staminate and pistillate flowers are borne on separate nodes. Depending on the genotype and growing condition,

flowering and fertilization happens between 35 and 55 days after sowing (Rasco and Castillo 1990; Reyes et al. 1994). Flowering continues about 6 months in the tropics (Reyes et al. 1994). Anthesis typically occurs in the early morning between 3:30 and 7:30 a.m., when flowers are completely open (Miniraj et al. 1993), and pollen viability is lost relatively rapidly (Desai and Musmade 1998). The stigma is usually receptive for 1 day before or after anthesis, and later on they start drying and turn brown (Rasco and Castillo 1990). Male flowers produce abundant quantity of pollen grains which are generally non-sticky and can spread easily with different insect vectors.

13.4 Floral Biology

Flowering behaviour in all cucurbitaceous vegetables including *Momordica* varies with cultivar, climatic conditions and cultural practices (Deshpande et al. 1979). In the monoecious lines, the average ratio of staminate to pistillate flowers in the peak flowering time is typically 50:1 (Rasco and Castillo 1990), but ratios can vary to a great extent based on the kind of genotypes and population (i.e. 9:1 to 48:1) (Dey et al. 2005). Long photoperiods result in the appearance of more staminate flowers, and short days result in increased proportion of female flowers (Huyskens et al. 1992). It is found that nearly 90% of pistillate flowers borne on the first 40 nodes, and majority of them mature at 21st to 30th nodes. Judicious pruning of lower lateral branches stimulates subsequent lateral branch production, which results in increased number of total number of flowers per plant (Rasco and Castillo 1990). Bees are important pollinators of bitter gourd in India (Behera 2004). The predominant bee species in India is *Apis florea*, followed by *A. cerana* and *A. dorsata*. The pistillate flower of bitter gourd consists of an inferior ovary and a three-lobed, wet stigma which is attached to a columnar, hollow style (Pillai et al. 1978). The ovary contains 3 carpels typical of many cucurbits, each with 14 to 18 ovules, surrounded by an ovary wall. However the number of ovules per ovary goes up to 60 with an average of 40. Anatropous ovules are attached to parietal placenta in two irregularly aligned rows in each carpel. Unlike other cucurbits, no more than four ovules can be seen in ovary cross-section. Typically, pollen tubes penetrate papillae tissue within 1 hour of pollination, arriving at the ovary cavities about 6 hours after pollination, and thus fertilization is accomplished within 18 to 24 hours post-pollination (Chang et al. 1999).

13.5 Flowering and Pollination

M. charantia, *M. balsamina* and *M. cymbalaria* are monoecious with male and female flowers borne separately on the same plant, while other remaining species are dioecious with male and female flowers borne in different plants. Yield in bitter

gourd can be enhanced by increasing the number of female flowers or decreasing the number of male flowers which can be achieved by spraying growth hormones to alter the sequence of flowering and sex ratio. Spraying of BA @ 25 ppm or ethephon @ 200 ppm or GA @ 25–100 ppm after six to eight true leaf stage (Yonemori and Fujieda 1985) and 500 ppm ethrel at germination or 100 ppm GA3 in adult plants (Thomas 2008) increases the female flowers. Production of male flowers was significantly reduced with 200–600 ppm of ethrel (Ravindran 1971), and female flower production was increased with B-9 (500–5000 ppm) and CCC (500–2000 ppm) (Ghosh and Basu 1982, 1983). Nearly 2.5 times yield increase in bitter gourd was reported with spraying of 500 ppm CCC in the variety HK-8 (Mangal et al. 1981). Application of boron @ 4 ppm (Verma et al. 1984) and a-NAA @ 100 ppm (Bisaria 1974) was also reported to increase the female flower production. In vitro hormone application during seed germination was much more successful than spraying of field grown plants (Thomas 2008). Soaking of seed at 25–100 ppm NAA, kinetin and ethrel for 24 h and keeping at 5C for 5–15 days before sowing increased the ratio of pistillate flower to staminate flower (Prakash 1976).

13.6 Sex Expression and Modification

All the cucurbitaceous vegetable showed a wide range of sex types and sex expression (Behera et al. 2006). Majority of the cucurbits are predominantly monoecious, but dioecism occurs in pointed gourd (*Trichosanthes dioica*), kakrol (*Momordica dioica*), ivy gourd (*Coccinia indica*) and some feral forms. Wang et al. (1997) found that plants bear hermaphroditic bud primordial that can produce either staminate or pistillate flowers at later stage in *M. charantia*. This process is correlated with RNA and protein synthesis, where soluble protein profiles of hermaphrodite flower buds and staminate and pistillate flowers differ at three early developmental stages (7, 10 and 13 days after initial bud formation) (Wang and Zeng 1998). Predominant 11 and 30 kD proteins are present in pistillate and staminate flowers, respectively, and it is speculated that these proteins may be associated directly with sex expression (Wang and Zeng 1996). Sex expression is also affected by environmental conditions under which *M. charantia* seedlings grow (Wang et al. 1997). Short-day cultivars, when grown under short photoperiods, exhibit rapid development and comparatively high percentage of pistillate flowers. To encourage a high frequency of pistillate flowers, short-day treatments should start at seedling emergence and proceed to sixth-leaf stage (20 days post emergence under growing optimal conditions). Pistillate flower production under short photoperiods is increased by low temperatures (20 °C) and night-time chilling (25 °C day/15 °C night) (Yonemori and Fujieda 1985). Similarly, it is found that optimal conditions for gynoecious *M. charantia* seedling growth are short days and low temperatures (Wang et al. 1997) for expression of consistent gynoecious sex forms. The concentration and relative proportions of endogenous growth regulators and polyamines (e.g. spermine, spermidine, cadaverine and

putrescine) in shoot meristems of bitter gourd change during plant development (Wang and Zeng 1997a). For instance, decrease in the concentration of indoleacetic acid (IAA) and zeatin after anthesis is associated with increased number of pistillate flower (Wang and Zeng 1997b). Cadaverine content is also higher in staminate and pistillate flowers when compared to vegetative tissues (e.g. leaf and stem), suggesting a possible role in sex determination (Wang and Zeng 1997a). It has been hypothesized that the variation in spermidine content is related to the initiation and development of pistillate flowers, while increases in endogenous putrescine concentrations are related to staminate flower initiation (Wang and Zeng 1997a).

Foliar application of growth regulators is very effective in the modification of sex expression (Ghosh and Basu 1982). It was found that foliar application of gibberellic acid (GA_3) at 25–100 mgL^{-1} can dramatically increase percentage of pistillate flowers in bitter gourd, while cycocel (CCC; chlormequat) at concentrations of 50–200 mg L^{-1} promotes staminate flower development (Wang and Zeng 1998). Moreover, the appearance of the first staminate flower is delayed, and pistillate flower initiation is promoted by relatively low concentrations of GA_3 (0.04–4 mg L^{-1}) (Wang and Zeng 1997c). Likewise, foliar application of CCC promotes staminate flower development at 50–200 mg L^{-1} and gynoecy at 500 mg L^{-1}. Foliar application of (2-chloroethyl) phosphonic acid (ethephon), maleic hydrazide (MH), GA_3, naphthaleneacetic acid (NAA), kinetin, IAA, 3-hydroxymethyl oxindole (HMO), morphactin, silver nitrate and boron, when applied at 2- and 4-leaf stage of bitter gourd plants, can dramatically affect sex expression (Prakash 1976). Foliar application of silver nitrate (i.e. 250 mgL^{-1} at the 5-leaf stage or 400 mgL^{-1} at the 3-leaf stage) induces bisexual flower formation, where ovaries and petals are larger than typical pistillate flowers (Iwamoto and Ishida 2005). Likewise, dramatic increases in early pistillate flower appearance can result from foliar application of MH (250 ppm) and ethephon (200 ppm), and staminate flower development can be promoted by application of GA_3 (i.e. 50–75 ppm) (Damodar et al. 2004). Interestingly, foliar treatment of bitter gourd plants with IAA or HMO at 35 mgL^{-1} increases total flower formation, which may be due in part to increased ethylene evolution (Damodar et al. 2004). Regarding such ethylene-dependent sex determination processes, foliar application of ethephon at relatively low concentrations (255 mgL^{-1}) enhances pistillate flowering, while application of moderately high concentrations (100 mgL^{-1}) depresses pistillate flower development. Likewise, although exogenous application of GA_3 (20–40 mgL^{-1}) increases pistillate and staminate flower number, comparatively high concentrations of GA_3 (60 mgL^{-1}) increase only pistillate flower number (Ghosh and Basu 1983). Finally, foliar sprays containing 50 ppm NAA stimulate early and abundant pistillate flower development (Shantappa et al. 2005); boron at 4 ppm enhances pistillate flower production and fruit number and weight (Verma et al. 1984).

13.7 Molecular Marker Studies in Bitter Gourd

Molecular markers are the variations in the genomic DNA sequences of different individuals. Theses variations in the genome arise due to mutation, insertion/deletion (InDel) and variations in the number of tandem repeats (VNTRs). Molecular or DNA-based markers are identified in a population either using restriction enzymes and PCR-based methods or by sequencing. Restriction fragment length polymorphism (RFLP) was the first DNA-based marker used for physical mapping of a gene in adenovirus. After that a number of other DNA markers were developed like randomly amplified polymorphic DNAs (RAPDs) (Williams et al. 1990), amplified fragment length polymorphism (AFLP) (Vos et al. 1995), sequence-characterized amplified regions (SCAR) (Paran and Michelmore 1993), sequence-tagged sites (STS) (Olson et al. 1989), simple sequence repeat (SSR) polymorphisms (Litt and Luty 1989; Weber and May 1989), cleaved amplified polymorphic sequences (CAPSs) (Konieczny and Ausubel 1993), inter-SSR (ISSR) markers (Godwin et al. 1997) and single-nucleotide polymorphism (SNP) (Lander 1996). DNA-based markers have several advantages like uniform distribution in the genome, abundance, reproducibility and amenability to automation. Molecular markers have various applications in crop improvement programmes like DNA fingerprinting, genetic diversity analysis, mapping and tagging of genes/QTLs, marker-assisted selection (MAS), etc.

The first attempt to estimate genetic diversity in bitter melon was done using random amplified polymorphic DNA (RAPD) markers (Dey et al. 2006). A total of 38 genotypes of *M. charantia* were analysed for diversity study. In another experiment of diversity analysis with ISSR markers, high polymorphism was recorded among 38 genotypes of *M. charantia* (Singh et al. 2007). The study indicated the high degree of dissimilarity between the accessions suggesting they are genetically distant to each other (Table 13.1). To further classify the genetic relationships of cultigens studied by Singh et al. (2007), genetic assessment of bitter gourd germplasm was done using AFLP markers (Gaikwad et al. 2008). Paul et al. (2010) analysed the genetic relationship of 12 accessions of bitter gourd using RAPD and SCAR markers. SSR markers have several advantages over other markers like uniform distribution in the genome, PCR based, codominant nature and high degree of polymorphism. Wang et al. (2010) developed and tested 16 microsatellite loci for *M. charantia* for genetic variation and diversity studies. These SSR loci were also found useful for its cross amplification in related species like *Cucurbita pepo* L., *Luffa cylindrica* L., *Lagenaria siceraria* L. and *Cucumis sativus* L. (Wang et al. 2010). Another 25 microsatellite loci were developed from the genome of bitter melon using the Fast Isolation by AFLP of Sequences COntaining Repeats (FIASCO) method (Guo et al. 2012). Among 25, 10 SSR loci were found polymorphic in *M. charantia* and in the related species *M. cochinchinensis* and *Cucurbita pepo*. Ji et al. (2012) developed 11 SSR loci from bitter gourd genome using SSR-enriched fragments. These SSR markers were found highly polymorphic for

Table 13.1 Details of the studies in *Momordica* conducted using different types of molecular markers

Species	No. of accessions/ genotypes	Type of markers	No. of primers	PIC	Marker development	References
M. charantia L.	38	RAPD	29	–	–	Dey et al. (2006)
M. charantia L.	38	AFLP	6	0.20–0.25	–	Gaikwad et al. (2008)
M. charantia L.	12	RAPD and SCAR	23	–	–	Paul et al. (2010)
M. charantia L.	38	ISSR	15	–	–	Singh et al. (2007)
M. charantia L.	36	SSR	16	–	FIASCO method	Wang et al. (2010)
M. charantia L.	40	SSR	10	–	FIASCO method	Guo et al. (2012)
M. charantia L.	114	SSR	50	–	Seed transcriptome	Dhillon et al. (2016)
M. charantia L.	55	SSR	11	0.57	SSR-enriched fragments or libraries	Ji et al. (2012)
M. charantia L.	54	SSR	51	0.13–0.77	SSR-enriched fragments or libraries	Saxena et al. (2015)
M. charantia L.	211	SSR	21	0.54	Whole genome	Cui et al. (2017)

studying genetic diversity in *M. charantia* and related species. Further, 151 SSR loci were identified, and 51 were tested for polymorphism in bitter gourd and related species (Saxena et al. 2015). Using transcriptome approach, Dhillon et al. (2016) designed 50 SSR loci in bitter gourd and used them for the characterization of 114 bitter gourd accessions widely grown in Asia. Recently, using genome-wide analysis for simple sequence repeats in bitter gourd genome, a large number of SSR motifs have been identified and characterized for SSR content and motif lengths (Cui et al. 2017). Moreover, 138,727 unique in silico SSR primer pairs were designed for bitter gourd (Cui et al. 2017). Among these, 21 SSR markers were used for diversity analysis of 211 bitter gourd lines collected from all over the world. These markers were able to classify 211 bitter gourd lines in 3 different populations with clear geographic separation (Cui et al. 2017).

Fig. 13.2 A gynoecious line of bitter gourd

Identification of molecular marker(s) linked with the trait of interest has enormous potential for the crop improvement through molecular breeding. Kole et al. (2012) used 108 AFLP markers to generate first genetic linkage map in bitter melon. One RAPD marker (OPZ 13, 700 bp) linked to (*gy-1*) gene at 22 cM distance has been identified (Mishra et al. 2014). Identification of the markers linked with gynoecy is of great significance to ascertain purity of gynoecious lines (Fig. 13.2) at an early stage of development for a cost-effective hybrid seed production. Wang and Xiang (2013) generated a linkage map in bitter gourd in F2 progenies derived from inbred lines gynoecia Z-1-4 and 189-4-1. A total of 194 polymorphic loci with 26 EST-SSR loci, 28 SSR loci, 124 AFLP loci and 16 SRAP loci were assigned in genetic linkage map with 12 linkage groups. In addition a total of 43 putative QTLs loci located on 9 chromosomes were identified. The identified QTLs were linked with 13 horticulture traits including flower-related traits like female flower ratios (FFR) and first female flower node (FFFN). Gaikwad et al. (2014) reported identification of one ISSR marker (AC)8T associated with the gynoecious trait in bitter gourd.

Using a RAD-seq (restriction-associated DNA tag sequencing) analysis, Matsumura et al. (2014) have constructed a linkage map using 552 codominant

markers in F_2 population derived from a cross between a gynoecious line (OHB61-5) and a monoecious line. In this study five SNP loci (bi-allelic tags) designated as GTFL-1, GTFL-2, GTFL-3, GTFL-11 and GTFL-13 genetically linked to putative gynoecious locus (*Mcgy*) were identified. GTFL-1, the closest SNP marker to *Mcgy*, was converted to an invader assay marker which can be used for MAS of gynoecious lines. Rao et al. (2018) employed genotyping-by-sequencing (GBS) technology to discover SNPs in bitter gourd. A population of 90 F_2 segregated and 65 $F_{2:3}$ families (individual plants of F_2 selfed) progenies derived from a cross of DBGy-201 (PVGy-201) and Pusa Do Mousami (PDM) was used for GBS. A total of 2013 high-quality SNP markers was used to generate 20 linkage groups (LGs) spanning a cumulative distance of 2329.2 cM. The gynoecious (*gy-1*) gene was mapped on LG-12 and flanked by TP_54865 and TP_54890 markers with a distance of 3.04 cM. A total of 22 QTLs related to 3 traits, sex ratio, node and days to first pistillate flower appearance, were mapped across 20 LGs.

13.8 Genomic Studies in Bitter Gourd

The draft genome sequence of bitter gourd was analysed through Illumina next-generation sequencing and de novo assembly (Urasak et al. 2016). A monoecious bitter gourd inbred line, OHB3-1, was used for sequencing. Sequencing libraries, i.e. paired-end (PE) and mate-pair (MP; with 2, 4, 6 and 8 kbp inserts), were generated from genomic DNA and sequenced using the Illumina MiSeq or HiSeq2500 DNA sequencer (Urasak et al. 2016). The final assembly had scaffolds of 285.5 Mb in length which matches to 84% of the estimated genome size of bitter gourd (339 Mb) (Urasak et al. 2016). Using ab initio prediction, total 45,859 protein-coding gene loci were identified from the OHB3-1 scaffold sequence. Comparative analysis of assembled genome sequences of bitter gourd with known genome sequences of other Cucurbitaceae species showed bitter gourd is near to watermelon (*Citrullus lanatus*) compared to cucumber (*Cucumis sativus*) or melon (*C. melo*). Moreover, using restriction site-associated DNA sequencing (RAD-seq) analysis, a linkage map consisting of 11 linkage groups was generated. Further, synteny mapping analysis of bitter gourd sequences with other cucurbits genomes identified two gene classes. Putative genes encoding trypsin inhibitor-like proteins were found repeatedly in the bitter gourd genome than other cucurbits genomes. Similarly, ribosomal inactivating protein (RIP) genes were recorded in multiple copies in bitter gourd scaffold sequences than other cucurbits genomes (Urasak et al. 2016). To identify the sex determination governing genes in bitter gourd, BLAST analysis was performed with melon and cucumber. Gene *CmAcs11* codes for aminocyclopropane-1-carboxylic acid (*ACC*) *ACC synthase* which governs female flower determination in melon. BLAST analysis showed that the MOMC3_649 in bitter gourd as an ortholog of *CmAcs11.CmAcs-7* gene was also reported to regulate unisexual flower

development in melon. Similarly, *CmWip1* gene encoding the zinc-finger domain protein was reported in melon for male flower differentiation. Two proteins (MOMC46_189, MOMC518_1) similar to *CmAcs-7* were found in bitter gourd through BLAST searches. Comparative analysis to identify unique genes in the bitter gourd genome showed MOMC1_984 corresponding to *Momordica charantia* insulin receptor (IR)-binding protein (mcIRBP). mcIRBP was obtained from bitter gourd seeds and reported as a novel IRBP which regulates blood glucose levels in mice suggesting its relevance to cure diabetes (Lo et al. 2014). The draft genome sequence of bitter gourd is a source for identification and characterization of genes for important agronomic traits, marker development to assist MAS programme and evolutionary studies.

13.9 Transcriptomic Studies in Bitter Gourd

Transcriptomics is the study of an organism transcriptome – the whole set of RNA transcripts expressed by the genome in various tissues, conditions and time. Study of transcriptome gives a glimpse of differentially expressed transcripts under specific spatiotemporal conditions or in different treatments in a particular cell type. Transcriptome analysis is done using high-throughput methods like microarray, serial analysis of gene expression (SAGE) and RNA sequencing (RNA-seq). RNA-seq, the sequencing of a transcriptome using NGS, has become the method of choice for gene expression analysis compared to other techniques like microarrays and serial analysis of gene expression (SAGE). RNA-seq provides complete transcriptome details of a sample under study along with rare transcripts due to great sequencing depth (Strickler et al. 2012). Transcriptome analysis has emerged as a powerful tool to obtain transcript/gene sequences, characterize genes, study gene expression, identify transcript variants and develop molecular markers for crop improvement programme especially in less researched species (Strickler et al. 2012). The first report on transcriptome sequencing in bitter gourd came in 2010, to study the functional genomics of eleostearic acid synthesis in bitter melon seeds (Yang et al. 2010) (Table 13.2). Deep sequencing using 454 technology of non-normalized and normalized cDNAs from developing bitter melon seeds was done to identify transcripts for the vast majority of known genes involved in fatty acid and triacylglycerol biosynthesis. Approximately 14,000 unique gene transcripts have been identified from normalized and non-normalized cDNA samples along with transcripts for genes encoding enzymes of lipid biosynthesis and metabolism. Further the transcriptome analysis has identified a divergent class of *FAD2* gene that was demonstrated to have Δ12-oleic acid desaturase activity. This data is a useful resource for the study of unusual fatty acid metabolism in plants and for the engineering of conjugated fatty acid production in oilseed crops. Another study on de novo transcriptome sequencing was to develop functional markers for gynoecy (Shukla et al. 2015) (Table 13.2). Samples from root, flower buds, stem and leaf of

Table 13.2 Description of transcriptomic and genomic studies published in bitter gourd (*Momordica charantia* L.)

S.No.	Plant organ	Objective of study	Sequencing platform	Raw reads (M)	Final assembly	Marker discovery	NCBI accession	References
(i)	Leaf	Draft genome sequence	Illumina sequencing and de novo assembly	37 Gb 285.5 Mb	Scaffolds of 285.5 Mb	1507 marker loci	DRA004548 BDCS01000001–BDCS01001052 (1052 entries)	Urasak et al. (2016)
(ii)	Root, flower, buds, stem and leaf	De novo transcriptome sequencing	Illumina next-generation sequencer	31.83 millions monoecious 29.56 millions gynoecious	130,000 unigenes	28,964 SSRs	SRX206126GANF00000000 and GANG00000000	Shukla et al. (2015)
(iii)	Female and hermaphrodite flower buds	Whole transcriptome sequencing	Roche 454 parallel pyrosequencing	858 Mbp	6459 unigenes	115 SSRs		Behera et al. (2016)
(iv)		Whole genome sequencing				138,727 SSRs		Cui et al. (2017)
(v)	Seeds	Transcriptome profile	Deep sequencing using 454 technology	0.66 millions	345 contigs		SRX030203, SRX030204	Yang et al. (2010)

gynoecious line (Gy323) and a monoecious line (DRAR1) were taken to see differentially expressed transcripts involved in the floral differentiation. A total of 65,540 transcripts for gynoecious line (Gy323) and 61,490 transcripts for a monoecious line (DRAR1) have been identified. Transcripts for 80 WRKY transcription factors involved in various biotic and abiotic stresses and 56 ARF genes related to auxin-regulated gene expression and development have been reported. To see the genes associated with sex determination in bitter gourd, whole transcriptome sequencing of female and hermaphrodite flower buds of bitter gourd was performed using Roche 454 parallel pyrosequencing technology (Behera et al. 2016) (Table 13.2). More than 2,000,000 ESTs have been identified from the female and hermaphrodite flower buds of the gynoecious bitter gourd inbred line, DBGY-201, treated with silver thiosulphate. These ESTs were clustered and assembled into 6459 unigenes, which were further aligned with predicted genes. Based on comparative digital gene expression, a total of 477 annotated unigenes have been found to have significant differential expression between the female and hermaphrodite flower buds of line DBGY-201. About 237 unigenes were down-regulated and 59 were up-regulated in hermaphrodite flower buds. A total of 115 putative SSRs motifs were reported from 108 unigenes.

13.10 Future Strategies

Only a limited number of traits have been investigated in this crop till date. There is a need to investigate most of the yield-related and biotic stress- and abiotic stress-related traits in this crop to complement the on-going improvement programme. Rapid generation advancement under protected and in vitro condition needs to be optimized using the genotypes under different regions. The number of molecular markers reported is very limited, and there is a need to develop molecular markers uniformly distributed throughout the genome for undertaking fine mapping of important traits. The sex expression is a very important trait influencing the final yield of this crop; therefore, there is a need to understand these traits and underlying physiological and molecular mechanism. Besides, this crop is prone to several biotic and abiotic stresses, and till date no reports are available to characterize the stress tolerance genges and identification of closely linked molecular markers associated with resistance or tolerance to these traits.

References

Behera TK (2004) Heterosis in bitter gourd. In: Singh PK, Dasgupta SK, Tripathi SK (eds) Hybrid vegetable development. The Haworth Press, New York, pp 217–221

Backer CA, Bakhuizen van den Brink RC (1963) Flora of Java, vol 1. Rijks Herbarium, Leiden, p 99

Behera TK, Dey SS, Sirohi PS (2006) DBGy-201 and DBGy-202: two gynoecious lines in bitter gourd (Momordica charantia L.) isolated from indigenous source. Indian J Genet 66:61–62

Behera TK, Rao AR, Amarnath R, Kumar RR (2016) Comparative transcriptome analysis of female and hermaphrodite flower buds in bitter gourd (Momordica charantia L.) by RNA sequencing. J Hortic Sci Biotechnol 91(3):250–257

Bisaria AK (1974) The effect of foliar sprays of alpha naphthalene acetic acid on sex expression in Momordica charantia L. Sci Cult 40:70–80

Chakravarty HL (1982) Cucurbitaceae. Fascicles of flora of India 2. Botanical Survey of India, Howrah, p 94

Chakravarty HL (1990) Cucurbits of India and their role in the development of vegetable crops. In: Bates DM, Robinson RW, Jeffrey C (eds) Biology and utilization of Cucurbitaceae. Cornell University Press, Ithaca, pp 325–334

Chang YM, Liou PC, Hsiao CH, Shii CT (1999) Observation of fruit anatomy and development of bitter gourd. I. Fruit anatomy and fertilization of bitter gourd. J Agric Res China 48:23–31

Clarke CB (1879) Cucurbitaceae. In: Hooker JD (ed) Flora of British India. Reeve, London, pp 604–635. Cogniaux A (1881) Cucurbitacees. In: de Candolle ALPP College, Tiruchirapally, p 648

Cui J, Cheng J, Nong D, Peng J, Hu Y, He W et al (2017) Genome-wide analysis of simple sequence repeats in bitter gourd (Momordica charantia). Front Plant Sci 8:1103

Damodar VP, Ghode PB, Nawghare PD, Sontakke MB, Pawar PM (2004) Studies on after-effects of foliar application of PGR on sex-expression and sex-ratio in bitter gourd (Momordica charantia L.) cv. Hirkani. Karnataka J Hortic 1:86–88

De Wilde WJJO, Duyfjes BEE (2002) Synopsis of *Momordica* (Cucurbitaceae) in South East Asia and Malaysia. Bot Zhurn 57:132–148

Desai UT, Musmade AM (1998) Pumpkins, squashes and gourds. In: Salunkhe DK, Kadam SS (eds) Handbook of vegetable science and technology: production, composition, storage and processing. Marcel Dekker, New York, pp 273–298

Deshpande AA, Venkatasubbaiah K, Bankapur VM, Nalawadi UG (1979) Studies on floral biology of bitter gourd (Momordica charantia L.). Mysore J Agric Sci 13:156–159

Dey SS, Behera TK, Pal A, Munshi AD (2005) Correlation and path coefficient analysis in bitter gourd (Momordica charantia L.). Veg Sci 32:173–176

Dey SS, Singh AK, Chandel D, Behera TK (2006) Genetic diversity of bitter gourd (Momordica charantia L.) genotypes revealed by RAPD markers and agronomic traits. Sci Hortic 109(1):21–28

Dhillon NP, Sanguansil S, Schafleitner R, Wang YW, McCreight JD (2016) Diversity among a wide Asian collection of bitter gourd landraces and their genetic relationships with commercial hybrid cultivars. J Am Soc Hortic Sci 141(5):475–484

Gaikwad AB, Behera TK, Singh AK, Chandel D, Karihaloo JL, Staub JE (2008) Amplified fragment length polymorphism analysis provides strategies for improvement of bitter gourd (Momordica charantia L.). HortScience 43(1):127–133

Gaikwad AB, Saxena S, Behera TK, Archak S, Meshram SU (2014) Molecular marker to identify gynoecious lines in bitter gourd. Indian J Hortic 71(1):142–144

Gamble JS, Fischer CEC (1919) Flora of the presidency of Madras, vol 1–3, repr. edn. Botanical Survey of India, Calcutta. Genomics 20(2):176–183, 1957

Ghosh S, Basu PS (1982) Effect of some growth regulators on sex expression of *Momordica charantia*. Sci Hortic 17:107–112

Ghosh S, Basu PS (1983) Hormonal regulation of sex expression in *Momordica charantia*. Physiol Plant 57:301–305

Godwin ID, Aitken EA, Smith LW (1997) Application of inter simple sequence repeat (ISSR) markers to plant genetics. Electrophoresis 18(9):1524–1528

Guo DL, Zhang JP, Xue YM, Hou XG (2012) Isolation and characterization of 10 SSR markers of Momordica charantia (*Cucurbitaceae*). Am J Bot 99(5):e182–e183

Henderson MR (1974) Malayan wild flowers. Dicotyledons. The Malayan Nature Society, Kualalampur, p 156

Hooker J (1871) Momordica L. In: Oliver D (ed) Flora of tropical Africa, vol 2. Reeve & Co, London

Huyskens S, Mendlinger S, Benzioni A, Ventura M (1992) Optimization of agro-techniques for cultivating Momordica charantia (karela). J Hortic Sci 67:259–264

Iwamoto E, Ishida T (2005) Bisexual flower induction by the application of silver nitrate in gynoecious balsam pear (Momordica chorantio L.). Hort Res (Japan) 4:391–395

Jeffrey C (1980) A review of the Cucurbitaceae. Bot J Linn Soc 81:233–247

Ji Y, Luo Y, Hou B, Wang W, Zhao J, Yang L et al (2012) Development of polymorphic microsatellite loci in *Momordica charantia* (*Cucurbitaceae*) and their transferability to other cucurbit species. Sci Hortic 140:115–118

Joseph JK (2005) Studies on ecogeography and genetic diversity of the genus Momordica L. in India. Dissertation, Mahatma Gandhi University, Kottayam

Joseph JK, Antony VT (2007) Momordica sahyadrica sp. nov. (Cucurbitaceae), an endemic species of Western Ghats of India. Nord J Bot 24:539–542

Joseph JK, Antony VT (2010) A taxonomic revision of the genus Momordica L. (Cucurbitaceae) in India. Indian J Plant Genet Resour 23:172–184

Keraudren AM (1975) Flore du Combodge du Laos et du Viet-Nam. 15. Cucurbitaceae, pp 36–44

Kole C, Olukolu BA, Kole P, Rao VK, Bajpai A, Backiyarani S (2012) The First Genetic Map and Positions of Major Fruit Trait Loci of Bitter Melon (Momordica charantia). Journal of Plant Science and Molecular Breeding 1:1. https://dx.doi.org/10.7243/2050-2389-1-1

Konieczny A, Ausubel FM (1993) A procedure for mapping Arabidopsis mutations using co-dominant ecotype-specific PCR-based markers. Plant J 4(2):403–410

Kumar N, Pandey AK (2002) Genus Momordica in India: diversity and conservation. In: Das AP (ed) Perspectives of plant biodiversity. Bishen Singh Mahendra Pal Singh Publishers, Dehradun, pp 35–43

Lander ES (1996) The new genomics: global views of biology. Science 274(5287):536–539

Litt M, Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44(3):397

Lo HY, Ho TY, Li CC, Chen JC, Liu JJ, Hsiang CY (2014) A novel insulin receptor-binding protein from Momordica charantia enhances glucose uptake and glucose clearance in vitro and in vivo through triggering insulin receptor signaling pathway. J Agric Food Chem 62(36):8952–8961

Mangal JL, Pandita ML, Singh GR (1981) Effect of various chemicals on growth, flowering and yield of bitter gourd. Indian J Agric Sci 15:185–188

Matsumura H, Miyagi N, Taniai N, Fukushima M, Tarora K, Shudo A, Urasaki N (2014) Mapping of the gynoecy in bitter gourd (Momordica charantia) using RAD-seq analysis. PLoS One 9(1):e87138

Miniraj N, Prasanna KP, Peter KV (1993) Bitter gourd Momordica spp. In: Kalloo G, Bergh BO (eds) Genetic improvement of vegetable plants. Pergamon Press, Oxford, pp 239–246

Mishra S, Behera TK, Munshi AD, Gaikwad K, Mohapatra T (2014) Identification of RAPD marker associated with gynoecious trait ('gy-1'gene) in bitter gourd ('Momordica charantia'L.). Aust J Crop Sci 8(5):706

Oliver D (1979) Flora of tropical Africa. Buckinghamshire AJ Reprint Agency, pp 417–418

Olson M, Hood L, Cantor C, Botstein D (1989) A common language for physical mapping of the human genome. Science 245(4925):1434–1435

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85(8):985–993

Paul A, Bandyopadhyay S, Acharyya P, Raychaudhuri SS (2010) Studies on genetic diversity of twelve accessions of Momordica charantia L. using morphological, RAPD and SCAR markers. Asian J Plant Sci 9(8):471–478

Pillai OA, Irulappan I, Jayapal R (1978) Studies on the floral biology of bitter gourd (Momordica charantia L.) varieties. Madras Agric J 65:168–171

Prakash G (1976) Effect of plant growth substances and vernalization on sex expression in Momordica charantia L. Indian J Exp Biol 14:360–362

Rao G, Behera TK, Gaikwad A, Munshi AD, Singh G, Krishnan B (2018) Mapping and QTL analysis of gynoecy and earliness in bitter gourd (Momordica charantia L.) using genotyping-by-sequencing (GBS) technology. Front Plant Sci 9:1555

Rasco AO, Castillo PS (1990) Flowering patterns and vine pruning effects in bittergourd (Momordica charantia L.) varieties 'Sta. Rita' and 'Makiling'. Philipp Agric 73:3–4

Ravindran DN (1971) Effect of photopeiod and growth substances on sex expression in snake gourd (Trichosanthes anguina L.). South Indian Hort. 15:1–21

Reyes MEC, Gildemacher BH, Jansen GJ (1994) Momordica L. In: Siemonsma JS, Piluek K (eds) Plant resources of South-East Asia: vegetables. Pudoc Scientific Publishers, Wageningen, pp 206–210

Robinson RW, Decker-Walters DS (1997) Cucurbits. CABInternational, Wallingford/Oxford

Saxena S, Singh A, Archak S, Behera TK, John JK, Meshram SU, Gaikwad AB (2015) Development of novel simple sequence repeat markers in bitter gourd (*Momordica charantia L.)* through enriched genomic libraries and their utilization in analysis of genetic diversity and cross-species transferability. Appl Biochem Biotechnol 175(1):93–118

Shantappa T, Gouda MS, Reddy BS, Adiga JD, Kukanoor L (2005) Effect of growth regulators and stages of spray on growth and seed yield in bitter gourd (Momordica charantia L.). Karnataka J Hortic 1:55–62

Shukla A, Singh VK, Bharadwaj DR, Kumar R, Rai A, Rai AK et al (2015) De novo assembly of bitter gourd transcriptomes: gene expression and sequence variations in gynoecious and monoecious lines. PLoS One 10(6):e0128331

Singh AK, Behera TK, Chandel D, Sharma P, Singh NK (2007) Assessing genetic relationships among bitter gourd (Momordica charantia L.) accessions using inter-simple sequence repeat (ISSR) markers. J Hortic Sci Biotechnol 82(2):217–222

Strickler SR, Bombarely A, Mueller LA (2012) Designing a transcriptome next-generation sequencing project for a nonmodel plant species1. Am J Bot 99(2):257–266

Thomas TD (2008) The effect of in vivo and in vitro applications of ethrel and GA3 on sex expression in bitter melon (Momordica charantia L.). Euphytica 164:317–323

Thwaites GHK (1864) Enumeratio plantarum zeylaniae (an enumeration of Ceylon plants). Dulau, London, p 126

Trimen H (1893–1900) A hand book of flora of Ceylon, vol 1–5. Dulau& Co, London, pp 248–250

Urasak N, Takagi H, Natsume S, Uemura A, Taniai N, Miyagi N et al (2016) Draft genome sequence of bitter gourd (*Momordica charantia*), a vegetable and medicinal plant in tropical and subtropical regions. DNA Res 24(1):51–58

Verma VK, Sirohi PS, Choudhry B (1984) Chemical sex modification and its effect on yield in bitter gourd (Momordica charantia L.). Progress Hortic 16:52–54

Vos P, Hogers R, Bleeker M, Reijans M, Lee TVD, Hornes M et al (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23(21):4407–4414

Wang Z, Xiang C (2013) Genetic mapping of QTLs for horticulture traits in a F 2–3 population of bitter gourd (Momordica charantia L.). Euphytica 193(2):235–250

Wang QM, Zeng GW (1996) Effects of gibberellic acid and cycocel on sex expression of *Momordica charantia*. J Zhejiang Agric Univ 22:541–546

Wang QM, Zeng GW (1997a) The effect of phytohormones and polyamines on sexual differentiation of Momordica charantia. Acta Hortic Sin 24:48–52

Wang QM, Zeng GW (1997b) Hormonal regulation of sex differentiation on Momordica charantia L. J Zhejiang Agric Univ 23:551–556

Wang QM, Zeng GW (1997c) Morphological and histochemical study on sex differentiation on Momordica charantia. J Zhejiang Agric Univ 23:149–153

Wang QM, Zeng GW, Jiang YT (1997) Effects of temperature and photoperiod on sex expression of Momordica charantia. China Veg 1:1–4

Wang QM, Zeng CW (1998) Study of specific protein on sex differentiation of Mom ojydica charantia. Arta Botanica Sinica 40:241–246
Wang SZ, Pan L, Hu K, Chen CY, Ding Y (2010) Development and characterization of polymorphic microsatellite markers in Momordica charantia (Cucurbitaceae). Am J Bot 97(8):e75–e78
Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44(3):388
Wight R, Arnott GAW (1841) Prodromus florae peninsulae, India orientalis, p 348
Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18(22):6531–6535
Yang SL, Walters TW (1992) Ethnobotany and the economic role of the Cucurbitaceae of China. Econ Bot 46:349–367
Yang P, Li X, Shipp MJ, Shockey JM, Cahoon EB (2010) Mining the bitter melon (Momordica charantia L.) seed transcriptome by 454 analysis of non-normalized and normalized cDNA populations for conjugated fatty acid metabolism-related genes. BMC Plant Biol 10(1):250
Yonemori S, Fujieda K (1985) Sex expression in Momordica charantia L. Sci Bull College Agric Univ Ryukyus, Okinawa 32:183–187

Chapter 14
Principles and Techniques for Rapid Improvement of Muskmelon for Yield, Fruit Quality and Resistance to Biotic Stresses

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14.1 Introduction

Muskmelon (*Cucumis melo* L.) is an important dessert fruit with enormous diversity which is a product of consumer preferences from different countries, ecologies and cultures (Tomason et al. 2013) with an estimated annual yield of about 32 million tons worldwide (http://faostat3.fao.org). It is highly relished because of its attractive fruit with unique aromatic musky flavour and sweet taste and considered as a healthy food (Munshi and Choudhary 2014) because it has low calories, sodium and fat and is rich in minerals (K), vit. C and vit. A (orange fleshed) leading to its increasing consumption worldwide. One cup (236 g) serving of muskmelon fruit provides the recommended daily dietary allowance (RDA) for vit. A (160%) and vit. C (130%) for a normal healthy person. Bio-accessibility comparison on dry weight basis showed nearly similar results for carrots (12 μg β-carotene/g) and orange-fleshed melon (8 μg β-carotene/g) (Fleshman et al. 2011). *Cucumis melo* is considered as one of the most diverse and highly polymorphic species in Cucurbitaceae family which comprises a large number of botanical varieties or horticultural groupings which may be a consequence of higher genetic diversity. There is tremendous variation in muskmelon fruit traits such as size, shape, colour, taste, texture and nutritional composition. Based on the fruit's traits and uses, the classification of *Cucumis melo* into six horticultural groups, *cantalupensis* (cantaloupe and muskmelon), *inodorous* (winter melon), *flexous* (long melon), *conomon* (pickling melon), *dudaim* (pomegranate melon) and *momordica* (snapmelon), is widely accepted.

Commercial melon varieties, with sweet, non-bitter and low-acidic fruits, carry three genes (*suc/suc*, *so/so*, *bif/bif*), which control high-quality fruit traits, in recessive

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form (Burger et al. 2002, 2003). Higher levels of resistance to diseases and pests are found in wild relatives or landraces of other cultivated species. Therefore, any intraspecific crosses, using traditional breeding methods, between melon landraces and commercial melon cultivars will produce hybrid fruit with low-quality characteristics, because of the effect of dominant genes controlling low-sweetness, high-acid and high-bitterness levels in the landraces of melon fruits. Consequently, in order to obtain a commercial melon product, it will take a long time to go through the backcrossing route. In order to increase the rate and diversity of new traits in melon, it would be advantageous to introduce new genes needed to enhance both melon productivity and melon fruit quality. Applications of biotechnological interventions such as genomic-assisted breeding and plant transformation techniques to introduce new or foreign genes into commercial varieties for improvement of specific traits especially disease resistance and fruit quality traits have been discussed here to gain an insight about different biotechnological tools for the rapid improvement of muskmelon.

14.2 Origin and Evolution

Melon was earlier considered to be originated in Africa; as the wild species of *Cucumis* occur in Africa, it was likely that it originated in that continent more specially in the eastern region of south Sahara desert. Whitaker and Davis (1962) and Whitaker (1979) thought that Central Asia comprising some parts of Southern Russia, Iran, Afghanistan and North west India may be regarded as a secondary centre of muskmelon. Recent studies support Asian origin (Sebastian et al. 2010) of *Cucumis* including both cucumber and muskmelon, and a recent study established that Indian and African germplasm of melon are genetically distinct and supported independent domestication events (Gonzalo et al. 2019).

14.3 Horticultural Groupings of Melon

This grouping is based on fruit characteristics (Fig. 14.1) and uses, not botanical varieties based on phylogeny (Munger and Robinson 1991). These cultivar-groups are:

1. Cantalupensis group (cantaloupe and muskmelon): The main characteristic of this group is that its fruits get separated/detached from the peduncle at maturity. Fruits are generally medium sized with netted, warty or scaly surface, flesh usually orange but sometimes green and also aromatic or musky flavour. Its sex form is generally andromonoecious.
2. Inodorous group (winter melon): Fruits of this group do not separate/detach from the peduncle when they get mature. Fruits are generally larger later in maturity and longer keeping than those of the cantalupensis group. Its rind surface is smooth or wrinkled but not netted. Its flesh is typically white or green and does not have a musky odour. Its sex form is generally andromonoecious.

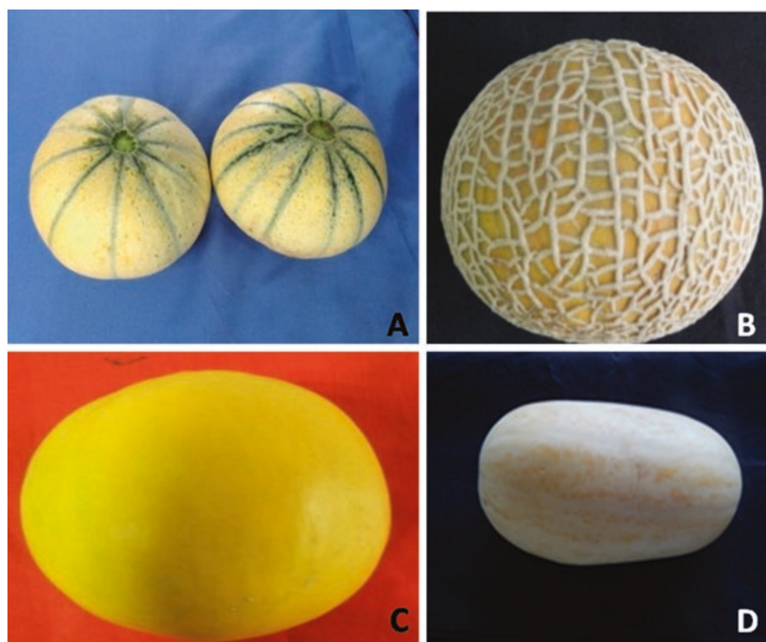

Fig. 14.1 Fruit characteristics of different cultivar-groups of melon. (**a**) Cantalupensis(non netted) group. (**b**) Cantalupensis(netted) group. (**c**) Inodorous group. (**d**) Momordica group

3. Flexuous group (snake melon): Fruits are used immature unlike other groups of melon which is similar to cucumber. Fruits are very long, slender and often ribbed. Its sex form is monoecious in nature.
4. Conomon group (pickling melon): Fruits are small with smooth, tender skin, flesh white, early maturity and usually little sweetness or odour. They are not often pickled and are also eaten fresh or cooked. Its sex form is generally andromonoecious.
5. Dudaim group (pomegranate melon): It includes members of previous members of Chito group (mango melon). It has small round to oval fruits with white flesh and thin rind.
6. Momordica group (snap melon): Fruits are generally ovoid to cylindrical in shape and its flesh is white or pale orange, low in sugar content, mealy and insipid or rather sour tasting. Fruit surface gets cracked or bursts as maturity approaches. Its sex form is generally monoecious in nature.

14.4 Genetic Diversity for Fruit Traits

Melon is highly polymorphic for different fruit traits, providing seemingly endless possibilities for genetic improvement through introgression and recombination. Fruit quality is determined mainly by appearance of fruits (size, shape, rind colour, netting, etc.),

flesh colour (white, green, orange), juiciness, flavour, acidity and taste, and a major component of taste is the content of sugar, particularly sucrose. Muskmelon shows enormous variation in fruit traits such as size, shape, colour, taste, texture and nutrient composition. Improvement in yield and quality is normally achieved by selecting genotypes with desirable character combinations existing in the nature or by hybridization. Large genetic diversity for various fruit traits in melon germplasm (Choudhary et al. 2011; Reddy et al. 2005; Dhillon et al. 2012; Pitrat 2016), and characterization of germplasm from different horticultural groups into various classes based on fruits quality and its nutritional compositions (Bhimappa et al. 2018, 2019) have been reported.

14.5 Breeding Objectives and Commercial Varieties

High total yields realizable through more number of medium-sized fruits rather large fruits are more desirable for packing, transport as well as consumer preference. Earliness in terms of total early yield, fruit quality in terms of T.S.S. and flesh thickness are important for this dessert fruit crop. Attractive fruit shape having small seed cavity thick salmon orange flesh, pleasant aroma and netted and thick skin are also major considerations. Round fruit with orange juicy, thick flesh and rough, tough, netted skin will be an attraction in the market and also help in long-distance transportation. Shelf-life of fruits is an important trait, and non-climacteric fruit ripening leads to better shelf-life. Resistance to common diseases like *Fusarium* wilt, powdery mildew, downy mildew and viruses is also important for melon improvement. Some of the popular commercial varieties of muskmelon developed by different institutions in India are Pusa Madhuras (IARI, New Delhi), Pusa Madhurima (IARI, New Delhi), Pusa Sarda (IARI, New Delhi)(Choudhary et al. 2019b, Hara Madhu (PAU, Ludhiana), Punjab Sunehri (PAU, Ludhiana), Punjab Hybrid (F_1 hybrid, PAU, Ludhiana), Punjab Rasila (PAU, Ludhiana), Arka Rajhans (IIHK, Bangalore), Arka Jeet (IIHR, Bangalore) and Kashi Madhu (IIVR, Varanasi).

14.6 Molecular Mapping of Important Horticultural Traits

Genetic maps in melon were constructed with a wide range of dominant (RAPD and AFLP) and codominant (RFLP, SSR and SNP) markers employing F_2 and backcross populations (Liou et al. 1998; Danin-Poleg et al. 2002; Silberstein et al. 2003). These maps were unsaturated and define the position of only relatively few horticulturally important traits. Later, genetic maps were constructed using recombinant inbred lines (RILs) (Perin et al. 2002a, b; Zalapa et al. 2007) and doubled haploid lines (Monforte et al. 2004) for the analysis of economically important qualitatively and quantitatively inherited horticultural traits. For instance, a 181-point (114 RAPD, 35 SSR and 32 AFLP) genetic map constructed by Zalapa et al. (2007) using RIL derived from Group Cantalupensis US Western Shipping germplasm spanned 1032 cM with a mean marker interval of 5.7 cM on 15 linkage groups. Although this map was useful for

identifying many yield and quality QTL (Zalapa et al. 2007; Paris et al. 2008), it must, along with other currently constituted maps, be considered relatively unsaturated.

14.7 Molecular Breeding for Fruit Quality Improvement

Melon (*Cucumis melo* L.) is an interesting model for fruit ripening studies due to the existence of both climacteric and non-climacteric genotypes within the species (Ezura and Owino 2008), and genetic and genomic tools developed in recent years such as doubled haploid lines (DHLs) (Gonzalo et al. 2011), near isogenic lines (NILs) (Vegas et al. 2013), saturated genetic maps (Diaz et al. 2011, 2015), reverse genetic platforms (Gonzalez et al. 2011) and whole genome sequence (Garcia-Mas et al. 2012) make possible the comprehensive studies related to the complex genetic process. The varieties from *cantalupensis* and *reticulatus* group showed climacteric ripening and short shelf-life, whereas *inodorus* varieties are non-climacteric and show long shelf-life (Saladie et al. 2015). *Cantalupensis* melons were studied for fruit ripening, and the role of ethylene was demonstrated by reducing its biosynthesis in antisense CmACO1 "Vedrantais" plants (Ayub et al. 1996; Pech et al. 2008). It was confirmed that fruit softening, aroma profile, fruit abscission, rind colour, etc. were ethylene-dependent processes (Nishiyama et al. 2007), whereas flesh colour, accumulation of sugars and carotenoids and loss of acidity are independent of ethylene (Pech et al. 2008), but detailed studies for ripening processes in non-climacteric types of melon were very few (Vegas et al. 2013). Two QTLs, ETHQB3.5 and ETHQV6.3, involved in the regulation of climacteric ripening were identified (Moreno et al. 2008; Vegas et al. 2013), but these QTLs were different from the earlier study of Perin et al. (2002), suggesting that the genetic basis of fruit ripening in melon is complex and variety-specific. The climacteric response QTL ETHQV6.3 was mapped to a 4.5-Mb region of melon genome LG VI (Vegas et al. 2013), and its causal gene MELO3C016540 (CmNAC-NOR) was identified, characterized and functionally validated (Rıos et al. 2017) which showed similarities with the well-studied tomato gene Nor. Many recent studies also suggest the involvement of miRNAs (Gao et al. 2015) and epigenetic regulation (Zhong et al. 2011; Liu et al. 2015) in fruit ripening. Despite these recent advances, the full complexity of the ethylene-dependent and ethylene-independent regulation of fruit ripening remains to be resolved. Identification of large numbers of SNPs through re-sequencing has made it possible to locate and refine candidate genomic regions for fruit quality traits in melon more efficiently compared to traditional QTL mapping approaches (Galpaz et al. 2018; Pereira et al. 2018).

14.8 Molecular Breeding for High β-Carotene Content

Beta-carotene presence or absence in muskmelon is controlled by two genes, green flesh *gf* and white flesh *wf*. In its dominant form, the *wf* gene is responsible for orange flesh colour; however, the epistatic interactions of *gf* and *wf* can create three

flesh colours: orange, white and green. Two F_2 populations, consisting of 77 greenhouse grown and 117 field grown plants, from the cross of 'Sunrise' (white fleshed) by 'TAM Uvalde' (orange fleshed), were used to examine the relationships of beta-carotene content, flesh colour and flesh colour intensity. Bulk segregate analysis was used with RAPD markers to identify molecular markers associated with high β-carotene content. Flesh colour and flesh colour intensity both had significant relationships with β-carotene content. A significant correlation between total soluble solids and β-carotene content was also found. Molecular markers were identified in both F_2 populations, and all significant, associated markers from 'TAM Uvalde' were linked with *WF*. A single QTL was also found to be linked with the *WF* locus. The identified QTL can be used to screen potential breeding lines for high β-carotene. It was also confirmed that the visual ratings of flesh colour intensity can be reliably used to select high β-carotene content melons (Napier 2006). A set of 81 recombinant inbred lines (RIL) derived from Group *Cantalupensis* US Western Shipper market-type germplasm was examined in two locations [Wisconsin (Wisc.) and California (Calif.), USA] for 2 years to identify quantitative trait loci (QTL) associated with ß-carotene accumulation in mature fruit. Three hundred fifty-eight melon simple sequence repeats (SSR), 191 cucumber (*Cucumis sativus* L.) expressed sequence tag (EST) and 42 cucumber EST-SSR markers were evaluated to enhance saturation of a resident 181-point map. Additionally, genomic information from diverse plant species was used to isolate partial nucleotide sequences of eight putative genes coding for carotenoid biosynthesis enzymes, identify single nucleotide polymorphisms (SNP) and perform candidate gene analysis. Mapping parent analyses detected 64 SSR polymorphisms, 7 SNP using cucumber EST and 4 SNP in putative carotenoid candidate genes, and these markers were used to create a moderately saturated 256-point RIL-based map [104 SSR, 7 CAPS, 4 SNP, 140 dominant markers and 1 morphological trait (a) spanning 12 linkage groups (LG)] for ß-carotene QTL analysis. Eight QTLs were detected in this two-location RIL evaluation that were distributed across four LG that explained a significant portion of the associated phenotypic variation for ß-carotene accumulation (R2 = 8 to 31.0%). Broad-sense heritabilities for ß-carotene accumulation obtained from RIL grown in Wisc. and Calif. were 0.56 and 0.68, respectively, and 0.62 combining both locations. Although genotypes x environment interactions were confirmed in 2-year experiments, relative RIL performance rankings remained consistent. QTL map positions were not uniformly associated with putative carotenoid genes. One QTL (ß-car 6.1) interval was located 10 cM from a ß-carotene hydroxylase gene, and this region was colinear with previously reported QTL for colour pigmentation. These results suggest that accumulation of ß-carotene in melon is under complex genetic control, where epistasis plays an important role in trait expression. This study provides the initial step for defining the genetic control of ß-carotene accumulation in melon leading to the development of varieties with enhanced ß-carotene content (Cuevas et al. 2008). In order to study the inheritance of β-carotene, a cross was made between the monoecious, early maturing Chinese line 'Q 3-2-2' (non-carotene accumulating, white mesocarp) and the andromonoecious, comparatively late maturing US line 'Top Mark' (carotene accumulating; orange mesocarp) to

determine the inheritance of fruit maturity and quantity of β-carotene in melon. Parents and derived cross-progenies (F_1, F_2, F_3, BC_1P_1 and BC_1P_2) were evaluated for fruit maturity and quantity of β-carotene at Hancock, Wisconsin, over 2 years. Estimates of narrow-sense heritability (h_N^2) for quantity of β-carotene and fruit maturity as defined by F_1, F_2 and BC (by individuals) were 0.55 and 0.62, respectively, while estimates based on F_3 families were 0.68 and 0.57, respectively, for these traits. Mesocarp colour segregation (F_2 and BC_1P_2) fit a two-gene recessive epistatic model, which, in turn, interacts with other minor genes. Although the inheritance of quantity of β-carotene and fruit maturity is complex, introgression (e.g. by backcrossing) of early fruit maturity genes resident in Chinese germplasm into US market types is possible. Such introgression may lead to increased yield potential in US market types while retaining relatively high β-carotene fruit content (i.e. orange mesocarp); if stringent, multiple location and early generation family selection ($F_{3–4}$) are practised for fruit maturity with concomitant selection for quantity of β-carotene (Cuevas et al. 2010b).

The orange-fleshed melon gets higher β-carotene accumulation in fruit mesocarp governed by the golden SNP of the Orange (CmOr) gene (Tzuri et al. 2015). Allelic variation of CmOr gene has no effect on the transcript levels of carotenogenesis genes (Chayut et al. 2015), but its mode of action in melon fruit is still not clear (Chayut et al. 2017). The orange mutation in CmOr is considered to be more recently evolved which enabled the transition of chloroplast to chromoplast with increased accumulation of carotenoids (Chayut et al. 2015, 2017; Tzuri et al. 2015; Galpaz et al. 2018) in orange-fleshed melon. Integrative strategy of QTL mapping, the predicted effect of variants using genomics databases and analysis of gene expression have been successful in the identification of potential candidate genes for higher β-carotene, and maximum LOD position of the QTL CARQU9.1 was within the CmOr gene (MELO3C005449) (Pereira et al. 2018).

14.9 Gene Expression and Molecular Changes During Ripening

The cloning and characterization of two melon ethylene receptor genes have allowed their use as molecular genetic tools in heterologous systems with promising results). The overexpression of a missense mutated melon ethylene receptor gene, *Cm-ETR1/H69A*, in a heterologous plant, *Nemesia strumosa,* conferred reduced ethylene sensitivity (Cui et al. 2004), making transgenic plants that had a significantly extended flower longevity compared with the wild-type counterpart. Because ethylene inhibits the establishment of symbiosis between *Rhizobium* spp. and legumes, a point mutated *Cm-ERS1/H70A* gene was used to transform *Lotus japonicus* plants in order to examine how and when endogenous ethylene inhibits that rhizobial infection and nodulation.

14.10 In Vitro Culture

Melon varieties (*reticulatus*, *cantalupensis*, *inodorus*, *flexuosus*, etc.) and commercial cultivars have differences in their regeneration ability under the same in vitro protocol and environmental conditions (Gray et al. 1993; Molina and Nuez 1997; Galperin, et al. 2003). Likewise, organogenesis and somatic embryogenesis responses in melon cultures are also genotype dependent. For instance, Oridate et al. (1992) and Gray et al. (1993) reported that *reticulatus* varieties were more prone to produce in vitro somatic embryos than *inodorus* varieties.

14.11 Genetic Engineering for Fruit Quality Improvements

The first transgenic melon plants carrying genes involved in fruit ripening process were obtained by Ayub et al. (1996). Using the *Agrobacterium*-mediated transformation system and cotyledons of the Charentais-type cantaloupe melon cv. Vedrantais, these authors were able to transfer the 1-aminocyclopropane-1-carboxylic-acid oxidase gene (ACC oxidase from melon under the control of a constitutive promoter) in antisense orientation to reduce the level of ethylene production. Different ripening parameters were evaluated in transgenic melon fruits, such as internal and gas space ethylene production, total soluble solids, titratable acidity, flesh pigment content, flesh firmness, rind and flesh colour, harvest maturity (timing from anthesis to full slip) and reversion to wild-type behaviour by exogenous ethylene treatment (Ayub et al. 1996; Guis et al. 1997). Clendennen et al. (1999) utilized the product of the S-adenosylmethionine hydrolase (SAMase) gene (from T3 bacteriophage) to catalyse the degradation of SAM, the initial precursor of ethylene. Unlike the T-DNA construct used by Ayub et al. (1996), Clendennen et al. (1999) used a fruit-specific promoter (chimeric ethylene-responsive E8/E4 promoter) aimed to overexpress the SAMase gene in two American cantaloupe (i.e. netted muskmelon) lines, which were proprietary inbred lines from Harris Moran Seed Company, Inc. These authors evaluated several postharvest fruit quality parameters, such as fruit size and weight, firmness, decay susceptibility, external and internal colour, soluble solids, harvest maturity (timing from anthesis to full slip, measured as heat units) and ethylene production in wild-type and transgenic melon fruits from plants grown under field conditions. Transgenic melon fruit from both lines A and B did not differ in horticultural traits from wild-type fruits, except for the intended goal of SAMase expression on ethylene biosynthesis and related events. In lab experiments, transgenic fruits produced half of the ethylene accumulated by wild-type fruits. However, in field trials, the onset of maturity, measured on four different dates, was not significantly delayed in transgenic fruit compared to wild-type, but transgenic fruits ripened more uniformly in the field. Firmness was also measured on transgenic and wild-type fruits from three different field trial locations. Significant differences were found in fruit mesocarp firmness between transgenic and wild-type melons, but only from one location. Clendennen et al. (1999) claimed that by expressing SAMase in a regulated manner by a

fruit-specific promoter, transgenic fruits produced less ethylene than non-transgenic fruit ensuing in a modified ripening phenotype. Silva et al. (2004) obtained transgenic cantaloupe melon plants cv. Vedrantais by inserting and overexpressing ACC oxidase from apple and not from melon as in the Ayub et al. (1996) protocol. These authors reported the characterization of ripening melon fruits, and their experimental comparison between transgenic and control fruit provided very similar results in almost all the evaluated parameters, such as harvest maturity, total soluble solids content, rind colour and internal ethylene production, to those previously reported by Ayub et al. and Guis et al. (1997). Nunez-Palenius et al. (2008) used a *reticulatus* melon cultivar, Krymka, to reduce the ethylene production from fruits by introducing a single copy of the ACC oxidase gene in antisense orientation (Cantliffe et al. 2006). Transgenic ACC oxidase antisense Krymka fruit had a greater firmness than wild-type fruit, GUS-transgenic and azygous counterparts at full-slip developmental stage. Likewise, ethylene production and ACC oxidase activity in half-slip antisense fruit were almost ten times lower, than those from wild-type, azygous and GUS-transgenic fruit. All fruit quality-oriented transgenic melon plants have been obtained using just four melon cultivars, such as Vedrantais belonging to *cantalupensis* (Ayub et al. 1996; Silva et al. 2004), and lines A and B (Clendennen et al. 1999) and Galia male parental line, Krymka, which are *reticulatus* melon varieties (Nunez-Palenius et al. 2008).

14.12 Biotic Stress Resistance in Muskmelon

Disease resistance was among the first breeding objectives for the development of new cultivars in muskmelon. Powdery Mildew Resistant 45 (PMR-45), resistant to powdery mildew, was bred in the 1930 in California. Three general comments should be done which are valid not only for melon but for all crops:

(i) Breeding for insect-pest and disease resistance is much more successful when methods of artificial inoculations on plantlets have been developed.
(ii) Variability of the causal organism of the disease and durability of the resistance could be obtained by pyramiding of genes for resistance and/or association of resistance with management practices and/or management in time and space of the resistances.
(iii) Multiple disease-resistant cultivars should be developed. For example, modern F_1 hybrids can cumulate resistance to four races of *Fusarium* wilt, to several races of powdery mildew and to *Aphis gossypii.*

14.12.1 Powdery Mildew

Powdery mildew is probably the most widespread devastating disease of Cucurbits, including melon. It can be caused by two pathogens, namely, *Golovinomyces cichoracearum* (DC) V.P. Heluta (formerly *Erysiphe cichoracearum*) and *Podosphaera*

xanthii (Castagne) Braun & Shishkoff (formerly *Sphaerotheca fuliginea*). The most prevalent species is *P. xanthii* especially under high temperature, but in some countries *G. cichoracearum* can also be found with a high frequency. Different races have been described mainly on *P. xanthii*, A notable milestone in the plant breeding history occurred in 1937 when I.C. Jagger and G.W. Scott reported research that led to the release of 'Powdery Mildew Resistant Cantaloupe-45, but a new race overcoming its resistance was described in 1938 (Jagger et al. 1938). Since then many races have been published by McCreight (2006). Resistance to different races of *P. xanthii* is generally found in accessions from India; resistance to *G. cichoracearum* is quite frequent in *inodorus* botanical group (Amarillo, Piel de sapo). Inheritance studies concluded generally to monogenic dominant controls but recessive control has also been observed (Jahn et al. 2002; McCreight 2003). Some genes can control several races of *P. xanthii*; some are specific of *P. xanthii* (e.g. *Pm*-1 in PMR 45), and some are specific of *G. cichoracearum* (e.g. *Pm-H* in Nantais oblong), while some can control some races of both *P. xanthii* and *G. cichoracearum*. All the allelism tests have not been performed and this situation is quite confused. The differential interactions of some powdery mildew races and melon accessions are presented in Table 14.1.

The susceptibility of the accession Iran H to races 0 of *P. xanthii* and of *G. cichoracearum* should be considered as an exception, as almost all melon accessions are resistant to races 0. The strains belonging to races 0 have been isolated from cucumber. This situation is similar to that described by Robinson and Provvidvnti (1975) where all watermelon accessions were resistant to the powdery mildew strain used in their test except PI-269677 which was susceptible.

Many lines with a high level of resistance to powdery mildew, for example, PMR-5, can present in some conditions (low light intensity, short day length, heavy fruit load) a severe leaf necrosis. These symptoms are not a hypersensitive reaction as they can be observed in the absence of the fungus. The linkage has not been broken and is more probably a pleiotropic effect than a true genetic linkage. The necrosis has a recessive genetic control, while the powdery mildew resistance is dominant in most cases. F_1 hybrids between a resistant necrotic parent and a susceptible line are resistant without the risk of necrotic reactions.

Genetic control of powdery mildew resistance is generally quite simple with major dominant genes. But modifier genes are also commonly observed. For example, F_1 hybrids between one resistant parental line and several susceptible lines do not have the same level of resistance, since PMR-45 and many powdery mildew-resistant cultivars have been released with resistance to one or several races.

14.12.2 Downy Mildew

Downy mildew, caused by *Pseudoperonospora cubensis* (Berk. & Curtis) Rostovzev, can be found in temperate and tropical areas with high relative humidity. Different pathotypes have been described, and good levels of resistance have been found in

Table 14.1 Reaction of powdery mildew *Podosphaera xanthii* (Px) and *Golovinomyces cichoracearum* (Gc) races on melon differential lines

Accessions	Px 0	Px 1	Px 2US	Px 2F	Px 3	Px 4	Px 5	Gc 0	Gc 1
Iran H	S	S	S	S	S	S	S	S	S
Vedrantais, Topmark	R	S	S	S	S	S	S	R	S
PMR 45	R	R	S	S	S	S	S	R	S
WMR 29	R	R	Het	R	R	S	S	R	S
EDISTO 47	R	R	S	R	R	R	S	R	S
PMR 5	R	R	R	R	S	R	R	R	R
Nantais oblong	R	S	S	S	S	S	S	R	R

S susceptible, *R* resistant, *Het* heterogeneous

Indian accessions like PI-124111 and its derivative MR-1 (Thomas 1986; Kenigsbuch and Cohen 1989) or PI-124112 (Kenigsbuch and Cohen 1992). Major genes have been described (Kenigsbuch and Cohen 1989, 1992) but also a polygenic control (Epinat and Pitrat 1994a, b; Perchepied et al. 2005a). Very few improved cultivars have been released with a high level of resistance to downy mildew in muskmelon.

14.12.3 Fusarium *Wilt*

Fusarium oxysporum Schltdl. f. sp. *melonis* Snyder & Hansen is a soil-borne pathogen causing *Fusarium* wilt. Four races have been described (Risser et al. 1976) (Table 14.2); strains belonging to race 1.2 can induce yellowing symptoms followed by the death of the plant or wilting without yellowing. A first major gene (*Fom*-1) has been described segregating in populations in France, and open pollinated cultivars homogeneous for resistance have been selected such as Doublon or Vedrantais. These cultivars were observed susceptible in some fields where race 1 was present. Screening of the genetic resources led to the discovery of an independent dominant gene (*Fom*-2) in accessions from the Far East (CM 17187) (Risser 1973). The third gene (*Fom*-3) controlling resistance to races 0 and 2 like *Fom*-1 has also been observed in few accessions, for example, Ogon 9, from the Far East (Perchepied et al. 2005b); this partial resistance is effective against all races, including race 1.2. A non-commercial breeding line, Isabelle, cumulating *Fom*-1, *Fom*-2 and the polygenic recessive resistance of Ogon 9 has been released.

Almost all the F_1 hybrids have *Fom*-1 and *Fom*-2. A few have also a good level of resistance to race 1.2 coming from Isabelle, but it is difficult to cumulate a high level of resistance and good horticultural characteristics. Control of *Fusarium* wilt includes also grafting on resistant rootstocks. Resistant melon accessions can be used but usually other cucurbits are preferred. *Benincasa hispida* (Thunb.) Cogn. has first be proposed but today *Cucurbita* rootstocks are more commonly used.

Table 14.2 Genetic control of resistance to races of *Fusarium oxysporum* f. sp. *melonis*

Accessions	Genotype	Race 0	Race 1	Race 2	Race 1.2
Charentais T		S	S	S	S
Doublon, Charentais Fom 1	*Fom-1*	R	S	R	S
CM 17187, Charentais Fom-2	*Fom-2*	R	R	S	S
Perlita FR	*Fom-3*	R	S	R	S
	Polygenic recessive	r	r	r	r
Isabelle	Fom-1, Fom-2, *polygenic recessive*	R	R	R	r

S susceptible, *R* resistant, *r* partially resistant

14.12.3.1 Marker-Assisted Breeding for Resistance Against *Fusarium* Wilt

Four physiological races of Fom designated 0, 1, 2 and 1.2 have been identified based on their ability to differentially infect melon hosts. The single dominant genes Fom-2 and Fom-1 confer resistance to races 0, 1 and 0, 2, respectively. The bulk segregant analysis (BSA) strategy was used to detect random amplified polymorphic DNA (RAPD) markers linked to Fom-2 (Wechter et al. 1995). These fingerprint markers have been transformed to more stable and convenient single-locus ones, such as cleaved amplified polymorphic sequences (Zheng et al. 1999), or sequence characterized amplified region (SCAR) (Zheng and Wolf 2000). Wang et al. (2000) found some AFLP markers linked to the Fom-2 locus and converted them into codominant SCAR markers, designated “AM” and “FM”. Joobeur et al. (2004) revealed that the amino acid sequences from three susceptible cultivars (‘Ve’drantais’, ‘AnanasYokneum’ and ‘Durango’) were identical to each other; however, when compared with the amino acid sequences deduced from resistant genotypes (MR-1 and PI 161375), 25 amino acids out of 541 were different. The sequences of the LRR fragment from the two resistant lines were identical, except of three nucleotides. These differences resulted in the substitution of the amino acid residues V and K in MR-1 by M and E in PI 161375, respectively. So far, however, functional validation of the Fom-2 gene by transgenic complementation/silencing of resistance, or by TILLING, has not been reported. Recently, Oumouloud et al. (2012) reported the cloning and sequencing of a partial LRR fragment of Fom-2 from 11 melon-resistant accessions from various geographic regions. They identified 3 alleles of Fom-2, and their results revealed that the structure of the Fom-2 LRR domain is highly conserved, since 8 of the 11 resistant genotypes showed similar alleles to the resistant one characterized in the PI 161375 line. Conversely, PI 124111 was the only line that presented the same resistant allele previously described in MR-1. The information generated from the Fom-2 LRR region sequences allowed the systematic development of “functional markers” that were developed based on the nucleotide polymorphisms detected between the susceptible and resistant Fom-2 alleles (Wang et al. 2011; Oumouloud et al. 2012). Sources of resistance from

Cucumis melo var. *momordica* (snapmelon) germplasm (DSM-11) have been identified at IARI, New Delhi, which could be utilized for the development of resistant genotype of muskmelon against this serious disease (Choudhary et al. 2013, 2015). Two functional SCAR markers Fom2-R_{408} and Fom2-S_{342} developed from LRR domain of Fom-2 gene were validated which could be used for the selection of resistant plant during backcrossing for introgression of fom-2 gene into commercially susceptible cultivars (Choudhary et al. 2019) (Fig. 14.2).

Fusarium wilt resistance gene Fom-2 has also been successfully introgressed through marker-assisted backcrossing to commercial muskmelon cultivar by Sousaraei et al. (2018).

14.12.4 Gummy Stem Blight

Didymella bryoniae (Fuckel) Rehm [syn. Mycosphaerella melonis (C.O. Sm.) Grossenb.] affects leaves, fruits and stems. The disease is observed mainly in hot and humid conditions (tropical and subtropical areas or greenhouses). Five genes (four dominant *Gsb*-1 to *Gsb*-4 and one recessive *gsb*-5) have been described in accessions from China, Mexico and Zimbabwe (Frantz and Jahn 2004). Up to now, no study on the interactions between these resistant accessions and *D. bryoniae* strains has been conducted, and no races have been clearly defined.

14.12.5 Viruses

More than 30 viruses can infect Cucurbitsand melon (Lecoq 2003). Some of them can induce very severe losses. They are very difficult to control. Symptoms are not always easy to recognize, particularly in the case of mixed infections which are common.

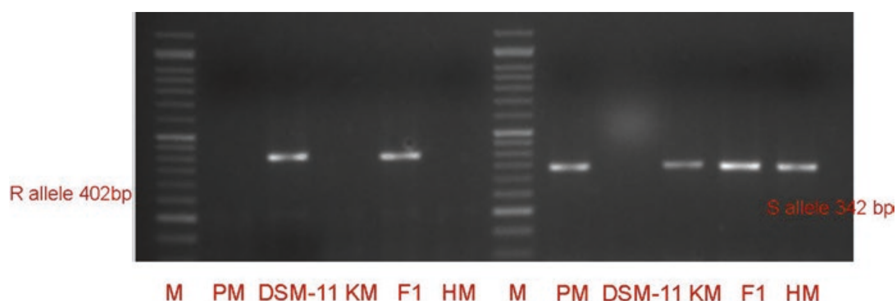

Fig. 14.2 Validation of functional markers associated with Fom-2 gene

The prevalence of the different viruses varies according to agro-system such as vectors, weeds and other crops as vectors and virus reservoirs. For example, in some parts of the world, the substitution of *Bemisia tabaci* Gennadius by the silverleaf whitefly *Bemisia argentifolii* Bellows & Perring (= biotype B of *B. tabaci*) led to the near disappearance of *Lettuce infectious yellows virus* (LiYV). In Southern Europe, the development of *B. tabaci* has been rapidly followed by the whiteflies transmitted viruses *Cucurbit yellow stunting disorder virus* (CYSDV) and *Cucumber vein yellowing virus* (CVYV). In France, it seems that the incidence of *Cucumber mosaic virus* (CMV) is decreasing and that the incidence of *Watermelon mosaic virus* (WMV) is increasing. In the beginning of the 1980s, *Zucchini yellow mosaic virus* (ZYMV) is a good example of emerging disease.

One of the best approaches to control the viruses is breeding for resistance, starting with the evaluation of genetic resources. Sources of resistance to several of them have been described, and their inheritance has been studied in many cases (Table 14.3). Sources of resistance are mainly found in accessions from India (PI 414723, PI 180280, PI 180283, PI 1214112 and PI 313970) but also from the Far East (PI 161375, Freeman's cucumber) or Zimbabwe (TGR 1551). Novel strategies have also been developed using the virus coat protein or ribozymes in transgenic plants.

Up to now, few improved cultivars with virus resistance have been released. Techniques for screening resistant plants in segregating progenies are available, at least with mechanically transmitted viruses. This screening is more difficult when the vectors, aphids or whiteflies, for instance, must be used. There are often mixed virus infections in melon crop, and resistance to one of the viruses of the complex would not bring any horticultural interest. Seed company strategy could be to have a cultivar with resistance to several viruses which would bring a commercial advantage over competitors and not to release cultivars with resistance to only one of the viruses.

14.12.6 Insect-Pests

Resistance to insect-pests has received less attention than resistance to fungi or viruses (Robinson 1992). Nevertheless resistances to aphid *Aphis gossypii* Glover (Kishaba et al. 1971; Bohn et al. 1973; Lecoq et al. 1979; Soria et al. 2003), to the whitefly *Bemisia tabaci* Gennadius (Boissot et al. 2003) and to leafminers *Liriomyza sativae* Blanchard (Kennedy et al. 1978) and *L. trifolii* Burgess (Dogimont et al. 1999) have been described. Resistance to *A. gossypii* is particularly interesting because resistance to colonization of the plant by the aphid is linked with resistance to transmission by *A. gossypii*. This resistance to virus transmission is specific of the aphid species but not of the virus (Lecoq et al. 1979, 1980; Soria et al. 2003). It brings a few days delay in the development of the epidemics which could be cumulated with partial virus resistance and technical practices such as weeding and using repulsive plastic mulches.

Table 14.3 Resistance to some virus infecting melon

Genus: Species	Source of resistance (genetic control)	References
Polerovirus: *Cucurbit aphid borne yellow virus* (CABYV)	PI 414723, 90625, PI 124112 (2 recessive genes, *cab-1 cab-2*)	Dogimont et al. (1997)
Carmovirus: *Melon necrotic spot virus* (MNSV)	PI 161375, Gulfstream, Planters Jumbo, PMR 5(*nsv*) Doublon (*Mnr1 Mnr2*)	Coudriet et al. (1981) and Mallor et al. (2003)
Cucumovirus: *Cucumber mosaic virus* (CMV)	Freeman's cucumber PI 161375, Ginsen makuwa (oligogenic recessive)	Karchi et al. (1975), Risser et al. (1977) and Dogimont et al. (2000)
Tobamovirus: *Cucumber green mottle mosaic virus* (CGMMV)	Phoot, VRM-10, VRM 29-1, etc. (inheritance?)	More (2001)
Tobamovirus: *Kyuri green mottle mosaic virus* (KGMMV)	Mawatauri, PI 161375 (inheritance?)	Doryono et al. (2005)
Crinivirus: *Lettuce infectious yellows virus* (CYSDV)	TGR 1551 (*Cys*)	Lopez-Sese and Gomez-Guillamon (2000)
Crinivirus: *Lettuce infections yellows virus* (LIYV)	PI 313970 (*Liy*)	McCreight (2000)
Crinivirus: *Beet pseudo-yellows virus* (BPYV)	Nagata Kin makuwa (*My* partially dominant)	Nuez et al. (1999)
Potyvirus: *Zucchini yellow mosaic virus* (ZYMV)	PI 414723 (*Zym*, *Zym-2*, *Zym-3*)	Pitrat and Lecoq (1984) and Danin-Poleg et al. (1997)
Potyvirus: *Papaya ringspot virus* (PRSV-W)	PI 180280 (*Prv*^{1}) PI 180283(*Prv*^{2}) PI 124112 (*Prv-2*)	Kaan (1973), Webb (1979), Pitrat and Lecoq (1983) and McCreight and Fashing-Burdette (1996)
Potyvirus: *Watermelon mosaic virus* (WMV).	PI 414723 (*Wmr*), TGR 1551 (one recessive and one dominant)	Gilbert et al. (1994) and Diaz-Pendon et al. (2005)
Begomovirus (Geminiviridae) Tomato leaf curl New Delhi virus (ToLCNDV)	Mom-Khalnd/Kharbuja Mom-PII24Ind/PI 124112 Mom-PI4I4InD/PI 414723 *Cucumis melo* subsp. *agrestis* var.*momordica*, WM-7,WM-9	Saez et.al. (2017)

Resistance to many other diseases has been described including among others *Alternaria cucumerina* (Ellis & Everh.) Elliot (Thomas et al. 1990), *Monosporascus cannonballus* Pollack & Uecker (Crosby et al. 2000; Dias et al. 2004) and *Diaphania hyalinata* L. (Guillaume and Boissot 2001).

Finally, there are many biotic stresses against which no resistance has been found, for example, root-knot nematodes (*Meloidogyne* sp.) or bacterial wilt [*Erwinia tracheiphila* (Smith) Bergey et al.].

Sources of resistance to insect-pests and diseases are mainly found in accessions from India and the Far East belonging to the *momordica*, *acidulous*, *conomon* and *makuwa* varieties. Some accessions are particularly interesting as they cumulate resistance to several diseases. For example, PI 414723 from India is resistant to *Fusarium* wilt, powdery mildew, *A. gossypii*, ZYMV, PRSV and CABYV; MR-1 from India is resistant to *Fusarium* wilt, powdery and downy mildew and *Alternaria*; PI 161375 from Korea is resistant to CMV, *A. gossypii*, MNSV and *Fusarium* wilt. Other geographical origins could also be interesting, for instance, TGR 1551 from Zimbabwe which is resistant to powdery mildew, CYSDV, *A. gossypii* and WMV. All these accessions are cultivated, and even if wild melons could be resistant to some disease, no resistance has been found only in non-cultivated melons. This could be also due to the fact that wild melons are underrepresented in collections.

14.13 Genetic Engineering for Disease Resistance

C. melo is attacked by a number of viral, bacterial, mycoplasmal and fungal organisms, which cause severe diseases. These diseases can affect at any plant developmental stage, resulting in enormous economic losses. More than 30 viruses are supposed to induce disease symptoms in melon plants. *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV) and *Watermelon mosaic virus* (WMV) are the most prevalent (Gaba et al. 2004). The first virus-resistant transgenic melon plants were obtained by Yoshioka et al. (1993). These authors transferred and overexpressed the gene for CMV coat protein via *Agrobacterium tumefaciens* using “Prince”, “EG360” and “Sunday Aki” melon cotyledons. Transgenic melon plants, which overexpressed the CMV-CP gene, were found to be resistant to infection after inoculation with a low dose of CMV grown under greenhouse conditions (Yoshioka et al. 1993). These plants did not develop symptoms of disease during a 46-day observation period, whereas control plants had mosaic symptoms 3 days after inoculation. When the virus dose was increased by tenfold, only a delayed appearance of symptoms was observed in transgenic plants. Other group of researchers were able to obtain transgenic melon plants overexpressing the CMV-CP for other melon cultivars, such as “Burpee Hybrid”, “Halest Best Jumbo” and “Topmark” (Gonsalves et al. 1994) and “Don Luis”, “Galleon”, “Hiline”, “Mission” and a distinct inbred line (Clough and Hamm 1995). In addition, transgenic plants overexpressing either CMV-CP for specific viral strains (White Leaf strain) or multi-virus resistance (CMV, WMV and ZYMV) (Fuchs et al. 1997) were described. Gonsalves et al. found strong resistance to CMV-White Leaf strain in 5

of 45 transgenic melon plants. High-level resistance to ZYMV, as measured by lack of symptom development and virus accumulation for a 30-dayin the greenhouse, also was achieved with the ZYMV-CP gene (Fang and Grumet 1993). Gaba, Zelcer and Gal-On stressed that CP protection gave effective field resistance, but not 100% protection. Clough and Hamm (1995) tested the level of resistance of five transgenic melon varieties to WMV and ZYMV. Transgenic melon plants had little or no virus infection, whereas more than 60% of the control plants developed virus symptoms. Regarding the resistance to fungal diseases, transgenic melon (BU21/3 line) plants were obtained by Taler et al. (2004). These transgenic melons overexpressed the enzymatic resistance (*eR*) genes *At1* and *At2* and displayed enhanced activity of glyoxylate aminotransferases and noteworthy resistance against *Pseudoperonospora cubensis* (downy mildew disease causal agent). These cloned *eR* genes will make available a new reserve for developing downy mildew-resistant melon varieties.

References

Ayub R, Guis M, Ben Amor M, Gillot L, Roustan JP, Latché A, Bouzayen M, Pech JC (1996) Expression of an antisense ACC oxidase gene inhibits ripening in cantaloupe melons fruits. Nat Biotechnol 14:862–866

Bhimappa BB, Choudhary H, Sharma VK, Behera TK (2018) Genetic diversity analysis for fruit quality traits and nutrient composition in different horticultural groups of muskmelon (*Cucumis melo* L.). Indian J Hortic 75(1):58–63. https://doi.org/10.5958/0974-0112.2018.00010.5

Bhimappa BB, Choudhary H, Behera TK, Sharma VK, Hussain Z, Tomar BS (2019) Study of genetic diversity in muskmelon (Cucumis melo) from different horticultural groups. Indian J Agric Sci 89:1293–1297

Bohn GW, Kishaba AN, Principe JA, Toba HH (1973) Tolerance to melon aphid in *Cucumis melo* L. J Am Soc Hortic Sci 98:37–40

Boissot N, Lafortune D, Pavis C, Sauvion N (2003) Field resistance to Bemisia tabaci in Cu*cumis melo*. HortScience 38:77–80

Burger Y, Sa'ar U, Katzir N, Paris HS, Yeselson Y, Levin I, Schaffer AA (2002) A single recessive gene for sucrose accumulation in *Cucumis melo* fruit. J Am Soc Hortic Sci 127:938–943

Burger Y, Sa'ar U, Distelfeld A, Katzir N,Yeselson Y, Shen A, Schaffer AA (2003) Development of sweet melon (*Cucumis melo* L.) genotypes combining high sucrose and organic acid contentJ. Amer. Soc. Hort. Sci.128537540

Chayut N, Yuan H, Ohali S, Meir A, Yeselson Y, Portnoy V, Zheng Y, Fei Z, Lewinsohn E, Katzir N et al (2015) A bulk segregant transcriptome analysis reveals metabolic and cellular processes associated with Orange allelic variation and fruit b-carotene accumulation in melon fruit. BMC Plant Biol 15:274

Chayut N, Yuan H, Ohali S et al (2017) Distinct mechanisms of the ORANGE protein in controlling carotenoid flux. Plant Physiol 173:376–389. https://doi.org/10.1104/pp.16.01256

Choudhary H, Ram HH, Singh DK (2011) Genetic variability study in muskmelon. Progress Hortic 43(2):231–233

Choudhary H, Dhar S, Kalia P (2013) Identification of new sources of resistance for *Fusarium* wilt of muskmelon. In: *Book of abstract of national symposium on abiotic and biotic stress management in vegetable crops*. IIVR, Varanasi, India, p 23

Choudhary H, Beesanakoppa BB, Behera TK, Dhar S, Shanmugam V (2015) Genetic characterization of novel source of resistance against *Fusarium* wilt of muskmelon and validation of Fom-2 gene. In: Book of abstract of national symposium on Germplasm to gene: harnessing biotechnology for food security and health, New Delhi, India

Choudhary HS, Kalia P (2013) Identification of new sources of resistance for *Fusarium* wilt of muskmelon. In: Book of abstract of national symposium on abiotic and biotic stress management in vegetable crops. IIVR, Varanasi, p 23

Choudhary H, Beesanakoppa BB, Dhar S, Tomar BS (2019a) Potential of genomics assisted breeding for introgression of resistance gene and fruit quality improvement in melon. In: Book of abstract of 1st vegetable science congress on emerging challenges in vegetable research & education, Jodhpur, 1–3 Feb, p 86

Choudhary H, Behera TK, Munshi AD (2019b) Breeding approaches and achievements in vegetable crops for protected cultivation. In: Singh SK, Patel VB, Goswami AK, Prakash, JP, Kumar C (eds) Breeding of perennial horticultural crops. Biotech Books, New Delhi

Clendennen SK, Kellogg JA, Wolff KA, Matsumura W, Peters S, Vanwinkle JE, Copess B, Pieper W, Kramer MG (1999) Genetic engineering of cantaloupe to reduce ethylene biosynthesis and control ripening. In: Kanellis AK, Chang C, Klee H, Bleecker AB, Pech JC, Grierson D (eds) Biology and biotechnology of the plant hormone ethylene. Kluwer, Dordrecht, pp 371–379

Clough GH, Hamm PB (1995) Coat protein transgenic resistance to watermelon mosaic and zucchini yellows mosaic virus in squash and cantaloupe. Plant Dis 79:1107–1109

Coudriet DL, Kishaba AN, Bohn GW (1981) Inheritance of resistance to muskmelon necrotic spot virus in a melon aphid resistance breeding lines of muskmelon. J Am Soc Hortic Sci 106:789–791

Crosby K, Wolff D, Miller M (2000) Comparisons of root morphology in susceptible and tolerant melon cultivars before and after infection by *Monosporascus cannonballus*. Hortic Sci 35:681–683

Cui ML, Takada K, Ma B, Ezura H (2004) Over-expression of a mutated melon ethylene receptor gene CmETR1/H69A confers reduced ethylene sensitivity in a heterologous plant, Nemesia strumosa. Plant Sci 167:253–258

Cuevas HE, Staub JE, Simon PW, McCreight JD, Zalapa JE (2008) Mapping of genetic loci that regulate accumulation of Beta-Carotene in fruit of U.S. Western shipping melon (*Cucumis melo* L.) and their association with putative carotenoid biosynthesis genes. Theor Appl Genet 117:1345–1359

Cuevas HE, Staub JE, Simon PW (2010b) Inheritance of beta-carotene-associated mesocarp color and fruit maturity of melon (*Cucumis melo* L.). Euphytica 173:129–140

Danin-Poleg Y, Paris HS, Cohen S, Rabinowithc HD, Karchi Z (1997) Oligogenic inheritance of resistance to zucchini yellow mosaic virus in melons. Euphytica 93:331–337

Danin-Poleg Y, Tadmor Y, Tzuri G, Reis N, Hirschberg J, Katzir N (2002) Construction of a genetic map of melon with molecular markers and horticultural traits, and localization of genes associated with ZYMV resistance. Euphytica 125:373–384

Daryono BS, Somowiyarjo S, Natsuaki KT (2005) Screening for resistance to Kyuri green mottle mosaic virus in various melons. Plant Breed 124:487–490

Dhillon NPS, Monforte AJ, Pitrat M, Pandey S, Singh PK et al (2012) Melon landraces of India: contributions and importance. Plant Breeding Rev 35:85–150

Dias RDS, Pico B, Espinos A, Nuez F (2004) Resistance to melon vine decline derived from *Cucumis melo* spp. *agrestis*: genetic analysis for root structure and root response. Plant Breed 123:66–72

Diaz A, Fergany M, Formisano G, Ziarsolo P, Blanca J, Fei Z, Monforte AJ (2011) A consensus linkage map for molecular markers and quantitative trait loci associated with economically important traits in melon (*Cucumis melo* L.). BMC Plant Biol 11:111–125

Diaz-Pendon JA, Fernandez-munoz R, Gomez-Guillamon ML, Moriones E (2005) Inheritance of resistance to watermelon mosaic virus in *Cucumis melo* that impairs virus accumulation, symptom expression and aphid transmission. Phytopathology 95:840–846

Diaz A, Forment J, Argyris J, Fukino N, Tzuri G, Harel-Beja R, Katzir N, Garcia-Mas J, Monforte A (2015) Anchoring the consensus ICuGI genetic map to the melon (*Cucumis melo* L.) genome. Mol Breed 35:1–7

Dogimont C, Bussemakers A, Martin J, Slama S, Lecoq H, Pitrat M (1997) Two complementary recessive genes conferring resistance to cucurbit aphid borne yellows luteovirus in an Indian melon line (*Cucumis melo* L.). Euphytica 96:391–395

Dogimont C, Bordat D, Pages C, Boissot N, Pitrat M (1999) One dominant gene conferring the resistance to the leafminer *Liriomyza trifolii* (Burgess) Diptera: Agromyzidae in melon (*Cucumis melo* L.). Euphytica 105:63–67

Dogimont C, Lecotmte L, Perin C, Thabuis A, Lecoq H, Pitrat M (2000) Identification of QTLs contributing to resistance to different strains of cucumber mosaic cucumovirus in melon. In: Cucurbitaceae 2000, 7th EUCARPIA meeting on cucurbit genetics and breeding, pp 391–398

Dwivedi NK, Bhandari DC, Neelam B (1998) Collecting wild species of some crop plants from Aravalli hills, Rajasthan, India. Indian J Pl Genet Resour 11(1):41–48

Dwivedi NK, Dhariwal OP, Gopala Krishnan S, Bhandari DC (2010) Distribution and extent of diversity in Cucumis species in the Aravalli ranges of India. Genet Resour Crop Evol 57:443–452

Epinat C, Pitrat M (1994a) Inheritance of resistance to downy mildew (*Pseudopernospora cubensis*) in muskmelon (*Cucumis melo* L.). I. Analysis of a 8×8 diallel table. Agronomie 14:239–248

Epinat C, Pitrat M (1994b) Inheritance of resistance to downy mildew (*Pseudopernospora cubensis*) in muskmelon (*Cucumis melo L.*). II. Generation means analysis of 5 genitors. Agronomie 14:249–257

Ezura H, Owino WO (2008) Melon, an alternative model plant for elucidating fruit ripening. Plant Sci 175:121–129

Fang GW, Grumet R (1993) Genetic-engineering of potyvirus resistance using constructs derived from the zucchini yellow mosaic-virus coat protein gene. Mol Plant-Microbe Interact 6:358–367

Fleshman MK, Lester GE, Reidl KM, Kopec RE, Narayanasamy S, Curley RW, Schwartz SJ, Harrison EH (2011) Carotene and novel apocarotenoid concentrations in orange-fleshed Cucumis melo melons: determinations of β-carotene bioaccessibility and bioavailability. J Agric Food Chem 599:4448–4454

Frantz JD, Jahn MM (2004) Five independent loci each control monogenic resistance to gummy stem blight in melon (*Cucumis melon* L.). Theor Appl Genet 108:1033–1038

Fuchs M, McFerson, JR, Tricoli D, McMaster JR, Deng RZ, Boeshore ML, Reynolds JF, Russell PF, Quemada HD, Gonsalves D (1997) Cantaloupe line CZW-30 containing coat protein genes of cucumber mosaic virus, zucchini yellow mosaic virus, and watermelon mosaic virus-2 is resistant to these three viruses in the field. Mol Breed 3:279–290

Gao Y, Xue Q, Wang D, Du M, Zhang Y, Gao S (2015) miR-873 induces lung adenocarcinoma cell proliferation and migration by targeting SRCIN1. Am J Transl Res 7:2519–2526

Galperin M, Zelcer A, Kenigsbuch D (2003) High competence for adventitious regeneration in the BU-21/3 melon genotype is controlled by a single dominant locus. HortScience 38:1167–1168

Gaba V, Zelcer A, Gal-On A (2004) Cucurbit biotechnology – the importance of virus resistance. In Vitro Cell Dev Biol-Plant 40:346–358

Galpaz N, Gonda I, Shem-Tov D, Barad O, Tzuri G, Lev S et al (2018) Deciphering genetic factors that determine melon fruit-quality traits using RNA-Seq based high-resolution QTL and e QTL mapping. Plant J 94:169–191. https://doi.org/10.1111/tpj.13838

Garcia-Mas J, Benjak A, Sanseverino W, Bourgeois M, Mir G, González VM et al (2012) The genome of melon (*Cucumis melo* L.). Proc Natl Acad Sci U S A 109:11872–11877

Gilbert RZ, Kyle MM, Munger HM, Gray SM (1994) Inheritance of resistance to watermelon mosaic virus in *Cucumis melo* L. Hortic Sci 29:107–110

Gonsalves C, Xue B, Yepes M, Fuchs M, Ling KS, Namba S, Chee P, Slightom JL, Gonsalves D (1994) Transferring cucumber mosaic virus-white leaf strain coat protein gene into *Cucumis melo* L. and evaluating transgenic plants for protection against infections. J Am Soc Hortic Sci 119:345–355

Gonzalo MJ, Claveria E, Monforte AJ, Sanjuan RD (2011) Parthenogenic haploids in melon: generation and molecular characterization of a doubled haploid line population. J Am Soc Hortic Sci 136(2):145–154. https://doi.org/10.21273/JASHS.136.2.145

González M et al (2011) Towards a TILLING platform for functional genomics in Piel de Sapo melons. BMC Res Notes 4:289

Gonzalo MJ, Aurora D, Dhillon NPS, Reddy UK et al (2019) Re-evaluation of the role of Indian germplasm as center of melon diversification based on genotyping by-sequencing analysis. BMC Genomics 20:448. https://doi.org/10.1186/s12864-019-5784-0

Gray DJ, McColley DW, Compton ME (1993) High frequency somatic embryogenesis from quiescent seed cotyledons of *Cucumis melo* cultivars. J Am Soc Hortic Sci 118:425–432
Guis M, Botondi R, Ben Amor M, Ayub R, Bouzayen M, Pech JC, Latché A (1997) Ripening-associated biochemical traits of cantaloupe charentais melons expressing an antisense ACC oxidase transgene. J Am Soc Hortic Sci 122:748–751
Guillaume R, Boissot N (2001) Resistance to *Diaphania hyalinata* (Lepidoptera: Crambidae) in *Cucumis* species. J Econ Entomol 94:719–723
Jagger IC, Whitaker TW, Porter DR (1938) A new biotic form of powdery mildew on muskmelon in the Imperial valley of California. Plant Dis Rep 22:275–276
Jahn M, Munger HM, McCreight JD (2002) Breeding cucurbit crops for powdery mildew resistance. In: The powdery mildews. John wiley, location USA, A comprehensive treatise, pp 239–242
Joobeur T, King JJ, Nolin SJ, Thomas CE, Dean RA (2004) The Fusarium wilt resistance locus *Fom-2* of melon contains a single resistance gene with complex features. Plant J 39:283–297
Kaan JF (1973) Recherché sur la resistance du melon aux maladies, notamment a la mosaique de la pasteque et au Pseudopernospora, appliqués au type varietal “Cantaloup”. In: Rsser G (eds) EUCARPIA meeting on melon, Saint Paul, MN: APS Press, pp 41–49
Karchi Z, Cohen S, Govers A (1975) Inheritance of resistance to cucumber mosaic virus in melons. Phytopathology 65:479–481
Kenigsbuch D, Cohen Y (1989) Inheritance of resistance to downy mildew in a gynocious muskmelon. Plant Dis 73:994–996
Kenigsbuch D, Cohen Y (1992) Inheritance of resistance to downy mildew in *Cucumis melo* PI 124112 and commonality of resistance genes with PI 124111F. Plant Dis 76:615–617
Kennedy GG, Bohn GW, Stonar AK, Webb RE (1978) Leafminer resistance in muskmelon. J Am Soc Hortic Sci 103:571–574
Kishaba AN, Bohn GW, Toba HH (1971) Resistance of *Aphis gossypii* in muskmelon. J Econ Entomol 64:935–937
Lecoq H (2003) Cucurbits. In: Loebenstein G, Thottappilly G (eds) Virus and virus-like diseases of major crops in developing countries. Kluwer Academic Publishers, Dordrecht, pp 665–688
Lecoq H, Cohn S, Pitrat M, Labonne G (1979) Resistance to cucumber mosaic virus transmission by aphids in *Cucumis melo*. Phytopathology 69:1223–1225
Lecoq H, Labonne G, Pitrat M (1980) Specificity of resistance to virus transmission by aphids in *Cucumis melo*. Ann Phytopathol 12:139–144
Liou PC, Chang YM, Hsu WS, Cheng YH, Chang HR, Hsiao CH (1998) Construction of a linkage map in Cucumis melo (L.) using random amplified polymorphic DNA markers. In: Drew RA (ed) Proceedings of the international symposium in biotechnology: tropical and subtropical species, pp 123–131
Lopez-Sese AI, Gomez-Guillamon ML (2000) Resistance to cucurbit yellowing stunting disorder virus (CYSDV) in *Cucumis melo* L. Hortic Sci 35:110–113
Liu J, Feng L, Li J, He Z (2015) Genetic and epigenetic control of plant heat responses. Front Plant Sci 6:267. https://doi.org/10.3389/fpls.2015.00267
Mallor C, Alvarez JM, Luis-artiga M (2003) Inheritance of resistance to systemic symptoms expression of melon necrotic spot virus (MNSV) in *Cucumis melo* L. ‘Doublon’. Euphytica 134:319–324
McCreight JD (2000) Inheritance of resistance to lettuce infectious yellows virus in melon. Hortic Sci 35:1118–1120
McCreight JD (2003) Genes for resistance to powdery mildew races 1 and 2 U.S. in melon PI 313970. Hortic Sci 38:591–594
McCreight JD (2006) Melon-powdery mildew interactions reveal variation in melon cultigens and *Podosphaera xanthii* races 1 and 2. J Am Soc Hortic Sci 131:59–65
McCreight JD, Fashing-Burdette P (1996) Resistance of PI 124112 and ‘Eldoardo-300’ melons (*Cucumis melo* L.) to papaya ring spot virus watermelon strain. In: Cucurbits toward 2000. VIth EUCARPIA meeting on cucurbit in genetic and breeding, pp 298–301

Molina RV, Nuez F (1997) Sexual transmission of the in vitro regeneration capacity via caulogenesis of Cucumis melo L. in a medium with a high auxin/cytokinin ratio. Sci Hort 70:237–241

Monforte AJ, Oliver M, Gonzalo MJ, Alvarez JM, Dolcet-Sanjuan R, Arus P (2004) Identification of quantitative trait loci involved in fruit quality traits in melon (Cucumis melo L.). *Theoretical and Applied Genetics* 108:750–758. https://doi.org/10.1007/s00122-003-1483-x

More TA (2001) Enhancement of muskmelon resistance to disease via breeding and transformation. In: Proceedings of the second international symposium, pp 205–211

Moreno E, Obando JM, Dos-Santos N, Fernandez-Trujillo JP, Monforte AJ, Garcia-Mas J (2008) Candidate genes and QTLs for fruit ripening and softening in melon. Theor Appl Genet 116:589–602

Munshi AD, Choudhary H (2014) Muskmelon. In Handbook of Vegetables Volume III (eds. K.V. Peter and P. Hazra), Studium Press, New Delhi 271–310

Munger HM, Robinson RW (1991) Nomenclature of Cucumis melo L. *Cucurbit Genet Coop Rep* 14:43–44

Napier AB (2006) Identification and confirmation of molecular markers and orange flesh color associated with major QTL for high beta-carotene content in muskmelon. M.Sc. Thesis, Texas A&M University

Nishiyama K, Guis M, Rose JKC, Kubo Y, Bennett KA, Wangjin L, Kato K, Ushijima K, Nakano R, Inaba A, Bouzayen M, Latche A, Pech J-C, Bennett AB (2007) Ethylene regulation of fruit softening and cell wall disassembly in Charentais melon. J Exp Bot 58:1281–1290

Nuez F, Pico B, Iglesias A, Esteva J, Juarez M (1999) Genetics of melon yellows virus resistance derived from *Cucumis melo* spp. agrestis. Eur J Plant Pathol 105:453–464

Nunez-Palenius GH, Gomez-Lim M, Ochoa-Alejo N (2008) Melon fruits: genetic diversity, physiology, and biotechnology features. Crit Rev Biotechnol 28:13–55

Oridate T, Atsumi H, Ito S, Araki H (1992) Genetic difference in somatic embryogenesis from seeds in melon (Cucumis melo L.). Plant Cell Tissue Organ Cult 29:27–30

Oumouloud A, Mokhtari M, Chikh-Rouhou H, Arnedo-Andrés MS, González-Torres R, Álvarez JM (2012) Characterization of the Fusarium wilt resistance Fom-2 gene in melon. Mol Breed 30:325–334

Paris KM, Zalapa JE, McCreight JD, Staub JE (2008) Genetic dissection of fruit quality components in melon (Cucumis melo L.) using a RIL population derived from exotic and elite US Western shipping germplasm. Mol Breed. https://doi.org/10.1007/S11032-008-9185-3

Pech JC, Bouzayen M, Latché A (2008) Climacteric fruit ripening: ethylene dependent and independent regulation of ripening pathways in melon fruit. Plant Sci 175:114–120

Perchepied L, Bardin M, Dogimont C, Pitrat M (2005a) Relationship between loci conferring downy mildew and powdery mildew resistance in melon assessed by QTL mapping. Phytopathology 95:556–565

Perchepied L, Dgimont C, Pitrat M (2005b) Strain-specific and recessive QTLs involved in control of partial resistance to *Fusarium oxysporum* f. sp. *melonis* race 1.2 in a recombinant inbred line population of melon. Theor Appl Genet 111:65–74

Pereira L, Ruggieri V, Pérez S et al (2018) QTL mapping of melon fruit quality traits using a high-density GBS-based genetic map. BMC Plant Biol 18:324

Perin C, Gomez-Jimenez M, Hagen L, Dogimont C, Pech J, Latché A et al (2002) Molecular and genetic characterization of a non- climacteric phenotype in melon reveals two loci conferring altered ethylene response in fruit. Plant Physiol 129:300–309

Perin C, Hagen LS, de Conto V, Katzir N, Danin-Poleg Y, Portnoy V, Baudracco-Arnas S, Chadoeuf J, Dogimont C, Pitrat M (2002a) A reference map of Cucumis melo based on two recombinant inbred line populations. Theor Appl Genet 104:1017–1034

Perin C, Hagen LS, Giovinazzo N, Besombes D, Dogimont C, Pitrat M (2002b) Genetic control of fruit shape acts prior to anthesis in melon (Cucumis melo L). Mol Gen Genomics 266:933–941

Pitrat M (2016) Melon genetic resources: phenotypic diversity and horticultural taxonomy. Springer, New York, pp 1–36

Pitrat M, Lecoq H (1983) Two alleles for watermelon mosaic virus 1 resistance in melon. Cucurbit Genet Coop Rep 6:52–53

Pitrat M, Lecoq H (1984) Inheritance of Zucchini yellow mosaic virus resistance in *Cucumis melo* L. Euphytica 33:57–61

Reddy ANK, Munshi AD, Behera TK, Kaur C (2005) Studies on genetic and biochemical parameters of introduced and indigenous germplasm in snap melon (*Cucumis melo*. L. var. *momordica* Duth. and Full.). Indian J Plant Genet Resour 18(1):91–93

Rıos P, Argyris J, Vegas J, Leida C et al (2017) ETHQV6.3 is involved in melon climacteric fruit ripening and is encoded by a NAC domain transcription factor. Plant J 91:671–683

Risser G (1973) Etude de 1′heredite de la resistance du melon (*Cucumis melo* L.) aux races. 1 et 2 de *Fusarium oxysporum* f. sp. *melonis*. Ann Amelior Plantes 23:259–263

Risser G, Banihashemi Z, Davis DW (1976) A proposed nomenclature of *Fusarium oxysporum* f sp. *melonis* races and resistance gene in *Cucumis melo*. Phytopathology 66:1105–1106

Risser G, Pitrate M, Rode JC (1977) Etude de la resistance du melon (C*ucumis melo*. L.) au virus de la mosaique du concombre. Ann Amelior Plantes 27:509–522

Robinson RW (1992) Genetic resistance in the Cucurbitaceae to insects and spider mites. In: Plant breeding reviews, John wiley, location USA, pp 309–360

Robinson RW, Provvidvnti R (1975) Susceptibility to powdery mildew in *Citrullus lanatus* (Thunb.) Matsum and Nakai. J Am Soc Hortic Sci 100:328–300

Saez C, Esteras C, Martinez C, Ferriol M, Dhillon NPS, Lopez C, Pico B (2017) Resistance to tomato leaf curl New Delhi virus in melon is controlled by a major QTL located in chromosome 11. Plant Cell Rep 36(10):1571–1584

Saladie M, Cañizares J, Phillips MA, Rodriguez-Concepcion M, Larrigaudiere C, Gibon Y, Stitt M, Lunn JE, Garcia-Mas J (2015) Comparative transcriptional profiling analysis of developing melon (Cucumis melo L.) fruit from climacteric and non-climacteric varieties. *BMC Genom* 16:440. https://doi.org/10.1186/s12864-015-1649-3

Sebastian P, Schaefer H, Telford IRH, Renner SS (2010) Cucumber (Cucumis sativus) and melon (C. melo) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia. Proc Natl Acad Sci U S A 107:14269–14273

Silva JA, da Costa TS, Luchetta L, Marini LJ, Zanuzo MR, Nora L, Nora FR, Twyman RM, Rom-baldi CV (2004) Characterization of ripening behaviour in transgenic melons expressing an antisense 1-aminocyclopropane-1-carboxylate (ACC) oxidase gene from apple. Postharvest Biol Technol 32:263–268

Silberstein L, Kovalski I, Brotman Y, Perin C, Dogimont C, Pitrat M, Klingler J, Thompson G, Portnoy V, Katzir N, Perl-Treves R (2003) Linkage map of Cucumis melo including phenotypic traits and sequence-characterized genes. Genome 46:761–773

Sousaraei N, Ramshini H, Lotfi M, Sharzei A (2018) Marker assisted backcrossing for introgression of Fusarium wilt resistance gene into melon. *Euphytica* 214(7):7

Soria C, Moriones E, Fereres A, Garzo E, Gomez-Guillamon ML (2003) New source of resistance to mosaic virus transmission by *Aphis gossypii* in melon. Euphytica 133:313–318

Taler D, Galperin M, Benjamin I, Cohen Y, Kenogsbuch D (2004) Plant eR genes that encode photorespiratory enzymes confer resistance against disease. Plant Cell 16:172–184

Thomas CE (1986) Downey and powdery mildew resistance musk melon breeding line MR-1. Hortic Sci 21:329

Thomas CE, McCreight JD, Jourdain EL (1990) Inheritance of resistance to *Alternaria cucumerina* in *Cucumis melo* line MR-1. Plant Dis 74:868–870

Tomason Y, Nimmakayala P, Levi A, Reddy UK (2013) Map based molecular diversity, linkage disequilibrium and association mapping of fruit traits in melon. Mol Breed 31:829–841. https://doi.org/10.1007/s11032-013-9837-9

Tzuri G, Zhou X, Chayut N, Yuan H, Portnoy V, Meir A et al (2015) A “golden” SNP in CmOr governs the fruit flesh color of melon (*Cucumis melo*). Plant J 82:267–279

Vegas J, Garcia-Mas J, Monforte AJ (2013) Interaction between QTLs induces an advance in ethylene biosynthesis during melon fruit ripening. Theor Appl Genet 126:1531–1544

Wang YH, Thomas CE, Dean RA (2000) Genetic mapping of a Fusarium wilt resistance gene Fom-2 in melon (Cucumis melo L.). Mol Breed 6:379–389

Wang S, Yang J, Zhang M (2011) Developments of functional markers for Fom-2-mediated *Fusarium* wilt resistance based on single nucleotide polymorphism in melon (Cucumis melo L.). Mol Breed 27:385–393

Webb RE (1979) Inheritance of resistance to watermelon mosaic virus in *Cucumis melo* L. Hortic Sci 14:265–266

Wechter WP, Whitehead MP, Thomas CE, Dean RA (1995) Identification of a randomly amplified polymorphic DNA marker linked to the Fom-2 *Fusarium* wilt resistance gene in muskmelon MR-1. Phytopathology 85:1245–1249

Whitaker TW (1979) The breeding of vegetable crop. Highlights of the past seventy five years. Hortic Sci 14:359–363

Whitaker TW, Davis GN (1962) Cucurbits cultivation and utilization. Leonard Hill, London

Yoshioka K, Hanada K, Harada T, Minobe Y, Oosawa K (1993) Virus-resistance in transgenic melon plants that express the cucumber mosaic-virus coat protein gene and in their progeny. Jpn J Breed 43:629–634

Zalapa JE, Staub JE, McCreight JD, Chung SM, Cuevas HE (2007) Detection of QTL for yield-related traits using recombinant inbred lines derived from exotic and elite US Western Shipping melon germplasm. Theor Appl Genet 114:1185–1201

Zheng XY, Wolf DW (2000) Development of a SCAR marker associated with *Fusarium* wilt resistance and the evidence of its segregation with the Fom-2 gene in melon (*Cucumis melo* L.). Subtrop Plant Sci 52:1–7

Zheng XY, Wolff DW, Baudracco-Arnas S, Pitrat M (1999) Development and utility of cleaved amplified polymorphic sequences (CAPS) and restriction fragment length polymorphisms (RFLPs) linked to the Fom-2 *Fusarium* wilt resistance gene in melon (*Cucumis melo* L.). Theor Appl Genet 99:453–463

Zhong S, Joung J-G, Zheng Y, Chen Y-R, Liu B, Shao Y, Xiang JZ, Fei Z, Giovannoni JJ (2011) High-throughput illumina strand-specific RNA sequencing library preparation. Cold Spring Harb Protoc:940–949. Pdb.prot5652. https://doi.org/10.1101/pdb.prot5652

Chapter 15
Accelerated Breeding of Cowpea [*Vigna unguiculata* (L.) Walp.] for Improved Yield and Pest Resistance

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15.1 Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is a legume crop cultivated worldwide as pulse, vegetable, forage, green manure and cover crop (Smartt 1990). Due to its high protein content in leaves, pods and grains, it is widely regarded as "poor man's meat" (Boukar et al. 2018). The primary centre of origin is Africa because it has high genetic diversity there. Cowpea can be grown easily in low fertility soils (Eloward and Hall 1987) and has the ability to fix atmospheric nitrogen like many other legumes (Ehlers and Hall 1996). Cowpea is one of the most tolerant legumes to drought because of its ability to grow in areas without irrigation and irregular rainfall (Agbicodo et al. 2009). It is one of the best crops that fit well in rice-wheat cropping systems.

The cowpea seed contains protein (23–32%), carbohydrate (17.50–60%) (Khalid and Elharadallou 2013; Kirse and Karklina 2015) and fat (1%) (Kirse and Karklina 2015) on dry weight basis. Compared to cereal and tuber crops, two- to fourfold more protein is present in cowpea (Sebetha et al. 2014; Trehan et al. 2015). Apart from this, it also contains soluble and insoluble fibre, phenolic compounds, miner-

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als and B group vitamins along with many other functional compounds which are health promoting (Mudryj et al. 2012; Liyanage et al. 2014) The tender green pods of cowpea are rich in crude protein (3.2%), iron (2.5 mg per 100 g), calcium (80 mg per 100 g), phosphorus (74 mg per 100 g), vitamin A (941 IU per 100 g), vitamin C (13 mg per 100 g) and dietary fibre (2 g per 100 g), making it an excellent vegetable (Singh et al. 2001).

The worldwide production of pulse cowpea is 7.41 million tonnes cultivated in an area of 12.58 million hectares with an average productivity of 589 kg/ha. The leading cowpea-producing countries (Table 15.1) are Nigeria (340,992 tonnes) followed by Niger (1,959,082 tonnes) grown in an area of 3,782,760 ha and 5,178,517 ha, respectively. In terms of productivity (Table 15.2), leading countries are Palestine (3929.40 kg/ha) followed by Egypt (3677.20 kg/ha) (FAOSTAT 2019).

Accelerated development of varieties should be combined with speedy dissemination of developed varieties and agile withdrawal of obsolete varieties. To reduce the risk of obsolete varieties which were developed a decade ago in a different climate than today's scenario should be replaced with varieties developed within one

Table 15.1 Leading cowpea-producing countries in the world (FAOSTAT 2019)

S. no.	Country	Area (ha)	Production (tonnes)	Productivity (kg/ha)
1.	Nigeria	3,782,760	3,409,992	901.50
2.	Niger	5,178,517	1,959,082	378.30
3.	Burkina Faso	1,254,934	603,966	481.30
4	United Republic of Tanzania	203,540	200,940	987.20
5.	Cameroon	222,625	198,201	890.30
6.	Myanmar	141,190	178,582	1264.80
7.	Kenya	260,036	146,342	562.80
8.	Mali	282,736	145,018	512.90
9.	Sudan	303,255	129,856	428.20
10.	Mozambique	331,213	87,723	264.90
11.	Democratic Republic of the Congo	170,208	72,580	426.40
12.	Senegal	159,321	59,157	371.30
13.	Malawi	100,684	48,168	478.40
14	Haiti	40,198	28,920	719.40
15.	Peru	17,912	20,341	1135.60
16.	USA	11,655	19,822	1700.70
17.	Serbia	4733	15,968	3373.60
18.	China, mainland	18,112	15,626	862.70
19.	Madagascar	15,000	13,000	866.70
20.	Uganda	25,892	12,015	464.00
21.	Sri Lanka	6807	8576	1259.90
22.	North Macedonia	2234	7974	3568.70
23.	Mauritania	22,193	7890	355.50

Table 15.2 Top five countries leading in cowpea productivity (FAOSTAT 2019)

S. no.	Country	Area (ha)	Production (tonnes)	Productivity (kg/ha)
1.	Palestine	38	148	3929.40
2.	Egypt	1932	7104	3677.20
3.	North Macedonia	2234	7974	3568.70
4.	Serbia	4733	15,968	3373.60
5.	Trinidad and Tobago	160	505	3161.30

decade. To achieve this cowpea breeding system is to be strengthened with free international exchange of germplasm, elite varieties, speed breeding, increasing the selection intensity, large-scale phenotyping and marker- and genomics-assisted selection for accuracy (Atlin et al. 2017).

15.2 Genetic Diversity and Taxonomy

Large amounts of cowpea landraces and cultivated cowpeas were present in West and Central Africa (Padulosi and Ng 1997) which is considered as the centre of origin of cowpea. Different organizations of the world hold around 36,383 cowpea germplasm (Table 15.3) under ex situ conservation (Dumet and Fatokun 2010). Apart from this, the National Bureau of Plant Genetic Resources, New Delhi, holds 5000 germplasm of cowpea under ex situ condition as exhibited in cowpea germplasm field day held on 22 October 2019. Out of the total germplasm stored under ex situ condition, the majority (60%) of the accessions were farmers' varieties/landraces, 5.2% are breeding lines, 2.0% are wild and the remaining up to 31% that were unknown are not documented (Dumet and Fatokun 2010).

Based on the characteristics of pod, seed and ovule, the cultivated types of cowpea (Table 15.4) have been divided into five cultivar groups (Pasquet 1998, 1999). Among them *unguiculata* is the largest cultivar group. The vegetable cowpea cultivar group *sesquipedalis* (also known as yardlong bean, asparagus bean, snake bean and long bean) has more than 16 ovules and seeds spaced apart within the pod (OECD 2016).

The *Vigna unguiculata* subspecies cultivated in India were *V. unguiculata* ssp. *unguiculata* and *V. unguiculata* ssp. *biflora* grown predominantly for pulse purpose, whereas *V. unguiculata* ssp. *sesquipedalis* (yardlong bean) is grown for its immature pods as vegetable. The vegetable cowpea is grown widely in India, China, Sri Lanka, Bangladesh, Indonesia and the Philippines (Pant et al. 1982; Chakraborti 1986; OECD 2016).

The classification and nomenclature of *Vigna unguiculata* species complex was done by several workers, viz. Verdcourt (1970), Marechal et al. (1978), Mithen and Kebblewhite (1993), Padulosi (1993) and Pasquet (1993/1998). Presently the *Vigna unguiculata* species complex has been divided into 11 subspecies (Padulosi 1993; Pasquet 1993a, b, 1997; Padulosi and Ng 1997) (Table 15.5). There exists a varying

Table 15.3 Number of cowpea accessions reported from various international holders under ex situ condition

Genebank	Number of cowpea accessions	Year of introduction
Angola (SADC)	172	Non-specified
AVRDC-Taiwan	322	1984
Belgium	331	1965
Benin	155	1978
Botswana (SADC)	49	Non-specified
Cote d'Ivoire	126	1990
Germany	291	1922
IITA	15,276	1971
Kenya	875	1979
Malawi (SADC)	83	Non-specified
Mauritius (SADC)	3	Non-specified
Mozambique (SADC)	29	Non-specified
Namibia (SADC)	57	Non-specified
Nigeria	384	1987
Russia	1945	1921
South Africa	886	2005
South Africa (SADC)	55	Non-specified
South Korea	910	1987
Spain	466	1981
Swaziland (SADC)	45	Non-specified
Tanzania	386	1993
Tanzania (SADC)	39	Non-specified
Togo	100	2004
USA (USDA)	8043	1936
USA (UCR)	550	1980
Zambia (SADC)	305	Non-specified
Total	36,383	

Adopted from Dumet and Fatokun (2010) document on Global Strategy for the Conservation of Cowpea (*Vigna unguiculata* subsp. *unguiculata*)

Table 15.4 The five cultivar groups of cultivated cowpea *V. unguiculata* ssp. *unguiculata* (OECD 2016)

Cultivar group	Main features
unguiculata	Contains most African grain and forage types. Pods contain more than 16 ovules/pod
melanophthalmus	These are black-eyed pea types with less than 17 ovules per pod. Americas are the main growing areas
biflora (Catiang)	The pods are short and erect with smooth seed and less than 17 ovules per pod. Common in India.
sesquipedalis	The pods are very long and fresh tender pods are consumed. Also called as yardlong beans or asparagus bean. Especially grown in China and India
textilis	This cultivar group is rare and has very long peduncles. In Africa this cultivar group was once used as fibre

Table 15.5 Classification of *Vigna unguiculata* (L.) Walp. and its subspecies complex (Pasquet 1993a, b, 1997)

S. no.	Subspecies	Growth habit	Domestication	Pollination
1.	*unguiculata*	Annual	Cultivated	Self-pollinated
2.	*dekindtiana*	Perennial	Wild	Self-pollinated
3.	*alba*	Perennial	Wild	Self-pollinated
4.	*baoulensis*	Perennial	Wild	Cross-pollinated
5.	*letouzeyi*	Perennial	Wild	Cross-pollinated
6.	*burundiensis*	Perennial	Wild	Cross-pollinated
7.	*pawekiae*	Perennial	Wild	Cross-pollinated
8.	*aduensis*	Perennial	Wild	Cross-pollinated
9.	*tenuis*	Perennial	Wild	Self-pollinated
10.	*stenophylla*	Perennial	Wild	Self-pollinated
11.	*pubescens*	Perennial	Wild	Self-pollinated

degree of crossability of the ten wild subspecies with the sole cultivated cowpea subspecies. The subspecies *dekindtiana*, *alba*, *tenuis* (and var. *spontanea*), *stenophylla* and *pubescens* were previously under *dekindtiana* subspecies, so-called conveniently as *dekindtiana* group. The subspecies *baoulensis*, *letouzeyi*, *burundiensis*, *pawekiae* and *aduensis* were previously under subspecies *mensensis* and conveniently called as *mensensis group*. The cultivated cowpea along with *dekindtiana* group was highly self-pollinated, whereas the *mensensis* group was cross-pollinated (OECD 2016).

The two botanical varieties of annual cowpea are *Vigna unguiculata unguiculata* var. *unguiculata* which is cultivated and *V.u.u* var. *spontanea* which is a wild form. The immediate progenitor of the cultivated cowpea is *V. unguiculata* ssp. *dekindtiana* sensu Verdc (*V. unguiculata* var. *spontanea* (Schweinf.) Pasquet) (Padulosi and Ng 1997).

15.3 Genetics

Cowpea is a diploid with a chromosome number of $2n = 22$. Genetics of cowpea were reviewed comprehensively by Fery (1980, 1985), Fery and Singh (1997), Singh (2002) and Boukar et al. (2018). The genetic control of various traits was presented (Table 15.6).

In vegetable cowpea breeding, both additive and dominance variances control the trait expression. High amount of variance was observed for number of pods per plant, pod yield, pod length and crude fibre content (Subbiah et al. 2013). Genetic analysis studies had shown that in vegetable cowpea, number of clusters per plant had high additive and additive × additive genetic component, while the pod weight had high broad and narrow-sense heritability suggesting that these traits should be focused during early generation selection. Selection for pod yield should be done in

Table 15.6 Genetic control of various traits in cowpea

S. no.	Trait	Number of genes involved	References
1.	Pod pigmentation	Digenic	Mustapha and Singh (2008)
2.	Pod tip pigmentation	Monogenic and digenic	Mustapha and Singh (2008)
3.	Growth habit	Monogenic	Lachyan et al. (2016)
4.	Flower colour	Monogenic	Lachyan et al. (2016)
5.	Seed coat colour	Monogenic	Lachyan et al. (2016)
6.	Seed coat colour pattern	Monogenic	Lachyan et al. (2016)
7.	100-seed weight	Five genes	Lopes et al. (2003)
8.	Stipules	Monogenic	Pandey and Dhanasekar (2004)
9.	Cowpea aphid-borne mosaic virus (CABMV)	More than one recessive gene	Orawu et al. (2013)
		Two dominant genes	Barro et al. (2016)
10.	Bacterial blight	One or two or three recessive genes	Patel (1981)
11.	Black eye cowpea mosaic virus	Single dominant gene	Fery (1985), Melton et al. (1987) and Ouattara and Chambish (1991)
12.	Cowpea aphid-borne mosaic virus	Single recessive gene with modifier genes with partial dominance	Patel et al. (1982)
13.	Cowpea mosaic virus	Single dominant gene	Eastwell et al. (1983), Bruening et al. (1987) and Ponz et al. (1988)
14.	Cowpea severe mosaic virus	Single recessive gene	de Jimenez et al. (1989)
15.	Southern bean mosaic virus	Two recessive genes	Melton et al. (1987)
16.	Southern root-knot nematode	Single dominant gene	Singh and Reddy (1986)
17.	Aphid resistance	Single dominant gene	Bata et al. (1987), Ombakho et al. (1987) and Pathak (1988)
18.	Bruchid resistance	Seed resistance controlled by two unlinked recessive genes and cytoplasmic factors	Rusoke and Fatunla (1987)
		Pod resistance controlled by partially dominant gene and cytoplasmic factors	Rusoke and Fatunla (1987)

later generations, and for multilocation testing of yield stability number of pods per plant may be used as a criterion (Pathmanathan et al. 1997). Green pod yield per plant showed positive significant correlation with pod length, ten pod weight and number of seeds per pod. The path coefficient analysis indicated that the highest positive direct effect on green pod yield per plant was exhibited by the number of

green pods per plant followed by days to 50% flowering, ash content and pod length (Hitiksha et al. 2014).

An effective cowpea breeding strategy involves combining the erect, determinate and early maturing characters of cv. *Unguiculata* (ssp. *unguiculata*) or *Biflora* (ssp. *cylindrica*) genotypes with the long, succulent and fleshy podded characters of cv. *Sesquipedalis* (ssp. *sesquipedalis*) genotypes. Crossing between genotypes of *sesquipedalis* and those of *unguiculata* and *cylindrica* revealed low success due to specific cross combinations, genetic divergence and environment. Additive genetic variance was predominant for pod length and weight and protein content in pods and seeds. Selection in the advanced generations should be based on bushy or less viny, high-yielding segregates with appreciable protein contents in pods and seeds (Hazra et al. 2007).

15.4 Improved Varieties of Cowpea

The International Institute for Tropical Agriculture (IITA) developed several pulse-type cowpea varieties (Table 15.7) with high yield ranging from 1.5 to 2 tonnes per hectare. The improved varieties of IITA *viz*., IT-16 (1400 kg/ha), IT-18 (1510 kg/ha), IT-04 K-321-2 (1460 kg/ha), IT-97 K-390-2 (1370 kg/ha) and IT-99 K-494-4 (1660 kg/ha) matures in about 90-94 days and are tolerant to drought, leaf spot and bacterial diseases and have a reddish-brown seed colour. All these IITA developed varieties have protein content of more than 25% (Lopez 2019). The variety IT99K-494-6 is an Alectra-resistant variety (Boukar et al. 2012). The pulse type of cowpea gives a maximum yield of 1.5 to 2.0 tonnes per hectare, whereas by cultivating vegetable cowpea bush varieties (Table 15.8), the maximum yield of up to 15–18 tonnes per hectare can be taken in 6–8 pickings based on the variety cultivated. But for cultivating vegetable cowpea, irrigation is required at regular intervals, and the first harvest of stringless tender pods is taken 55 days after sowing.

Table 15.7 Improved varieties of cowpea by IITA

Cowpea variety	Year of release	Country	References
IT97K-499-35	2008	Nigeria	Boukar et al. (2012)
IT89KD-288, IT89KD-391	2009	Nigeria	Boukar et al. (2012)
IT97K-499-35, IT97K-499-38, IT98K-205-8	2009	Niger	Boukar et al. (2018)
IT97K-499-35, IT93K-876-30	2010	Mali	Boukar et al. (2018)
IT99K-573-1-1	2010	Niger	Boukar et al. (2018)
IT99K-573-1-1, IT99K-573-2-1	2011	Nigeria	Boukar et al. (2012)

(continued)

Table 15.7 (continued)

Cowpea variety	Year of release	Country	References
IT97K-1069-6, IT00K-1263 and IT82E-16	2011	Mozambique	Boukar et al. (2012)
IT99K-494-6	2011	Malawi	Boukar et al. (2012)
IT99K-7-21-2-2-1, IT99K-573-1-1	2012	Tanzania	Boukar et al. (2018)
IT99K-573-2-1, IT98K-205-8	2013	Burkina Faso	Boukar et al. (2018)
IT95K-193-12	2013	Benin	Boukar et al. (2018)
IT-16, IT-18, IT-04 K-321-2, IT-97 K-390-2 and IT-99 K-494-4	2015	Swaziland	Lopez (2019)
IT00K-1263, IT99K-1122	2015	Tanzania	Boukar et al. (2018)
IT07K-292-10, IT07K-318-33	2015	Nigeria	Boukar et al. (2018)
IT99K-573-2-1, IT99K-573-1-1	2015	Sierra Leone	Boukar et al. (2018)
IT99K-573-2-1, IT99K-573-1-1	2016	Ghana	Boukar et al. (2018)
IT90K-277-2, IT07K- 211-1-8	2016	South Sudan	Boukar et al. (2018)
IT99K-573-2-1 and IT98K-205-8	2019	Burkina Faso	Lopez (2019)

Table 15.8 Improved varieties of vegetable cowpea in India

S. no.	Variety	Yield (q/ha)	Developing organization	Country	Breeding method
1.	Kashi Kanchan	150–175	IIVR, Varanasi	India	Back cross pedigree selection
2.	Kashi Nidhi	125–150	IIVR, Varanasi	India	Pedigree selection
3.	Kashi Gauri	100–125	IIVR, Varanasi	India	Pedigree selection
4.	Kashi Unnati	125–150	IIVR, Varanasi	India	Pedigree selection
5.	Kashi Shyamal	80–100	IIVR, Varanasi	India	Selection from local collection Kala Jhamla
6.	Arka Garima	75–100	IIHR, Bengaluru	India	Pedigree selection
7.	Arka Samrudhi	75–100	IIHR, Bengaluru	India	Pedigree selection
8.	Arka Suman	75–100	IIHR, Bengaluru	India	Pedigree selection
9.	Pusa Komal	75–100	IARI, New Delhi	India	Pedigree selection
10	Swarna Harita	100–125	HARP, Ranchi	India	Selection

Fig. 15.1 Symptoms of cowpea golden mosaic disease on cowpea line VRCP-195-2

15.5 Breeding Cowpea for Pest Resistance

15.5.1 Cowpea Golden Mosaic Disease Resistance

In cowpea, infections caused by viruses are the most important as they can reduce the production from 60% to 80% in susceptible varieties. Among them, cowpea golden mosaic disease (CPGMD) is of prime importance causing extensive losses of 40–78% in production (Santos and Freire-Filho 1984). This disease is caused by begomovirus of the Geminiviridae family. The main symptom (Fig. 15.1) was golden mosaic of the leaves which then coalesces and cause complete yellowing of the leaves. The vector for transmission is whitefly. Resistance to CPGMD is attributed to two dominant and independent genes (Sangwan and Rish 2004) and single dominant gene (Kumar et al. 1994; Rodrigues et al. 2012). In Brazil, three AFLP markers, E.AAC/M.CCC515, E.AGG/M.CTT280 and E.AAA/M.CAG352, were found linked to CGMV resistance gene at 50.4, 24.4 and 28.7 LOD scores, respectively (Rodrigues et al. 2012). The cowpea golden mosaic DNA A virus isolates from India and Nigeria has similarity of only 62% which indicates that there exists a great viral diversity in cowpea golden mosaic virus isolates globally (Winter et al. 2002).

To identify the cowpea genes that confer durable resistance to CPGMD, we should use defined gemini virus isolates for controlled inoculation of indicator cowpea genotypes where it produces typical golden mosaic symptoms consistently in proven susceptible genotypes and no symptoms in resistance genotypes (Singh et al. 1997). Another feasible method for transmission of the virus is by grafting the diseased plant scion onto host plant root stock by top cleft or side cleft grafting. For better success, the rootstock and scion should be of similar thickness (Green 1991).

15.5.2 Cercospora *Resistance*

In humid tropics, *Cercospora* leaf spot (CLS) (Fig. 15.2) is an important disease of cowpea causing a yield loss from 36% to 42% (Schneider et al. 1976; Fery et al. 1977). *Cercospora* leaf spot-causing pathogens in cowpea are *Pseudocercospora cruenta* (Deighton 1976) and *Cercospora apii s. lat. emend.* (Crous and Braun 2003). Booker and Umaharan (2008) developed four crosses from the above four resistant genotypes and two susceptible genotypes CB27 and Los Banos Bush Sitao no.1 and developed six populations (Parent 1, Parent 2, F1, F2, BC1 and BC2) for

Fig. 15.2 *Cercospora* infestation on leaves of CP2 vegetable cowpea variety

each cross combination to know the genetics of inheritance to Cercospora leaf spot disease caused by Pseudocercospora cruenta in cowpea. He also observed that there was a differential resistance to both the pathogens among the tested cowpea varieties. For *P. cruenta* alone, four genotypes, VRB-10, IT-86D-719, IT87D-939-1 and IT-87D-792, were found resistant. Booker and Umaharan (2008) developed four crosses from the above four resistant genotypes and two susceptible genotypes CB27 and Los Banos Bush Sitao no.1 and developed six populations (Parent 1, Parent 2, F1, F2, BC1 and BC2) from each cross to know the genetics of inheritance to *Cercospora* leaf spot disease caused by *Pseudocercospora cruenta* in cowpea. These populations were screened under induced epiphytotic conditions in four separate field experiments. The onset of CLS disease varied from 35 to 48 days after sowing. The results from this study showed that resistance to CLS is governed by genetic mechanisms varying from monogenic, oligogenic to polygenic inheritance. In the cross CB27 × IT86D-719, intermediate level of resistance was found in F1 generation, and normal distribution was observed in F2 generation for CLS disease which confers polygenic resistance. Oligogenic inheritance was observed in other three crosses. In the cross CB27 × IT87D-939-1, single gene model with incomplete dominance was observed followed by single gene model with complete dominance in the cross CB27 × VRB-10. A trigger model was observed in the cross Los Banos Bush Sitao × IT86D-792 where three major genes were involved. In all these crosses, the role of minor genes was also observed. Based on symptomatic to non-symptomatic plants' ratio, these probable inheritance mechanisms were observed.

15.5.3 Anthracnose Resistance

In cowpea, anthracnose is caused by *Colletotrichum lindemuthianum* which is one of the destructive diseases. Field cowpeas (*Vigna unguiculata* ssp. *cylindrica*) show various levels of resistance to this disease, whereas vegetable-type cowpeas (*Vigna unguiculata* ssp. *sesquipedalis*) are highly susceptible to this disease. The linked markers identified for this disease are ISSR primers UBC 810 and UBC 811 which have yielded markers at 1.4 and 1.5 kb in resistant genomes, respectively, whereas RAPD primer OPA02 has yielded a marker at 850 bp in susceptible genome (Pradhan et al. 2018). In cowpea, the genetics of anthracnose resistance is not reported, while in various legumes, the gene action was reported and confusing. Polygenic resistance to anthracnose was reported in common bean (Sousa et al. 2014), and the genes offering resistance were fine mapped (Sousa et al. 2015). In lupin, single dominant gene has conferred resistance to anthracnose (Yang et al. 2012).

15.5.4 Bruchid Resistance

The main storage pest of cowpea causing considerable loss is cowpea seed beetle (*Callosobruchus maculatus* (P.)) commonly known as bruchid. Apart from seed loss, it reduces the seed quality and affects germination. The bruchid resistance is

characterized by delayed and staggered infestation along with lower bruchid emergence (Singh and Singh 1989). It was observed that after infestation of 200 g cowpea seed sample in different cowpea varieties with 2 pairs of bruchid had 25–26% seeds damaged in resistant lines, while there was 95% damaged seeds in susceptible variety after storing for 103 days (Singh et al. 1985). The bruchid resistance in cowpea is governed by two pairs of recessive genes which showed that any outcrossing reduces the resistant plants' proportion in the succeeding generation. The line Tvu 2027 was identified as moderately resistant to bruchids. Apart from this, IT84S-2246-4 is another important line which has combined resistance to bruchids, aphids and thrips along with resistance to ten diseases. For bruchid resistant breeding plants should be selected in F2 based on plant type, maturity, seed type and resistance to diseases, and then the F3 seed from individual plant progeny of each F2 plant was tested for bruchid and aphid resistance. Then the selected progenies from subsequent F4, F5 and F6 generations were selected for insect and disease resistance along with yield (Singh and Singh 1985). A number of *Vigna* species were also screened for resistance to *Callosobruchus maculates* and were found that *V. luteola* and *V. adenantha* were immune and *V. oblongifolia* and *V. racemosa* were moderately resistant (Ofuya 1987). The most of these *Vigna* species do not cross with cultivated *Vigna*.

15.5.5 Pod Borer Resistance

Maruca vitrata also called as legume pod borer is an important cowpea pest that causes huge yield losses between 20% and 80% if no control measures are employed. The larva of *Maruca* is the most destructive stage that causes damage mainly during reproductive stage of the plant by feeding on the young shoots, floral parts, pods and seeds. In comparison with any other insect pests of cowpea, *Maruca* causes higher yield loss (Fatokun 2009). Through conventional breeding, varieties resistant to aphids and thrips and low levels of resistance to storage weevil were developed, less progress was observed while breeding resistance to *Maruca* in cowpea. After screening several cowpea accessions along with their wild relatives, it was found that *Vigna vexillata* accessions have resistance to *Maruca vitrata* (Fatokun 2009). Strong cross-incompatibility exists between *V. vexillata* and *V. unguiculata*, making the gene transfer impossible (Fatokun 2009). The best alternative is development of transgenic cowpea against legume pod borer by using crystal proteins (Cry) and vegetative insecticidal proteins (Vips) of the *Bacillus thuringiensis* (Bt) bacterium (Bett et al. 2017). Five Vip genes, *vip3Aa35*, *vip3Af1*, *vip3Ag*, *vip3Ca2* and *vip3Ba1*, for resistance to *Maruca* pod borer were identified, cloned and over-expressed in *Escherichia coli* to produce Vip3 protein. Among these Vip3Ba1 proteins was selected as a candidate gene for cowpea transformation because of its effective larval growth inhibition. Transgenic lines with Vip3Ba protein expression were found completely free from *Maruca* pod borer in insect feeding trials. From this, it was

proposed that combining existing *cry*-transgenic cowpea and *vip*-transgenic cowpea will provide additional resistance and the greatly delay the resistance development by *Maruca* (Bett et al. 2017).

To know the genetics of transgenic cowpea carrying *Cry1Ab transgene*, two lines of transgenic cowpea (TCL-709 and TCL-711) containing *transgene Cry1Ab* were crossed with three traditional cowpea genotypes (IT97K-499-35, IT93K-693-2 and IT86D-1010) and found monogenic segregation in F2 and BC1 with 3:1 and 1:1, respectively, by using *Bt* strips analysis and also by artificial infestation of legume pod borer. As there was stable transmission in sexual generations of *cry*-transgenic cowpea under lab and field conditions, transgenic cowpea varieties for insect resistance can be developed by combining conventional breeding with marker-assisted selection (Mohammed et al. 2015).

First genetically modified cowpea resistant to pod borer was introduced in Nigeria in 2011 (Klopez 2009; Abutu 2017) and then to Burkina Faso, Ghana and Malawi (Gomes et al. 2019). The Nigerian Biosafety Management Authority (NBMA) approved the commercial release of GM cowpea on 29 January 2019 to Nigeria farmers which facilitated the release of Pod Borer-Resistant Cowpea (PBR Cowpea)-event AAT709A (Lopez 2019).

15.6 Tissue Culture Plant Regeneration Protocols for Cowpea

In many tropical legumes, limited transformation protocols were reported due to their regeneration inability under tissue culture conditions (Somers et al. 2003). As phenolic levels are high that lead to explants' oxidation, the Leguminosae family is highly recalcitrant (Anthony et al. 1999). In spite of several numerous protocols for cowpea in vitro regeneration, there was no efficient protocol in vitro regeneration due to difficulty in reproducibility and very low regeneration frequency (Anand et al. 2000).

Raveendar et al. (2009) developed a rapid highly efficient system of organogenesis in cowpea, where the seeds were pretreated for 3 days with 13.3 μM BAP and were cultured for 2–3 weeks on MSB5 medium supplemented with 6.6 μM BAP for induction of multiple shoot buds. The multiple shoot buds were transferred onto a 0.5 μM BAP amended medium for shoot elongation. On a growth regulator-free medium, the elongated shoots were rooted and then the plantlets were transferred to soil after 12 days, with a survival success of 90-95%. Here MS medium (Murashige and Skoog 1962) with B5 (Gamborg et al. 1968) vitamins (MSB5) containing 3% (w/v) sucrose and 0.7% agar supplemented with growth regulators was used. The pH of the medium was adjusted to 5.8 by using 1 M NaOH or 1 M HCl and autoclaved at 1.06 kg cm^{-2} at 121 °C for 15 minutes. The incubation conditions for the culture include 25 ± 2 °C with irradiance of 50 μmol m^{-2} s^{-1} with 16 hours of photoperiod and 55% relative humidity.

15.7 Embryo Rescue

For *Vigna* species, the medium containing MS basal nutrients (Murashige and Skoog 1962) with sucrose (88 mM), casein hydrolysate (500 mg L^{-1}) and agar (8 g L^{-1}), but devoid of plant growth regulators (EGM), was found to be the best medium for successful germination of immature embryos in four *Vigna* species, *Vigna vexillata*, *V. lanceolata*, *V. marina*, *V. luteola*, and two mung bean subspecies, *V. radiata* ssp. *radiata* and *V. radiata* ssp. *sublobata* (Palmer et al. 2002).

15.8 Genomics-Assisted Breeding

The integration of new technologies into public plant breeding programs can make a powerful step change in agricultural productivity when aligned with principles of quantitative and Mendelian genetics (Cobb et al. 2019). Cowpea (*Vigna unguiculata* L.) has a chromosome number of $2n = 22$ and an estimated genome size of 640.6 Mbp (Lonardi et al. 2019). Initially Munoz-Amatriain et al. (2017) developed a highly fragmented draft assemblies and BAC sequence assemblies of cowpea genotype IT97K-499-35, but they lacked completeness required for genome annotation, candidate gene investigation and complete genome comparisons. So, Lonardi et al. (2019) developed an assembly of the single haplotype inbred genome of cowpea genotype IT97K-499-35 by exploiting the synergies between single-molecule real-time sequencing, optical and genetic mapping and an assembly reconciliation algorithm. Repetitive elements were present in about half of the sequences assembled in cowpea that propound that differences among genome size of *Vigna* species were mainly due to the changes in Gypsy retrotransposon quantity. Based on synteny with common bean (*Phaseolus vulgaris*), revised chromosome numbering has been adopted for cowpea chromosomes (Lonardi et al. 2019).

Molecular markers permit the indirect selection for desired alleles of genes of interest, independent of the conditions and stage of crop growth (Moose and Mumm 2008). Markers were adopted for breeding in cowpea which includes 1536-SNP GoldenGate assay (Muchero et al. 2009), which has enabled the linkage mapping and QTL analysis by Luca et al. (2011), Muchero et al. (2013) and Pottorff et al. (2014) (Amatriain et al. 2017). Timko et al. (2008) published gene space sequences in IT97K-499-35 genome approximately accounting for 160 Mb. Apart from this, in the software HarvEST:Cowpea, 29,728 unigene sequences were available (harvest.ucr.edu) (Muchero et al. 2009).

15.9 Conclusion

In cowpea, the pedigree selection should be combined with marker-assisted breeding, embryo rescue technology, genomics-assisted breeding and transgenic technology to develop multiple pest-resistant cowpeas in the present-day climate change

scenario. Apart from the above technologies used for cowpea improvement, speed breeding technology for cowpea is to be standardized so that 6–8 generations can be taken in a year making the accelerated development of cowpea varieties with improved yield and pest resistance.

References

Abutu A (2017) Brighter Days for African Farmers-As Pod-Borer Resistant Cowpea Edge Towards Commercialisation. Available from: https://aatfnews.aatfafrica.org/?p=669

Agbicodo EM, Fatokun CA, Muranaka S, Visser RGF, Der CGLV (2009) Breeding drought tolerant cowpea: constraints, accomplishments, and future prospects. Euphytica 167:353–370. https://doi.org/10.1007/s10681-009-9893-8

Amatriain M M, Mirebrahim H, Xu P et al. (2017) Genome resources for climate-resilient cowpea, an essential crop for food security. Plant J 89:1042–1054

Anand RP, Ganapathi A, Ramesh A, Vengadesan G, Selvaraj N (2000) High frequency plant regeneration *via* somatic embryogenesis in cell suspension cultures of cowpea (*Vigna unguiculata* L. Walp). In Vitro Cell Dev Biol Plant 36:475–480

Anthony JM, Senaratna T, Dixon KW, Sivasithamparam K, Bunn E (1999) *In vitro* regeneration of recalcitrant Australian plants. In Vitro Cell Dev Biol 35:1062

Atlin GN, Cairns JE, Das B (2017) Rapid breeding and varietal replacement are critical to adaptation of cropping systems in the developing world to climate change. Glob Food Sec 12:31–37

Barro A, De La Salle TJB, Dieni Z, Kiebre Z, Poda L, Sawadogo M (2016) Inheritance and the allelic relationship of resistance to Cowpea Aphid Borne Mosaic Virus (CABMV) in two cowpea genotypes, KVX640 and KVX396-4-5-2D, in Burkina Faso. Int J Curr Microbiol App Sci 5(8):285–292

Bata HD, Singh BB, Singh SR, Landeinde TAO (1987) Inheritance of resistance to aphid in cowpea. Crop Sci 27:892–894

Bett B, Gollasch S, Moore A, James W, Armstrong J, Walsh T, Harding R, Higgins TJV (2017) Transgenic cowpeas (*Vigna unguiculata* L. Walp) expressing *Bacillus thuringiensis* Vip*3Ba* protein are protected against the *Maruca* pod borer (*Maruca vitrata*). Plant Cell Tissue Org Cult 131:335–345

Booker HM, Umaharan P (2007) Identification of resistance to Cercospora leaf spot of cowpea. Eur J Plant Pathol 118:401–410

Booker HM, Umaharan P (2008) Quantitative resistance to Cercospora leaf spot disease caused by Pseudocercospora cruenta in cowpea. Euphytica 162:167–177

Boukar O, Abdoulaye T, Tam M, Agrama H, Tefera H, Fatokun C Boahen S (2012) A success tale on improving two legume crops in Africa. http://r4dreview.iita.org/index.php/tag/cowpea/

Boukar O, Belko N, Chamarthi S, Togola A, Batieno J, Owusu E, Haruna M, Diallo S, Umar ML, Olufajo O, Fatokun C (2018) Cowpea (*Vigna unguiculata*): genetics, genomics and breeding. Plant Breed 138:415. https://doi.org/10.1111/pbr.12589

Bruening G, Ponz F, Glascock C, Russell ML, Rowhani A, Chay C (1987) Resistance of cowpeas to cowpea mosaic virus and to tobacco ringspot virus. In: Evered D, Harnett S (eds) Plant resistance to viruses. Wiley, Chichester, pp 23–37

Chakraborti AK (1986) Cowpea. In: Bose TK, Som MG, Kabir J (eds) Vegetable crops. Naya Prakash, Calcutta, pp 603–611

Cobb JN, Juma RU, Biswas PS, Arbelaez JD, Rutkoski J, Atlin G, Hagen T, Quinn M, Ng EH (2019) Enhancing the rate of genetic gain in public-sector plant breeding programs: lessons from the breeder's equation. Theor Appl Genet 132:627–645

Crous PW, Braun U (2003) Mycospaerella and its anamorphs: 1. Names published in Cercospora and Passalora. Centraalbureau voor Schimmelcultures (CBS), Utrecht

de Jimenez CCM, Borges FOL, Debrot CEA (1989) Herencia de la resistancia del frijol (*Vigna Unguiculata* [L.] Walp.). al virus del mosaico severo del caupi. Fitopatologia Venezolana 2(1):5–9. (English summary)

Deighton FC (1976) Studies on *Cercospora* and allied genera. VI. *Pseudocercospora* Speg., Pantospora Cif. And Cercoseptoria Petr. Mycol Pap 140:1–168

Dumet D, Fatokun C (2010) Global strategy for the conservation of cowpea (*Vigna unguiculata* subsp. Unguiculata). Document by International Institute for Tropical Agriculture

Eastwell KC, Kiefer MC, Bruening G (1983) Immunity of cowpeas to cowpea mosaic virus. In: Goldberg RB (ed) Plant molecular biology. UCLA Symposia on molecular and cell biology, New series, vol XII. Alan R. Liss, New York, pp 201–211

Ehlers JD, Hall AE (1996) Genotypic classification of cowpea based on responses to heat and photoperiod. Crop Sci 36:673–679. https://doi.org/10.2135/cropsci1996.0011183X003600030026x

Eloward HO, Hall AE (1987) Influence of early and late nitrogen fertilization on yield and nitrogen fixation of cowpea under well-watered and dry field conditions. Field Crop Res 15:229–244

FAOSTAT (2019) FAOSTAT, Statistical data base. Food and Agricultural Organization of the United Nations, Rome

Fatokun C (2009) Designer (Cowpea) plants. Accessed on 16 Nov 2019 from http://r4dreview.iita.org/index.php/2009/03/08/designer-cowpea-plants/

Fery RL (1980) Genetics of *Vigna*. In: Janick J (ed) Horticultural reviews. AVI Publishing, Westport, pp 311–394

Fery RL (1985) The genetics of cowpea. A review of the world literature. In: Singh SR, Rachie KO (eds) Cowpea research, production and utilization. Wiley, Chichester, pp 25–62

Fery RL, Singh BB (1997) Cowpea genetics: a review of recent literature. In: Singh BB, Mohan Raj DR, Dashiell KE, Jackai LEN (eds) Advances in cowpea research. Co-publication of IITA and JIRCAS, IITA, Ibadan/Tsukuba, pp 13–29

Fery RL, Dukes PD, Cuthbert FP Jr (1977) Yield loss of southern pea (*Vigna unguiculata*) caused by Cercospora leaf spot. Plant Dis Rep 61(9):741–743

Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soyabean root cells. Exp Cell Res 50:151–158

Gomes AMF, Nhantumbo N, Pinto MF, Massinga R, Ramalho JC, Barros AR (2019) Breeding elite cowpea [Vigna unguiculata (L.) Walp] varieties for improved food security and income in Africa: opportunities and challenges. Conference Proceedings. Intech Open. http://dx.doi.org/10.5772/intechopen.84985

Green SK (1991) Guidelines for diagnostic work in plant virology. AVRDC technical bulletin, vol 15. AVRDC, Taipei

Hazra P, Chattopadhaya A, Dasgupta T, Kar N, Das PK, Som MG (2007) Breeding strategy for improving plant type, pod yield and protein content in vegetable cowpea (*Vigna unguiculata*). Acta Hortic 752:275–280

Hitiksha KP, Acharya RR, Patel SR (2014) Interrelationship among green pod yield and its yield contributing characters and biochemical characters vegetable cowpea [*Vigna unguiculata* L. (Walp.)]. Trends Biosci 7(23):3972–3976

Khalid II, Elharadallou SB (2013) Functional properties of cowpea (*Vigna unguiculata* L. Walp), and Lupin (*Lupinus termis*) flour and protein isolates. J Nutr Food Sci 3:1–6

Kirse A, Karklina D (2015) Integrated evaluation of cowpea (*Vigna unguiculata* (L.) Walp.) and maple pea (*Pisum sativum* var. *arvense* L.) spreads. Agron Res 13:956–968

Klopez (2009) Is genetically modified cowpea safe? Available from http://r4dreview.iita.org/index.php/2009/03/08/640/. Accessed 16 Nov 2019

Kumar K, Dahiya SB, Rish N (1994) Inheritance to cowpea yellow MOSAIc virus in cowpea (*Vigna unguiculata* (L.) Walp.). In: Rishi N, Ahuja KL, Singh BP (eds) Virology in the tropics. Malhotra Publishing House, New Delhi

Lachyan TS, Desai SS, Dalvi VV (2016) Inheritance study of qualitative and quantitative characters in cowpea varieties (*Vigna unguiculata* (L.) Walp.). Electron J Plant Breed 7(3):708–713. https://doi.org/10.5958/0975-928X.2016.00091.0

Liyanage R, Perera OS, Wethasinghe P, Jayawardana BC, Vidanaarachchi JK, Sivaganesan R (2014) Nutritional properties and antioxidant content of commonly consumed cowpea cultivars in Sri Lanka. J Food Legum Indian J Pulses Res 27:215–217

Lonardi S, Amatriaın MM, Liang Q, Shu S, Wanamaker SI, Lo S, Tanskanen J, Schulman AH, Zhu T, Luo MC, Alhakami H, Ounit R, Hasan AM, Verdier J, Roberts PA, Santos JRP, Ndeve A, Dolezell J, Vrana J, Hokin SA, Farmer AD, Cannon SB, Close TJ (2019) The genome of cowpea (*Vigna unguiculata* [L.] Walp.). Plant J 98:767–782

Lopes FCC, Gomes RLF, Filho FRF (2003) Genetic control of cowpea seed sizes. Sci Agric 60(2):315–318. https://doi.org/10.1590/S0103-90162003000200016

Lopez K (2019) News crop: Cowpea. https://www.iita.org/news-crop/cowpea/

Lucas M R, Diop N N, Wanamaker S, Ehlers J D, Roberts P A and Close T J (2011) Cowpea-soybean synteny clarified through an improved genetic map. Plant Genome 4: 218–224. https://doi.org/10.3835/plantgenome2011.06.0019

Marechal R, Mascherpa JM, Stainer F (1978) Etude taxonomique d'un groupe complexe d'especes des genres *Phaseolus* et *Vigna* (Papilionaceae) sur la base de donnees morphologiques et polliniques, traitees par l'analyse informatique. Boissiera 28:1–273

Melton A, Ogle WL, Barnett OW, Caldwell JD (1987) Inheritance of resistance to viruses in cowpeas. Phytopathology 77:642. (Abstract)

Mithen R and Kibblewhite H (1993) Taxonomy and ecology of *Vigna unguiculata* (Leguminosae-Papilionoideae) in South Central Africa. Kirkia 14(1):100–113

Mohammed BS, Ishiyaku MF, Abdullahi US, Katung MD (2015) Genetics of *cry1ab* transgene in transgenic cowpea. Prod Agric Technol 11(1):108–116

Moose SP, Mumm RH (2008) Molecular plant breeding as the foundation for 21st century crop improvement. Plant Physiol 147:969–977

Muchero W, Diop NN, Bhat PR et al (2009) A consensus genetic map of cowpea *Vigna unguiculata* (L) Walp. and synteny based on EST derived SNPs. Proc Natl Acad Sci U S A 106:18159–18164

Muchero W, Roberts PA, Diop NN, Drabo I, Cisse N, Close TJ, Muranaka S, Boukar O, Ehlers JD (2013) Genetic architecture of delayed senescence, biomass, and grain yield under drought stress in cowpea. PLoS ONE 8:e70041

Mudryj AN, Yu N, Hartman TJ, Mitchell DC, Lawrence FR, Aukema HM (2012) Pulse consumption in Canadian adults influences nutrient intakes. Br J Nutr 108:27–36

Munoz-Amatriain M, Mirebrahim H, Xu P, Wanamaker SI, Luo M, Alhakami H, Alpert M, Atokple I, Batieno BJ, Boukar O, Bozdag S, Cisse N, Drabo I, Ehlers JD, Farmer A, Fatokun C, Gu YQ, Guo YN, Huynh BL, Jackson SA, Kusi F, Lawley CT, Lucas MR, Ma Y, Timko MP, Wu J, You F, Barkley NA, Roberts PA, Lonardi S, Close TJ (2017) Genome resources for climate-resilient cowpea, an essential crop for food security. Plant J 89:1042–1054

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Mustapha Y, Singh BB (2008) Inheritance of pod colour in cowpea (*Vigna unguiculata* (L.) Walp.). Sci World J 3(2):39–42

OECD (2016) Cowpea (*Vigna unguiculata*). In: Safety assessment of transgenic organisms in the environment, Volume 6: OECD consensus documents. OECD Publishing, Paris

Ofuya TI (1987) Susceptibility of some *Vigna* species to infestation and damage by *CaJlosobruchus maculatus* (F.). J Stored Prod Res 23:137–138

Ombakho GA, Tyagi Ap, Pathak RS (1987) Inheritance of resistance to the cowpea aphid in cowpea. Theror Appl Genet 74:817–819

Orawu M, Melis R, Laing M, Derera J (2013) Genetic inheritance of resistance to cowpea aphid-borne mosaic virus in cowpea. Euphytica 189:191–201. https://doi.org/10.1007/s10681-012-0756-3

Ouattara S, Chambish OL (1991) Inheritance of resistance to blackeye cowpea mosaic virus in "White acre-BVR" cowpea. HortScience 26:194–196

Padulosi S (1993) Genetic diversity, taxonomy, and ecogeographic survey of the wild relatives of cowpea (*Vigna unguiculata* (L.) Walpers). Thesis, University Catholique de Louvain-La-Neuve, Louvain

Padulosi S, Ng NQ (1997) Origin, taxonomy and morphology of *Vigna unguiculata* (L.) Walp. In: Singh BB, Mohan-Raj DR, Dashiell KE, Jackai LEN (eds) Advances in cowpea research. Co-publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan

Palmer JL, Lawn RJ, Adkins SW (2002) An embryo-rescue protocol for *Vigna* interspecific hybrids. Aust J Bot 50:331–338

Pandey RN, Dhanasekar P (2004) Morphological features and inheritance of foliaceous stipules of primary leaves in cowpea (*Vigna unguiculata*). Ann Bot 94:469–471. https://doi.org/10.1093/aob/mch161

Pant KC, Chandel KPS, Joshi BS (1982) Analysis of diversity in Indian cowpea genetic resources. SABRAO J 14:103–111

Pasquet RS (1993a) Classification infraspecifique des formes spontanées de *Vigna unguiculata* (L.) Walp. à partir des données morphologiques. Bulletin du Jardin Botanique National deBelgique 62(1/4):127–173. https://doi.org/10.2307/3668271

Pasquet RS (1993b) Two new subspecies of *Vigna unguiculata* (L.) Walp. Kew Bull 48:805–806

Pasquet RS (1997) A new subspecies of *Vigna unguiculata*. Kew Bull 52(4):840. https://doi.org/10.2307/4117815

Pasquet RS (1998) Morphological study of cultivated cowpea *Vigna unguiculata* (L.) Walp. Importance of ovule number and definition of cv. gr Melanophthalmus. Agronomie 18(1): 61–70. http://dx.doi.org/10.1051/agro:19980104

Pasquet R S (1999) Genetic relationships among subspecies of *Vigna unguiculata* (L.) Walp. based on allozyme variation. Theoretical and Applied Genetics 98 (6): 1104–1119

Patel PN (1981) Pathogen variability and host resistance to three races of the bacterial pustule pathogen in cowpea. Trop Agric (Trinidad) 58:275–280

Patel PN, Mligo JK, Leyna HK, Kuwit C, Mmbaga ET (1982) Source of resistance, Inheritance and breeding of cowpea for resistance to a strain of cowpea aphid-born mosaic virus from Tanzania. Indian J Genet 42:221–229

Pathak RS (1988) Genetics of resistance to aphid in cowpea. Crop Sci 28:474–476

Pathmanathan U, Rasiah PA, Syed QH (1997) Genetic analysis of yield and its components in vegetable cowpea (*Vigna unguiculata* L. Walp). Euphytica 96(2):207–213

Ponz F, Russell ML, Rowhani A, Bruening G (1988) A cowpeas line has distinct genes for resistance to tobacco ringspot virus and cowpea mosaic virus. Phytopathology 78:1124–1128

Pottorff MO, Li G, Ehlers JD, Close TJ, Roberts PA (2014) Genetic mapping, synteny, and physical location of two loci for *Fusarium oxysporum* f. sp. *tracheiphilum* race 4 resistance in cowpea *Vigna unguiculata* (L.) Walp. Mol Breed 33:779–791

Pradhan D, Mathew D, Mathew SK, Nazeem PA (2018) Identifying the markers and tagging a leucine-rich repeat receptor-like kinase gene for resistance to anthracnose disease in vegetable cowpea [*Vigna unguiculata* (L.) Walp.]. J Hortic Sci Biotechnol 93(3):225–231

Raveendar S, Premkumar A, Sasikumar S, Ignacimuthu S, Agastian P (2009) Development of a rapid, highly efficient system of organogenesis in cowpea *Vigna unguiculata* (L.) Walp. S Afr J Bot 75:17–21

Rodrigues MA, Santos CAF, Santana JRF (2012) Mapping of AFLP loci linked to tolerance to cowpea golden mosaic virus. Genet Mol Res 11(4):3789–3797

Rusoke DG, Fatunla T (1987) Inheritance of pod and seed resistance to the cowpea seed beetle (*Callosobruchus maculatus* Fabr.). J Agric Sci (Camb) 108:655–660

Sangwan SR, Rish N (2004) Genetics of resistance to cowpea yellow mosaic virus in cowpea (*Vigna unguiculata* (L.) Walp.). Indian J Mycol Plant Pathol 34:620–622

Santos AAD, Freire-Filho FR (1984) Reducao da producao do feijao massacar causado pelo vírus do mosqueado amarelo. Fitopatol Bras 9:407

Schneider RW, Williams RJ, Sinclair JB (1976) Cercospora leaf spot of cowpea: models for estimating yield loss. Phytopathology 66:384–388

Sebetha ET, Modi AT, Owoeye LG (2014) Cowpea crude protein as affected by cropping system, site and nitrogen fertilization. J Agric Sci 7:224–234

Singh B B, Chambliss O L, Sharma B (1997). Recent advances in cowpea. In: Singh B.B., Mohan-Raj D.R., Dashiel K.E., Jackai L.E.N. (Eds.). Advances in cowpea research.Co-publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), Ibadan, Nigeria 30–49

Singh BB (2002) Recent genetic studies in cowpea. In: Fatokun CA, Tarawali SA, Singh BB, Kormawa PM, Tamo M (eds) Challenges and opportunities for enhancing sustainable cowpea production. Proceedings of the World cowpea conference III held at the International Institute of Tropical Agriculture (IITA), Ibadan, 4–8 Sept 2000. IITA, Ibadan, pp 3–13

Singh DB, Reddy PP (1986) Inheritance of resistance to root-knot nematode in cowpea. Indian J Nematol 16:284–285

Singh BB, Singh SR (1985) Breeding for bruchid resistance in cowpea. In: Fujii K, Gatehouse AMR, Johnson CD, Mitchel R, Yoshida T (eds) Bruchids and legumes: economics, ecology and coevolution. Proceedings of the second international symposium on bruchids and legumes (ISBL-2) held at Okayama, 6–9 Sept 1989

Singh BB, Singh SR, Adjadi O (1985) Bruchid of resistance in cowpea. Crop Sci 25:736–739

Singh J, Kalloo G, Singh KP (2001) Vegetable crops: nutritional security. Indian Institute of Vegetable Research, Varanasi, p 56

Smartt J (1990) The old world pulses: *Vigna* species. In: Grain legumes: evolution and genetic resources. Cambridge University Press, Cambridge, pp 140–175

Somers DA, Samac DA, Olhoft PM (2003) Recent advances in legume transformation. Plant Physiol 131:892–899

Sousa LL, Cruz AS, Vidigal Filho PS, Vallejo VA, Kelly JD, Gonçalves-Vidigal MC (2014) Genetic mapping of the resistance allele Co-52 to *Colletotrichum lindemuthianum* in the common bean MSU 7-1 line. Aust J Crop Sci 8:317–323

Sousa LL, Gonçalves AO, Gonçalves-Vidigal MC, Lacanallo GF, Fernandez AC, Awale H, Kelly JD (2015) Genetic characterization and mapping of anthracnose resistance of common bean landrace cultivar Corinthiano. Crop Sci 55:1900–1910. https://doi.org/10.2135/cropsci2014.09.0604

Subbiah A, Prabhu M, Rajangam J, Jagadeesan R, Anbu S (2013) Genetic analysis of vegetable cowpea [*Vigna unguiculata* (L.) Walp.]. Legum Res 36(1):1–9

Timko MP, Rushton PJ, Laudeman TW, Bokowiec MT, Chipumuro E, Cheung F, Town CD, Chen X (2008) Sequencing and analysis of the gene-rich space of cowpea. BMC Genomics 9:103

Trehan I, Benzoni NS, Wang AZ, Bollinger LB, Ngoma TN, Chimimba UK et al (2015) Common beans and cowpeas as complementary foods to reduce environmental enteric dysfunction and stunting in Malawian children: study protocol for two randomized controlled trials. Trials 16:520

Verdcourt B (1970) Studies in the leguminosae-Papilionoideae for the 'flora of tropical East Africa': IV. Kew Bull 24(3):507–569. www.jstor.org/stable/4102859

Winter S, Butgereitt A, Thottappilly G (2002) Cowpea golden mosaic virus and related geminiviruses associated with *Vigna* spp. in Nigeria. Poster presentation. Intern Virology Congress

Yang H, Tao Y, Zheng Z, Li C, Sweetingham MW, Howieson JG (2012) Application of next generation sequencing for rapid marker development in molecular plant breeding: a case study on anthracnose disease resistance in *Lupinus angustifolius* L. BMC Genomics 13:318–328. https://doi.org/10.1186/1471-2164-13-318

Chapter 16
Recent Trends in Sweet Pepper Breeding

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16.1 Introduction

Sweet pepper also called bell pepper or capsicum is a cultivar of the species *Capsicum* with fruits which are large and bell-shaped. Scientifically known as *Capsicum annuum* var. *grossum*, it originated in Mexico, Central America and northern South America. It is an important cash crop cultivated for blocky, round, conical, thick-fleshed, non-pungent fruits. Sweet pepper fruits can be of different colours including red, yellow and orange on ripening and green, white and purple at unripened stage. It is a popular exotic vegetable used in many continental food preparations. The fruits are used for salad, stuffing or cooked as vegetable. It is one of the best known luxury vegetable appreciated worldwide for its flavour, aroma and an excellent source of provitamin A and vitamin C (Chassy et al. 2006). Its fruits are important constituents of many special recipes. It is also rich in minerals like iron, potassium, calcium, magnesium, phosphorus, sodium and selenium (Aggarwal et al. 2007).

Sweet pepper breeding aims at delivering hybrids and varieties with superior performance in the most broadly grown group like blocky and conical types. Market sizes, growing area, condition of cultivation (open or protected) as well as planting dates are important factors which are taken into consideration while deciding the breeding goals. The biggest challenge in sweet pepper breeding is improving traits that are complex in nature, traits that constitute multiple genetic factors and/or are sensitive to environmental and growing conditions, yield, adaptability, abiotic stresses and shelf life. Further the market and consumer demand requires being

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innovative and faster than the competition. Breeding for new colours, new shapes, new tastes and higher digestibility are some examples for diversification of sweet pepper breeding programme. With new innovative interventions coming up, more molecular breeding tools are available which give breeders the opportunity to better understand and select for these complex traits. New areas that can be foreseen as opportunities for the growers are the use of rootstocks in pepper just as in case of tomato. Although rootstock breeding is still at its infancy, it has the potential to address many biotic and abiotic stresses.

16.2 Crop Biology

It is a cool season vegetable. Bell pepper can be grown both as an open field crop and as a protected greenhouse crop. Under open field conditions, bell pepper is grown in hills during summers and during winters in plains. Under protected conditions, bell pepper can be grown throughout the year. The protected cultivation is getting preference over open field cultivation for off-season quality production by extending availability of the quality produce, higher productivity and improved nutritional attributes of the polyhouse produce.

Sweet pepper normally has solitary flowers borne in the leaf axils. Calyx is five-lobed, while corolla is five-parted. It has a single style which is usually longer than the stamens. Self-pollination is main mode for fruit formation; however some amount of outcrossing due to insect activity has also been observed. Anthesis usually occurs after flower opening, and the flowers remain open for 2–3 days. In sweet pepper, flower opening commences at 7:0 a.m. which continues up to 11:00 a.m., but the peak time is between 7:15 a.m. and 10:00 a.m. The anthers dehisce after 30 min of flower opening. Pollen viability and stigma receptivity are maximum on the day of anthesis, but the stigma remains receptive up to 2 days after anthesis. During cold and cloudy days, flower opening is delayed.

For open field condition sweet pepper requires long and warm growing season, which is frost-free for 4–5 months. The crop is cultivated mainly in tropical and sub-tropical regions of the world during cooler season and in temperate regions during summers. The ideal temperature for growth is 21°C at night and 27°C during the day. Flower drop occurs and fruits develop very slowly if temperature deviates beyond this ideal range. Under protected cultivation, sweet pepper can be grown almost throughout the year. Efforts to breed varieties adapted to protected cultivation are underway.

16.3 Breeding Objectives

As in any other crop, the main objective for sweet pepper improvement is high yield coupled with improved fruit quality characters. Breeding sweet peppers involve developing varieties and hybrids with blocky, half long or conical shape having

medium to dark green fruits at unripe stage. Efforts to generate different colour sweet pepper genotypes like red, yellow or orange at maturity is also an area of focus. These coloured capsicums (red, orange and yellow) have vitamin A precursors like α- and β-carotene and β-cryptoxanthin which is desired for human health. Quality improvement in sweet pepper also includes selection and developing new breeding lines of peppers with high levels of antioxidants, vitamins and pleasing flavour. These include ascorbic acid (vitamin C) and flavonoids (phenolics). Breeding efforts are directed to improve agronomic traits like earliness, fruit weight, fruit number and yield both under open field condition and protected cultivation. Peppers are affected by a range of diseases and pests, and breeding for resistance to these stresses is also an important area of focus. Sweet pepper is affected by many pathogens, but diseases of importance are *Phytophthora* fruit rot, anthracnose, *Cercospora* leaf spot, viruses, powdery mildew and bacterial wilt under open field cultivation. Sweet pepper is also attacked by insect pests like thrips, mites, aphids and fruit borer, and developing resistance against them is also important objective. With changing climate and for wider adaptability of sweet pepper, genetic improvement for abiotic stresses like heat, frost, water stress, salinity, etc. also becomes important. Breeding sweet pepper genotypes with wider adaptability is another important objective as it is a cool season crop, and hence tropicalization is necessary. This will ensure availability of the crop in nontraditional areas during greater part of the year. Activities also include breeding for stability of carotenoid extracts under long-term storage conditions and against photooxidation. Under protected cultivation breeding, sweet pepper lines with indeterminate growth habit, amenability to training and pruning, blocky fruit and resistance to root knot nematode are the major breeding. Specific problems identified for protected cultivation are yield and earliness, obtaining fruit set at low temperatures without hormone application either through parthenocarpic varieties or by improving both the anther dehiscence and the pollen viability. Sweet pepper breeding also tries to explore the possibilities of transgenics or in vitro culture for the improvement of the crop.

16.4 Heterosis Breeding

In recent times the production scenario of capsicum has changed with the increasing popularity of hybrids cultivated on commercial scale. Hybrids not only led to a boom in productivity but also helped in improving the fruit quality. Sweet pepper presents much scope for its improvement through heterosis breeding (Sood and Kumar 2010). The systematic approach for developing F_1 hybrids in any crop depends primarily on magnitude of desirable heterosis. The major objective of increasing sweet pepper productivity can be achieved by utilizing heterosis breeding (Joshi and Singh 1980). The high economic heterosis for fruit yield and other traits in sweet pepper have been reported in cross combination PRC-1 × Rani Sel-1, California Wonder × SSP and Rani Sel-1 × Sel-12-2-1 over standard check (Vinod et al. 2013). Several researchers Zecevic (1997), Ahmed et al. (2003), Milerue and Nikornpun (2006) and Sujiprihati et al. (2007) have reported significant heterosis

for various traits such as earliness, fruit number per plant, fruit girth and fruit yield in sweet pepper. These cross combinations showing high yield potential could be utilized in hybrid breeding and can also be released as hybrids after further field testing. Some of the experimental hybrids HTCH-13Y, KTCH-155, KTCH-17 and KTCH-142 have revealed significant heterosis for fruit weight and fruit yield under polyhouse conditions at IARI, Regional Station Katrain (Fig. 16.1).

Hybrid cultivars are very popular among the farmers due to their superior performance. Seed of hybrids is produced by using hand emasculation and pollination method that is why seed cost per kilogram is very high ranging between Rs.1.0 Lac and 5.0 Lakh. The seed cost can be reduced by using genetic mechanisms like male sterility. Both genic male sterility and cytoplasmic genic male sterility can be used in sweet pepper hybrid seed production. Genic or nuclear male sterility in peppers was first reported by Martin and Crawford (1951) in *Capsicum frutescens*. About 20 ms genes that inherit independently have been reported in peppers. Male sterility is peppers controlled by single recessive nuclear gene. The allelic association between ms genes is still unknown (Lee et al. 2011). To construct a new genic male sterile line through backcross breeding method is tedious and time-consuming. At present, a very few genic male sterile lines are in use in hybrid development in peppers (Dhaliwal and Jindal 2014). The main limitation of using GMS line is that it produces 50 percent fertile plants and 50 percent sterile plants in the progeny. It requires a lot of skill to identify and maintain sterile plants through sib mating.

Fig. 16.1 Sweet pepper hybrid development under polyhouse conditions at Katrain

The rouging of male fertile plants in the hybrid seed production field is very tedious and labour-intensive. However, the system is stable over environments and can be used in bell pepper and hot pepper hybrid seed production.

Cytoplasmic male sterility was first reported by Peterson (1958) in Indian line PI164835. It is a maternally inherited trait encoded by the mitochondrial genome. The male sterile phenotype is determined by interaction of sterile cytoplasm and recessive nuclear gene *rf*. Fertility in CMS plants is restored by fertility restorer gene *Rf*. The fertility may also be restored temporarily when the plants are exposed to low temperature (Shifriss and Guri 1979; Shifriss 1997). Use of cytoplasmic male sterile lines in hybrid development requires A line (female parent/sterile line), B line (maintainer line for maintaining sterility in A line) and C line (male line for restoring fertility in A line). The CMS system has the advantage of producing 100% male sterile progeny but is influenced by the environmental factors especially the low temperature (Peterson 1958; Shifriss and Guri 1979), multiallelic inheritance (Min et al. 2008, 2009) and the modifier genes (Shifriss and Guri 1979; Lee et al. 2008). The effort to develop CMS lines in sweet pepper was started at Katrain in 2014. The backcross generations of three CMS lines (KCS1A, KCS2A and KCS3A) under conversion have been advanced up to BC5 for the development of stable indigenous CMS lines in sweet pepper (Fig. 16.2). These CMS lines possess bell shape, medium to large fruits and red to yellow colour along with other desirable horticultural traits.

Fertility restoration (*Rf)* allele is commonly found in hot pepper and the maintainer allele (*rf*) in bell pepper (Zhang et al. 2000; Kumar et al. 2007). Marker-assisted introgression of *Rf* allele in bell pepper background was carried out at IARI, Regional Station, Katrain. A marker CRF-S870 was screened across the lines for the presence of fertility restoration (*Rf*) allele in bell pepper and validated only in one bell pepper line KTCR15. Analysis of segregation for fertility behaviour in cross KTCA5 × KTCR15 (sweet pepper × sweet pepper) revealed the goodness of fit to the 3:1 and 1:1 (fertility/sterility) ratio in F2, and backcross populations) indicated that the fertility restorer gene trait is controlled by a single dominant gene.

Fig 16.2 Development of CMS lines in sweet pepper at Katrain

Markers linked to the modifier genes can be useful to breed C lines with the ability to restore full fertility of the hybrid population (Lee et al. 2008a), thus increasing commercial crop yields. The CMS linked markers improve efficiency of the system by ensuring 100% pure stand of the seed parent, thus reducing hybrid seed costs and increasing seed yield (Lee et al. 2010d).

16.5 Disease Resistance Breeding

Bell pepper is affected by a number of diseases such anthracnose (*Colletotrichum capsici*), fruit rot (*Phytophthora nicotianae*), damping off (*Pythium aphanidermatum*), powdery mildew (*Leveillula taurica*), stem rot (*Sclerotium rolfsii*), wilt (*Fusarium oxysporum* f.sp. *capsici* Riv.), bacterial wilt (*Ralstonia solanacearum*) and root rot (*Rhizoctonia solani*) out of which powdery mildew, anthracnose and fruit rot are most serious destructive diseases of bell pepper (Isaac 1992). Anthracnose causes yield losses up to 50 percent in sweet pepper (Pakdeevaraparn et al. 2005), powdery mildew causes heavy yield losses ranging from 14 to 20 percent due to severe defoliation and number of fruits per plant (Brand et al. 2002) and *Phytophthora* blight affects the plants at any growth stage and kills seedling (Erwin and Ribeiro 1996), while bacterial wilt caused by *R. solanacearum* (Hayward 1991) causes yield losses up to 100 percent (Kishun 1987) due to entire plant death.

16.5.1 Phytophthora Rot

Different models of resistance have been reported for resistance to *P. capsici*. Criollo de Morelos 334 (CM334), a resistant source to *P. capsici* has been reported to have two genes, while some report more genes conferring resistance (Guerrero-Moreno and Laborde 1980; Ortega et al. 1991, 1992; Reifschneider et al. 1992; Walker and Bosland 1999; Thabuis et al. 2003; Sy et al. 2005). Another source of resistance PI 201234 has been reported to have a single dominant gene (Saini and Sharma 1978; Kim and Hur 1990) or single gene with modifying genes (Barksdale et al. 1984) governing resistance, while from "Perennial" multiple genes with additive or epistatic effects are involved in resistance (Lefebvre and Palloix 1996). Polygenetic inheritance with high-order epistasis has also been identified by some workers (Pochard and Daubeze 1980; Palloix et al. 1988; Bartual et al. 1991, Pflieger et al. 2001; Lefebvre et al. 2002; Ogundiwin et al. 2005; Bonnet et al. 2007; Minamiyama et al. 2007; Truong et al. 2012). Efforts have been made to identify quantitative trait loci (QTL) linked with *P. capsici* resistance and transfer these QTLs into elite material (Thabuis et al. 2003, 2004a; Ogundiwin et al. 2005; Sugita et al. 2006; Jin et al. 2007; Minamiyama et al. 2007; Kim et al. 2008a; Truong et al. 2012; Liu et al. 2014). Introgression of genes for resistance to *P. capsici* is difficult using traditional breeding like backcross methods. It has been reported that resistance in introgressed genotypes is lower than the donor parent due to loss of other secondary genes con-

trolling resistance to Phytophthora (Palloix et al. 1990). Recurrent selection has been used to move polygenic resistance into elite material (Thabuis et al. 2004b). However, linkage drag associated with low yield, small and undesirable fruit and less vigorous plants is a major limitation to wide adoption of resistant cultivars. Growers would rather plant high-yielding, high-quality, more uniform cultivars that are susceptible to *P. capsici* and risk losing a portion of their crop than plant less adapted but resistant cultivars. Even cultivars that had field resistance to *P. capsici*, e.g., Paladin (Dunn et al. 2014), became susceptible within a decade as the pathogen

16.5.2 Powdery Mildew

In the past few decades, powdery mildew has been increasing in both greenhouse- and open field-grown sweet peppers worldwide (Damicone 2009; Sudha and Lakshmanan 2009; Cerkauskas et al. 2011). The disease is characterized by premature defoliation thereby reducing crop yields and making fruits unfavourable for marketing. Developing powdery mildew disease resistance in sweet pepper is one of the main objectives of genetic and breeding programmes. Pepper genotypes showing varied resistance levels against powdery mildew have been identified in *C. annuum*, *C. frutescens*, *C. baccatum* and *C. chinense* (Ullassa et al. 1981; Deshpande et al. 1985; Pochard et al. 1986; Anand et al. 1987; De Souza and Café-Filho 2003). Pepper genotypes 'H-V-12' and '4638' (*C. annuum*), 'IHR 703' (*C. frutescens*) and CNPH 36, 38, 50, 52, 279, and 288 (*C. baccatum*) are resistant to *L. taurica* (Anand et al. 1987; De Souza and Café-Filho 2003). According to De Souza and Café-Filho (2003), most *C. annuum* species are moderately to highly susceptible to powdery mildew, whereas *C. baccatum*, *C. chinense* and *C. frutescens* species are often resistant, suggesting that among *Capsicum* species, resistance to powdery mildew is primarily found in taxa other than *C. annuum*. Powdery mildew resistance in pepper is reported to be a dominant and polygenic trait (Anand et al. 1987; Murthy and Deshpande 1997; Blat et al. 2005). Genetic analyses have also indicated that relatively few genetic factors with significant additive and epistatic effects confer resistance to powdery mildew in different pepper genetic backgrounds (Daubèze et al. 1995; Murthy and Deshpande 1997; Blat et al. 2005, 2006). At least three pairs of incompletely dominant genes are thought to confer resistance to powdery mildew in the *C. frutescens* line 'IHR 703' (Anand et al. 1987). The most important and durable source of powdery mildew resistance reported is in the small-fruited pungent *C. annuum* accession 'H3' from Ethiopia (Daubèze et al. 1995; Lefebvre et al. 2003). In addition, the *L. taurica*-resistant Israeli pepper line 'H-V-12' was derived from a cross between the resistant cultivar 'H3' and the susceptible cultivar 'Vania'(Shifriss et al. 1992); at least three genes appear to control resistance to *L. taurica* in 'H3' (Shifriss et al. 1992; Daubèze et al. 1995). Lefebvre et al. (2003) identified seven genomic regions, including additive quantitative trait loci (QTLs) and epistatic loci that contribute to the resistance of the cultivar 'H3' using a double-haploid population derived from a cross between 'H3' and 'Vania'.

16.5.3 *Anthracnose in Sweet Pepper*

Reports say anthracnose is a seed-borne disease which causes a marketable yield loss of approximately 50% in Malaysia (Sariah 1994), 15% in Korea (Kim and Park 1988), 35% in Indonesia (Sastrosumarjo 2003) and an approximately 80% yield loss in Thailand (Poonpolgul and Kumphai 2007). This disease basically affects the quality of fruits (Azad 1991). The anthracnose disease caused by *C. truncatum* (synonym *C. capsici*) has been most commonly found in chilli (*C. annuum* L.) (Montri 2009). In the USA, *C. acutatum* is considered to be the most destructive species of *Colletotrichum* as it affects both ripe and unripe pepper fruit, while *C. gloeosporioides* only affects ripe pepper fruit (Harp et al. 2008). One of the most economical and significant strategies to reduce crop losses is to cultivate resistant varieties or hybrids. The success of the breeding programme in developing durable resistant varieties has been limited due to the association of multiple *Colletotrichum* species in anthracnose infection (Sharma et al. 2005; Than et al. 2008; Saxena et al. 2014), along with the differential capabilities of the pathogenic virulence (Montri 2009). The use of resistant varieties not only eradicates anthracnose but also removes the chemical and mechanical responses to the disease.

Breeding methods like pedigree, backcrossing and recurrent selection have been commonly utilized for disease resistance improvement in peppers. Quantitative trait locus (QTL) mapping approach was followed by Voorrips et al. (2004) to study the inheritance of anthracnose resistance in an F_2 population derived from the cross of *C. annuum* × *C. chinense*. Successful resistant lines have been reported to be derived from the backcrossing susceptible pepper genotype of *C. annuum* with resistant chilli pepper genotypes from *C. baccatum* and *C. chinense* (Lee et al. 2010a; Mahasuk et al. 2009; Sun et al. 2015; Suwor et al. 2015). Breeding for anthracnose resistance started in early 1990s (Park et al. 1990; Mahasuk et al. 2009), involving some *Capsicum* species with a potential resistance trait, such as *C. annuum*, *C. frutescens* and *C. baccatum*. Resistant sources to anthracnose found that *Capsicum baccatum* lines have been found to be better than those compared to other *Capsicum* spp. (Park et al. 2009) and have proven to be a useful genetic resources for anthracnose resistance.

16.6 Rootstock Breeding in Capsicum

Grafting in *Capsicum* species first began in Japan and Korea, but currently it is the least grafted among the solanaceous family (Lee et al. 2010b). The available rootstocks in pepper are not so beneficial, and there is need to develop new rootstocks to address the market opportunities. Popular rootstocks are from *Capsicum annuum*, but genotypes from cultivated species of *Capsicum* like *C. baccatum*, *C. chinense* and *C. frutescens* and their interspecific hybrids with *C. annuum* have also been tested as rootstocks for pepper scions (Lee et al. 2010b). Studies of Oka et al. (2004)

reveals that wild relatives of *Capsicum* such as *C. chacoense* may lead to the development of more robust rootstocks. Developing new rootstocks through breeding followed by selection needs to be continuous process as the soil pathogens may acquire resistance against a particular rootstock being utilized (Ros-Ibáñez et al. 2014). This starts with screening different accessions of *Capsicum* species for selection of the right rootstock (Gisbert et al. 2013). Rootstocks from *C. annuum* have been found to be more compatible for pepper production in comparison to other cultivated species of *Capsicum* as reflected through better fruit yield and quality (de Oliveira et al. 2009). Attempts to graft peppers on *S. scabrum* and *S. gilo*, 7 *Solanum* species and 13 eggplant lines have been found to be unsuccessful (Tai et al. 2004).

Grafting in peppers mainly targets soilborne pathogens like *Phytophthora capsici*, nematodes and viruses such as tobacco mosaic virus or potato Y virus. Other objectives for rootstock breeding include finding excellent scion compatibility, resistance to abiotic stresses, improving fruit yield and quality (Gisbert et al. 2010; Chávez-Mendoza et al. 2013; Jang et al. 2013). Rootstocks having moderate to high resistance to *M. incognita* and *M. javanica* have been identified in *C. annuum* and *C. frutescens* genotypes (Oka et al. 2004; Geboloğlu et al. 2011; Pinheiro et al. 2015). Further *C. annuum* genotypes and intraspecific hybrid rootstocks have been utilized to manage fungal diseases caused by *Fusarium oxysporum* and *Verticillium dahliae* (Geboloğlu et al. 2011). Interspecific pepper hybrids were created for use as rootstocks, and these exhibited exceptional tolerance to *Phytophthora* and viruses (Lee and Oda 2003). Saadoun and Allagui (2013) found wild serrano-type pepper SCM334, a successful rootstock against root rot and wilt (*Phytophthora nicotianae*). Improved agronomic performance and marketable yield have been achieved using different *C. annuum* rootstocks (Colla et al. 2008), but it depends also on the rootstock genotype used (Doñas-Uclés et al. 2014). *C. annuum* rootstocks have also been used to increase the nutritional fruit quality in terms of β-carotene, vitamin C and total antioxidant capacity (Chávez-Mendoza et al. 2013).

C. baccatum was evaluated as rootstock in terms of graft compatibility with sweet pepper and then tested for resistance to *Meloidogyne* nematodes. The graft showed resistance to *M. javanica* and susceptibility to *M. incognita* and *M. enterolobii* (Oka et al. 2004; Pinheiro et al. 2015). *C. baccatum* var. *pendulum* rootstock also exhibited tolerance to salinity (Penella et al. 2014, 2015) and flooding damage (Palada and Wu 2008a). Hybrid of C. annuum × C. baccatum rootstocks have been developed and patented for providing multiple disease resistances and high yield (Hennart 2014). Rootstock from *C. frutescens* genotypes provide resistance to *M. incognita* and *M. javanica* (Oka et al. 2004; de Oliveira et al. 2009; Gisbert et al. 2013; Pinheiro et al. 2015) but were susceptible to *M. enterolobii* (Pinheiro et al. 2015). *C. frutescens* rootstock have shown submergence tolerance but resulted in poor fruit yield and quality when compared with C. annuum rootstocks (de Oliveira et al. 2009). *C. chinense* has also been evaluated for resistance to nematodes as rootstock, but all elite genotypes of *C. chinense* were susceptible to *M. incognita*, *M. javanica* and *M. enterolobii* (Pinheiro et al. 2015) except few which showed resistance to *M. incognita* (Oka et al. 2004; de Oliveira et al. 2009; Gisbert et al. 2013). Graft compatibility studies have been conducted in *C. chinense* (Oka et al. 2004) where

one genotype has manifested highest tolerance to soil salinity (Penella et al. 2013). Rootstocks from *C. annuum* × *C. chinense* hybrids have been extensively utilized to increase growth and yield of green pepper (Lee et al. 2010).

Sweet pepper cultivar 'Shishito' was grafted on interspecific hybrids of *C. annuum* × *C. chinense*, and the grafted plants were found to be resistant to bacteria and more productive in nature (Yazawa et al. 1980). Similarly pepper hybrids 'Edo' and 'Lux' grafted on commercial rootstocks were taller and gave higher yield than self-rooted plants but were similar to self-rooted plants for quality traits like dry matter, ^{0}Brix and titratable acidity (Colla et al. 2008). Grafting in sweet peppers results in morphological changes like erect or pendant fruits in cluster or solitary habit (Yagishita and Hirata 1987; Ohta 1991), two-lobed fruits instead of four (Taller et al. 1998, 1999), dwarfism and plants with small leaves and fruit malformations (Hirata et al. 2003). There are physiological changes also like changes in fruit colour, e.g. yellow instead of red on ripening (Taller et al. 1998, 1999), reduced capsaicin content and changes in Brix value (Yagishita et al. 1985, 1990; Hirata et al. 2003). These morphological and physiological changes in the scion are due to the genetic material transported from rootstock to scion. These characteristics may even be carried over into the next generation via the seed.

Sweet peppers were grafted on nematode-resistant rootstocks of *C. annuum*, *Capsicum baccatum*, *C. chinense* and *Capsicum frutescens* and grown on nematode sick soil, and it was found that yield of grafted plants was double that of non-grafted plants (Ros et al. 2002; Oka et al. 2004). The use of resistant rootstocks decreases the incidence of nematode infection and reduces the nematode population in soil to a similar level to that brought about by crop rotation (Thies and Fery 2002). However, if grafted rootstocks are grown in the same soil for a number of years, nematode populations increase, and infection occurs (Ros et al. 2002). Although it has been reported that the resistance of *Capsicum* species to nematodes remains constant at soil temperatures of up to 38°C (Di Vito et al. 1995), others suggest that resistance ceases at soil temperatures above 32°C (Thies and Fery 2000). Grafted peppers inoculated with *Phytophthora infestans* and *Fusarium oxysporum* showed resistance and produced higher yields than similarly inoculated self-rooted plants (Piedra-Buena et al. 2007). By evaluating several pepper lines (*C. baccatum*, *C. frutescens* and *Capsicum chacoense* Hunz.), rootstocks have recently been identified that give increased production of sweet pepper scions under hot-wet and hot-dry seasons (Palada and Wu 2008b).

Though grafting in peppers was initiated to combat soilborne diseases but with development in research in rootstock breeding in *Capsicum*, rootstocks compatible with sweet peppers and providing resistance to *P. capsici*, bell pepper mosaic virus (BePMV) and tomato mosaic virus (ToMV) (Palada and Wu 2008; Louws et al. 2010) have been generated. Rootstocks have also been developed for improving crops under flood conditions, salinity, soil toxicity and nutrient use efficiency (Rouphael et al. 2008; He et al. 2009) and utilizing low levels of potassium (Schwarz et al. 2013) and low temperature tolerance (Palada and Wu 2008). However, the science of rootstock breeding in pepper is much behind the cucurbits. This has resulted in lack of information about grafting behaviour in pepper with respect to compat-

ibility of rootstock variety, development of grafted behaviour and tolerance to biotic and abiotic stresses. The lack of research related with the use of grafting in peppers has caused a lack of information about grafting behaviour, with respect to compatibility of rootstock variety, development of grafted plants and tolerance to biotic and abiotic stresses (Colla et al. 2008). However, recent works show that the use of some combinations of rootstock variety may provide advantages with respect to higher yield and higher pepper fruit quality (Tsaballa et al. 2013).

16.7 Marker-Assisted Breeding

Marker-assisted selection based on DNA markers is a powerful tool to select preferred individuals among segregating populations. More often, molecular breeding implies molecular marker-assisted breeding (MAB) and is defined as the application of molecular markers, in combination with linkage maps and genomics, to alter and improve traits on the basis of genotypic assays. This term is used to describe several modern breeding strategies, including marker-assisted selection (MAS), marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS) and genome-wide selection (GWS) or genomic selection (GS). MAS has been used for breeding of various traits in pepper including male sterility, biotic stress resistance and fruit quality.

Male sterility is one of the most important traits used in hybrid pepper breeding. Application of male sterility reduces hybrid production costs by excluding the need for manual emasculation of maternal line and elimination of impurities of the seed material originated from self-pollination. Reports in the literature describe pepper genic male sterility (ms), cytoplasmic male sterility (CMS) and nuclear cytoplasmic male sterility—a combination of CMS with nuclear fertility restoration genes (Shifriss 1997; Wang and Bosland 2006). At present, the nuclear cytoplasmic male sterility has been used primarily in chilli pepper hybrid breeding, while its use in sweet pepper breeding is still limited due to lack of stability of this trait (Shifriss 1997; Zhang et al. 2000; Wang et al. 2004). More than 20 nuclear genes responsible for male sterility in pepper have been reported so far (Wang and Bosland 2006), including the ms8 nuclear recessive gene that was obtained as a result of mutagenesis induced by gamma irradiation applied to the Bulgarian variety Zlaten Medal (Daskaloff 1973, 1974). Expression of male sterility determined by ms8 gene is very stable both in the field and plastic tunnels conditions (Sztangret 1998). Cytological investigations of meiosis showed that microspore formation in ms8/ms8 plants is aborted after the second telophase, as cytokinesis is blocked and no viable pollen is formed (Nikolova et al. 2010). Genic male sterility has the advantage of simple inheritance, thus allowing a relatively quick recovery of female parental lines that contain this characteristic. However, this system has a significant disadvantage in the fact that only 50% of maternal line plants are male sterile, necessitating the removal of remaining 50% of plants that are male fertile (Rao et al. 1990; Shifriss 1997). An advanced genic male sterility system based on two nuclear

male sterility genes that would increase the number of male sterile plants up to 75% was proposed by Shifriss and Pilovsky (1993). Alternatively, the fingerprinting of homozygous male sterile genotypes using molecular markers can help in screening seedlings before experimental trials. Various types of molecular markers used in pepper genetics and molecular breeding successively included restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), single nucleotide polymorphism (SNP) and transposon-based and locus specific PCR-based markers (Lefebvre 2004). In the last years, PCR-based orthologous gene markers referred to as conserved ortholog set COSII were developed (Wu et al. 2006). These markers proved to be widely applicable to genetic studies and molecular breeding in the Solanaceae family, including pepper. The use of COSII markers supported the development of one of the most advanced molecular maps of pepper (Wu et al. 2009). Recently, several molecular markers linked to genic male sterility genes in pepper have been described. In coloured sweet pepper CAPS marker linked to ms gene of unknown origin was identified (Lee et al. 2010a). In chilli pepper a codominant SCAR marker linked to the genic male sterility gene ms1 and three AFLP markers linked to the ms3 gene were found (Lee et al. 2010b, c). So far none of these genes has been located on the pepper chromosomes. Molecular markers tightly linked to genic male sterility (ms) genes would facilitate an efficient and rapid transfer of ms genes into different genetic backgrounds through marker-assisted backcrossing. The two non-allelic genic male sterility genes ms3 and msw in hot and sweet pepper backgrounds, respectively, are monogenic recessive. Naresh et al. (2018) carried out genotyping by sequencing (GBS) in an F2 population segregating for ms3 gene in hot pepper and in an F6 inbred near-isogenic line (NIL) population segregating for msw gene in sweet pepper which yielded 9713 and 7453 single nucleotide polymorphism markers, respectively. Four candidate SNPs cosegregating with ms3 gene and one cosegregating with msw gene were identified by bulk segregant analysis and physically mapped to chromosomes 1 and 5, respectively. In hot pepper, two markers [HPGMS2 (CAPS) and HPGMS3 (dCAPS)] located 3.83 cM away from the ms3 gene and in sweet pepper the dCAPS marker SPGMS1 cosegregated (completely linked) with the msw gene have been developed. These markers have the potential to increase the efficacy of the male sterility genes for pepper breeding, as they can be useful in developing the genic male sterile lines in parental inbred lines of commercial hybrids through marker-assisted backcrossing, hybrid seed production, and genetic purity testing of hybrid seeds. Bartoszewski et al. (2012) used F2 population resulting from a cross between the sweet pepper male sterile line 320 and the male fertile variety Elf to identify DNA markers linked to the nuclear male sterility gene ms8. With the use of RAPD-BSA technique, seven markers linked to the ms8 locus were found. Four of them were converted into SCAR markers. In addition, two COSII/CAPS markers linked to the ms8 locus were identified. Comparative mapping with reference pepper maps indicated that the ms8 locus is located on the lower arm of the pepper chromosome P4.

In addition to the ms8 locus, a number of other important traits of pepper have been mapped to the P4 chromosome: the pvr2 locus for PVY resistance (Caranta

et al. 1997; Lefebvre et al. 2002), the C2 locus for fruit colour (Thorup et al. 2000), QTLs for fruit weight and shape (Zygier et al. 2005; Barchi et al. 2009) and QTL for *Phytophthora capsici* resistance (Thabuis et al. 2004a). It has to be considered in the breeding programmes aimed to introgress ms8 gene into the different breeding lines. It seems that QTLs for fruit weight and shape are closely linked to ms8 locus and selection of recombinants may be necessary.

The advent of molecular markers enabled to identify the chromosomal regions involved in the variation of the components of the *Phytophthora capsici* resistance in pepper. Thabuis et al. (2004a) used 114 doubled haploid DH lines from the cross Perennial X Yolo Wonder. A total of five different genomic regions displayed an additive effect on resistance. Four major epistatic relationships were detected between either additive QTLs or between QTLs involved only in epistatic relationships (Lefebvre and Palloix 1996; Thabuis et al. 2003). Marker-assisted selection appeared as a promising tool for breeding quantitative resistance. Regarding the genetic distance between Perennial and bell pepper accessions (Lefebvre et al. 2001), the marker-assisted backcross strategy MAB appeared as the most suitable to transfer a limited number of QTLs. However, given the imprecision around the positions of the QTLs, Hospital and Charcosset (1997) showed that MAB needed to be optimized for a successful QTL transfer. They proposed a twofold strategy: selection for the donor alleles on the carrier chromosomes (foreground selection) and, in the remaining plants, selection for the return to the recipient parent (background selection). Through their theoretical study, they showed that three markers spread along the confidence interval of each QTL enabled an efficient control of the QTLs during the introgression. Once the interval lengths and marker locations were defined, they computed the minimal population size for recovering at least one plant having the entire donor segments for a given type I error. Thabuis et al. (2004a) conducted a MAB programme to transfer favourable alleles at the four main QTLs controlling resistance to *P. capsici* from Perennial accession into YW, a bell pepper line, by taking into account the optimisations from Hospital and Charcosset (1997). To speed up the breeding process, a DH line from the mapping population, having all the chromosomal regions to be transferred (Thabuis et al. 2003), was used as the donor parent to initiate the MAB programme. They examined the results of three MAB cycles conducted according to the theoretical optimisations, the additive and epistatic effects of the transferred segments in validation populations and the impact of the background selection step on the improvement of horticultural traits. Two populations, derived by selfing the plants selected after the first selection cycle, were genotyped and evaluated phenotypically for their resistance level. The additive and epistatic effects of the four resistance factors were re-detected and validated in these populations, indicating that introgression of four QTLs in this MAB programme was successful. A decrease of the effect for the moderate-effect QTLs and of the epistatic interaction was observed. Phenotypic evaluations of horticultural traits were performed on sample of each backcross generation. The results indicated an efficient return to the recipient phenotype using this MAB strategy.

Douglas Willian Nogueira et al. (2012) attempted to determine the presence of the Pvr4 allele, which controls the resistance to the PepYMV (Pepper yellow mosaic

virus), in sweet pepper genotypes commonly available in the Brazilian market, using a CAPS codominant molecular marker. The resistance to PepYMV, in the genotypes CM-334-INRA and Myr-29 and in genotypes derived from the hybrid Mônica-R, was found to be associated with the 444 bp band linked to the resistance allele Pvr4. Homozygous resistant plants (Pvr4/Pvr4) showed a single band of 444 bp, the susceptible ones (Pvr4+/Pvr4+) showed a band of 458 bp and the heterozygous resistant plants (Pvr4+/Pvr4) showed both bands. However, in the resistant accession CM-334-UFV and in the hybrids Magali-R and Martha-R, as well as in populations derived from this accession and these hybrids, the resistance to PepYMV was not associated to the CAPS marker. The accession CM-334-UFV ('Criollo de Morelos-334' from Viçosa, MG, Brazil) was distinct from CM-334-INRA ('Criollo de Morelos-334', from France); although both accessions were resistant to PepYMV, the association of resistance with the 444 bp band was found only in CM-334-INRA.

Previous genetic studies showed that capsinoid biosynthesis is determined by loss-of-function *p-AMT* alleles (Lang et al. 2009). Recent investigation has demonstrated that *Pun1* is also involved in capsinoid biosynthesis (Han et al. 2013). It is known that many cultivars for which the fruit can be eaten raw have loss-of-function allele *pun1* (Lee et al. 2005). Yoshiyuki Tanaka et al. (2014) used DNA markers of *p-AMT* and *Pun1* in an attempt to develop a new fresh cultivar containing capsinoids in a cross-breeding programme. The genotypes of each progeny derived from the cross of 'Murasaki' (AAbb) × 'CH-19 Sweet' (aaBB) were determined using the DNA markers. Specifically, *p-AMT* genotypes were determined using a dCAPS marker and *Pun1* genotypes using a SCAR marker. From the results of genotyping, aaBB or aaBb plants were selected and developed as a new cultivar, 'Maru Salad'.

Babak et al. (2016) worked on development of DNA markers to genes determining biosynthesis of pigments (*Ccs* and *cl*) and the fruit ripening process (*nor* and *rin*) for identification of their allelic composition in sweet pepper. There are three basic unlinked genes (*Y*, *c1* and *c2*) in pepper which regulate formation of ketocarotenoids and control accumulation of pigments in fruit chromoplasts. The locus *c2* encodes phytoene synthase enzyme (*Psy*). Its mutation often causes a serious decrease in the amount of carotenoids. DNA typing of the sweet pepper collection (31 varieties) using the markers, known in the literature, has not detected genotypes with mutations in this locus. The gene *Y* encoding bifunctional capsanthin-capsorubin synthase enzyme (*Ccs*) determines synthesis of red pigments in pepper: capsanthin and capsorubin. The research was conducted for identifying the *Ccs* gene. The locus *Y* was detected in all the pepper varieties with red colour of the fruit (the presence of amplicon of 1470 bp in length), but not in the varieties with orange and yellow colour. The research is under way for developing codominant STS marker for identification of the heterozygous allelic state of the *Ccs* gene. Mutation *cl* (*chlorophyll retainer*), blocking chlorophyll destruction in ripening, is described in pepper. Depending on the allelic combination of the *cl* and *Ccs* genes, fruits become brown or green-yellow. A dCAPS marker to the *cl* mutation has been developed. The application of the developed primers has detected this mutation in four sweet pepper varieties of the studied collection characterized by brown and green-yellow colour of the fruit. Carotenoid biosynthesis depends also on the terms of

ripening. Specific genes encoding cellulase and polygalacturonase enzymes are activated in plants during fruit ripening. The STS marker to the analogue of the *rin* mutation, specific to sweet pepper, was developed. Analysis of the sweet pepper collection for this locus has shown heterogeneity of the studied accessions. In tomato, the mutation *nor* was caused by a short deletion (2 bp) leading to the translational frameshift and formation of nonfunctional protein. Sequencing of the fragment, formed by the amplification of the total DNA of pepper with tomato primers to the mutation *nor*, has shown the difference in 7 bp between sequenced alleles. The mutant allele *norc162* was identified in three accessions and *norc169*—in the other part of the sweet pepper collection.

16.8 Haploid Production

Conventional method of varietal development is very tedious and time-taking process as it includes screening, development of lines, evaluation, recognition and crossing for getting highly homozygous lines. In addition infinite capitals of water, land and fertilizers are also required. The breeding can be accelerated by the use of double haploid production. The success rate of DHs varies crop to crop, and its success depends on the efficiency of the methods used for the production of the haploids (Iqbal and Yousaf 2018). Haploid plant technology involves producing haploid or doubled haploid (DH) plants using the method involving the culturing of gamete cells (male or female) (androgenesis and gynogenesis) or chromosomal elimination methods in which a parental genome is removed after fertilization. Haploids inherit their chromosomes from a single parent. By doubling their chromosomes, they skip many generations of inbreeding even in incompatible species and convert them into a doubled haploid, resulting in rapid production of completely homozygous lines.

The first studies in anther culture of pepper were reported in 1973 using semisolid media by Kuo et al. (1973), George and Narayanaswamy (1973) and Wang et al. (1973). Pepper is accepted to be the third solanaceous crop that could be defined as recalcitrant with regard to the response to androgenesis induction (Segui-Simarro et al. 2011). Since 1973, many studies have been conducted to overcome this bottleneck and elaborate the critical factors involved in anther culture response of peppers, such as media, culture system and genotype. The two-step anther culture system, first introduced by Sibi et al. (1979) and then optimized by Dumas de Vaulx et al. (1981), was used in various bell pepper breeding programmes (Abak et al. 1982; Hendy et al. 1985; Daubeze et al. 1990; Caranta et al. 1996). However, the two-step procedure did not produce positive results for many accessions in different pepper germplasms. Later, the double-layer anther culture system was developed by Dolcet-Sanjuan et al. (1997). This technique, also called shed-microspore culture, was successfully refined by Supena et al. (2006a, b) and Supena and Custers (2011) for Indonesian hot peppers. In shed-microspore cultures, anthers float on the liquid layer for a while, and then they open and release their microspores into the medium as the microspores grow and undergo dehiscence (Supena et al. 2006b). Among the

many factors influencing successful DH plant production in pepper, the genotype effect on haploid embryogenesis has been demonstrated to be the most critical one in many studies (Kristiansen and Andersen 1993; Qin and Rotino 1993; Mityko et al. 1995; Rodeva et al. 2004, 2006; Gemes Juhasz et al. 2009; Nowaczyk et al. 2009).

The medium used in anther or microspore cultures is generally one of the most critical factors affecting androgenetic induction. Besides the effectiveness of the double-layer system, the presence of maltose in the medium instead of sucrose, which was used in both semisolid media, could provide an explanation for our positive results in shed-microspore cultures. As an alternative carbohydrate source, maltose has been in use for haploidy studies of many plant species, especially cereals. Scott et al. (1995) attributed this effect to its slower metabolism and consequently the availability of sufficient oxygen in the medium, allowing cells to live longer. The employment of maltose in pepper androgenesis studies was first reported by Dolcet-Sanjuan et al. (1997), followed by Gyulai et al. (2000), Supena et al. (2006a, b), Kim et al. (2008b), Gemes Juhasz et al. (2009) and Parra-Vega et al. (2013). Except for Kim et al. (2008), all of these researchers reported positive results by using maltose in their studies. Moreover, Vizintin and Bohanec (2004) inferred that the beneficial effect of maltose on androgenesis was dependent on the genotype. The effect of genotype on the success of anther or microspore cultures of vegetable peppers has been demonstrated as the main factor in previous studies (Kristiansen and Andersen 1993; Qin and Rotino 1993; Mityko et al. 1995; Rodeva et al. 2004, 2006; Gemes Juhasz et al. 2009; Nowaczyk et al. 2009). Ozsan and Onus (2018) reported that glutamine concentration has a positive effect on pepper androgenesis. Glutamine is known as an organic nitrogen source to supply energy for cells that cannot use energy sources effectively. They used 4 different pepper types (Erciyes, Filinta, Ergenekon and Bellisa F1) as plant materials and 12 different nutrient media combinations. To enhance embryo formation, they evaluated the effects of different combinations of MS and Gamborg B5 nutrient media with or without glutamine. They found best results with respect to pepper androgenesis on Gamborg B5 media fortified with 4.0 mg/l NAA, 0.1 or 1.0 mg/l BAP, 0.25% AC, 15.0 mg/l AgNO3 and 1.0 g/l glutamine.

Esin et al. (2016) carried out haploidy study with 48 ornamental pepper genotypes and found that androgenic induction is significantly related to the genotypes tested in shed-microspore culture protocol. Androgenic responses of 48 genotypes at the F2 or F3 generation were compared in three androgenesis protocols to determine the most effective method. Of the three protocols tested, anthers were placed on two different semisolid culture media and on a double-layer medium also called shed-microspore culture medium. The results revealed that the shed-microspore culture protocol was superior to both semisolid anther culture protocols. The average numbers of total and normal-looking embryos per bud of the most responsive genotype were 102.90 and 34.11, respectively. They regenerated 122 ornamental pepper plants including 63 DH, 52 haploid and 7 mixoploid plants.

The chromosome content of haploid plants can be doubled spontaneously or using colchicine. Keles et al. (2015) compared the rate of spontaneous doubled hap-

loidy of different pepper types. Seven charleston, six bell, eight capia and seven green pepper genotypes were used as plant material. Murashige and Skoog (MS) nutrient medium with 4 mg/L NAA, 0.5 mg/L BAP, 0.25% activated charcoal, 30 g/L sucrose and 15 mg/L silver nitrate (AgNO3) was used. Ploidy levels of plants obtained through anther culture were detected using both flow cytometry and simple sequence repeats (SSR) markers. The results showed that different spontaneous doubled haploidy rates were obtained from different pepper types. The highest rate was observed in bell pepper type with 53.4% (mean of six genotypes) of haploid plants undergoing spontaneous chromosome doubling. This was followed by charleston and capia types with 31.9% and 30.4% doubling, respectively. Green pepper type gave the lowest spontaneous doubled haploidy rate with 22.2% doubling. Esin et al (2016) in their study on 48 ornamental peppers observed a spontaneous diploidization rate of 51.6% based on flow cytometry analyses.

16.9 Transgenics

While many members of the Solanaceae family are facile with regard to cell culture and regeneration, pepper (*Capsicum annuum* L.) is considered to be recalcitrant to regeneration. So far, the most successful method of regeneration involves direct organogenesis from cotyledons and hypocotyls (Gunay and Rao 1978; Agrawal et al. 1989; Wang et al. 1991) and also from young leaves (Zhu et al. 1996). Pepper cultivars differ markedly in their regeneration requirements. The major problem during the in vitro regeneration process is shoot elongation. Regeneration is also severely limited due to the formation of ill-formed buds or shoot-like structures which either resist elongation or produce rosettes of distorted leaves that do not produce normal shoots (Husain et al. 1999). The most recent report for the regeneration of chilli pepper (*Capsicum annuum*) from cotyledon explants was developed by Husain et al. (1999) and is a highly efficient three-stage protocol. Wang et al. (1991) reported, for the first time, the recovery of transformed through *Agrobacterium*-method plantlets from cultured sweet pepper hypocotyls and cotyledons with transient GUS gene expression. However, the numbers of elongated buds and hence complete pepper plants were again limited. The development of novel direct DNA transfer methodology, such as particle bombardment (biolistics), bypassing limitations imposed by *Agrobacterium* host specificity and improving safety and quality of agricultural products, has allowed the engineering of almost all major crops, including formerly recalcitrant cereals, legumes and woody species (Christou 1995). Nianiou et al. (2002) had established a regeneration and transformation protocol for the sweet red pepper type 'Florinis' and for two pepper hybrids PO1 and C using hypocotyl explants. The rate of plant regeneration was found to depend on the types of explants cultured and the media used. Shoot bud initiation was found to more effective on MS media supplemented with IAA and BAP, and shoot bud development was promoted with addition of GA3. Rooted shoots were successfully established in soil. They applied two different methods to achieve

transformation in pepper, using *Agrobacterium* and the particle gun. Following the first method, fertile transgenic pepper plants were regenerated from hypocotyl explants that were cocultivated with *Agrobacterium tumefaciens* strain LBA4404 harbouring a plasmid that contained the gus reporter gene and the *nptII* selection gene or a plasmid with the Cu/Zn SOD gene of tomato that was expressed in chloroplasts. Transgenic pepper plants were developed, verified and characterized; however the percentage of transformed plants obtained using *Agrobacterium* was rather small. In the second method, pepper hypocotyls as explants were bombarded by the hand gene gun of Bio-Rad. The plasmid used in this transformation contained the gus reporter gene driven by the CaMV-35S promoter. They observed the particle gyn method to be more efficient as compared to *Agrobacterium*-mediated transformation protocol.

Despite several studies on establishing systems for regeneration of chilli and bell pepper cultivars, plant regeneration, especially elongation of buds produced by cultured explants, is seemingly a formidable job (Gunay and Rao 1978; Fari and Czako 1981; Diaz et al. 1988; Agrawal et al. 1989; Ochoa-Alejo and Ireta-Moreno 1990; Valera-Montero and Ochoa-Alejo 1991; Harini and Lakshmi 1993). Therefore, one of the major drawbacks of the *Agrobacterium*-mediated transformation is the recalcitrance of bell pepper, and it's difficult to regenerate nature in vitro and therefore the genetic transformation (Li et al. 2000). However, transgenic plants have been generated by regeneration from cotyledon explants (Li et al. 2003; Ko and Soh 2007), hypocotyl explants (Borychowski et al. 2002; Delis et al. 2005), cotyledonary leaves (Manoharan et al. 1998), callus (Lee et al. 2004) anther culture (Kim et al. 2007) and young leaves in C. annuum (Zhu et al. 1996) var. grossum but with low regeneration and transformation efficiencies.

There is a need to develop easy, reliable and efficient transformation protocols in bell pepper transformation for improvement, particularly in the Indian cultivars which are adapted to local conditions. It would be worthwhile to establish alternate protocols that minimize or bypass tissue culture steps for capsicum improvement. Research with *Arabidopsis* has benefited from the development of high throughput non-tissue culture in planta transformation methods that avoid plant tissue culture (Azipiroz-Leehan and Feldmann 1997). In particular, the development of the *Agrobacterium tumefaciens*-mediated vacuum infiltration method (Bechtold et al. 1993) and floral dip method (Clough and Bent 1998; Bent 2006; Zhang et al. 2006) has emerged as powerful tools and had a major impact on *Arabidopsis* research. In planta transformation methods that target the *Agrobacterium* to the differentiated embryos have also been standardized for different crops where *Agrobacterium* is directed towards either the apical meristem or the meristems of axillary buds. The strategy essentially involves in planta inoculation of embryo axes of germinating seeds and allowing them to grow into seedlings ex vitro. These in planta transformation protocols are advantageous over other methods because they do not involve regeneration procedures, and therefore the tissue culture-induced somaclonal variations are avoided.

Kumar et al. (2009) developed an in planta transformation protocol for successful transformation of bell pepper in two varieties, viz. Arka Gaurav and Arka

Mohini. They developed transgenic bell pepper plants by a tissue culture-independent *Agrobacterium tumefaciens*-mediated in planta transformation procedure. *Agrobacterium* strain EHA105 harbouring the binary vector pCAMBIA1301 that carries the genes for β-glucuronidase (*uid A*) and hygromycin phosphotransferase II (*hpt II)* was used for transformation. GUS histochemical analysis of T0 and T1 plants at various stages of growth followed by molecular analysis using PCR, southern analysis and RT-PCR allowed selection of transgenics. The method resulted in 17.8% and 11.4% of the T0 plants in Arka Gaurav and Arka Mohini being selected as chimeric, and 35.0% and 29.7%, respectively, were identified as stable transformants in the T1 generation based on PCR analysis.

References

Abak K, Pochard E, Dumas de Vaulx R (1982) Transmission of resistance to Phytophthora capsici on roots and stems of pepper plants: study of doubled haploid lines issued from the cross 'PM217'×'Yolo Wonder' through anther culture. Capsicum Newsl 1:62–63

Agrawal S, Chandra N, Kothari SL (1989) Plant regeneration in tissue cultures of pepper (Capsicum annuum L. cv. Mathania). Plant Cell Tissue Organ Cult 16:47–55

Anand N, Deshpande AA, Sridhar TS (1987) Resistance to powdery mildew in an accession of *Capsicum frutescens* and its inheritance pattern. Capsicum Eggplant Newsl 6:77–78

Agarwal A, Gupta S, Ahmed Z (2007) Influence of plant densities on productivity of bell pepper (Capsicum annuum L.) under greenhouse in high altitude cold desert of Ladakh. In: Int. Symposium on Medicinal & nutrceutical Plants. 756:309–314

Ahmed N, Hurra M, Wani SA Khan SH (2003). Gene action and combining ability for fruit yield and its component characters in sweet pepper Capsicum Eggplant Newslett 22:55–58

Azad P (1991) Fate and role of chemical constituents of chilli fruits during infection with *Colletotrichum capsici*. Indian Phytopathol 44:129–131

Azipiroz-Leehan R, Feldmann KA (1997) T-DNA insertion mutagenesis in Arabidopsis: going back and forth. Trends Genet 13:152–156

Babak OG, Nikitinskaya TV, Yatsevich KK, Nekrashevich NA, Kilchevsky AV (2016) Development of DNA-markers to fruit quality genes of sweet pepper (Capsicum annuum L.). In: Proceedings of XVIth EUCARPIA capsicum and eggplant working group meeting, Kecskemét, 12–14 Sept 2016, pp 137–144

Barchi L, Lefebvre V, Sage-Palloix AM, Lanteri S, Palloix A (2009) QTL analysis of plant development and fruit traits in pepper and performance of selective phenotyping. Theor Appl Genet 118:1157–1171

Barksdale TH, Papavizas GC, Johnson SA (1984) Resistance to foliar blight and crown rot of pepper caused by *Phytophthora capsici*. 68:506–509. https://doi.org/10.1094/PD-68-506

Bartoszewski G, Waszczak C, Gawronski P, Stepien I, Bolibok-Bragoszewska H, Palloix A, Lefebvre V, Korzeniewska A, Niemirowicz-Szczytt K (2012) Mapping of the ms8 male sterility gene in sweet pepper (Capsicum annuum L.) on the chromosome P4 using PCR-based markers useful for breeding programmes. Euphytica 186:453–461

Bartual R, Carbonell EA, Marsal JI, Tello JC, Campos T (1991) Gene action in resistance to peppers (Capsicum annuum) to Phytophthora stem blight (Phytophthora capsici L.). Euphytica 54:195–200

Bechtold N, Ellis J, Pelletier G (1993) In planta Agrobacterium-mediated gene transfer by infiltration of adult Arabidopsis thaliana plants. C R Acad Sci Paris Life Sci 316:1194–1199

Bent A (2006) Arabidopsis thaliana floral dip transformation method. Methods Mol Biol 343:87–103

Blat SF, da Costa CP, Vencovsky R, Sala FC (2005) Inheritance of reaction to Leveillula taurica (Lev.) Arn. in Capsicum baccatum. Crop Breed Appl Biotechnol 5:467–472. https://doi.org/10.1590/S0103-90162005000100008

Blat SF, da Costa CP, Vencovsky R, Sala FC (2006) Hot pepper (Capsicum chinense, Jacq.) inheritance of reaction to powdery mildew. Sci Agric 63:471–474. https://doi.org/10.1590/S0103-

Bonnet J, Danan S, Boudet C, Barchi L, Sage-Palloix AM, Caromel B et al (2007) Are the polygenetic architectures of resistance to *Phytophthora capsici* and *P. parasitica* independent in pepper? 115:253–264. https://doi.org/10.1007/s00122-007-0561-x

Borychowski A, Niemirowicz-Szczytt K, Jcdraszko M (2002) Plant regeneration from sweet pepper (Capsicum annuum L.) hypocotyls Explants. Acta Physiol Plant 24:257–264

Caranta C, Palloix A, Gebre-Selassie K, Lefebvre V, Moury B, Daubeze AM (1996) A complementation of two genes originating from susceptible Capsicum annuum lines confers a new and complete resistance to pepper veinal mottle virus. Phytopathology 86:739–743

Caranta C, Lefebvre V, Palloix A (1997) Polygenic resistance of pepper to potyviruses consists of a combination of isolatespecific and broad-spectrum quantitative trait loci. Mol Plant-Microbe Interact 10:872–878

Cerkauskas RF, Ferguson G, Banik M (2011) Powdery mildew (*Leveillula taurica*) on greenhouse and field peppers in Ontario: host range, cultivar response and disease management strategies. Can J Plant Pathol 33:485–498. https://doi.org/10.1080/07060661.2011.619828

Chassy AW, Bui L, Renaud ENC, Horn MV, Mitchell AE (2006) Three-year Comparison of the Content of Antioxidant Microconstituents and Several Quality Characteristics in Organic and Conventionally Managed Tomatoes and Bell Peppers. J Agric Food Chem18;54(21):8244–52

Chávez-Mendoza C, Sánchez E, Carvajal-Millán E, Munoz-Márquez E, GuevaraAguilar A (2013) Characterization of the nutraceutical quality and antioxidant activity in Bell pepper in response to grafting. Molecules 18:15689–15703

Christou P (1995) Strategies for variety-independent genetic transformation of important cereals, legumes and woody species utilizing particle bombardment. Euphytica 85:13–27

Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium mediated transformation of Arabidopsis thaliana. Plant J 16:735–743

Colla G, Rouphael Y, Cardarelli M, Temperini O, Rea E, Salerno A, Pierandrei F (2008) Influence of grafting on yield and fruit quality of pepper (Capsicum annuum L.) grown under greenhouse conditions. Acta Hortic 782:359–363

Damicone J (2009) Fungicide resistance management. Fact Sheets EPP-7663. Oklahoma State University Division of Agricultural Sciences and Natural Resources website. Available online at: http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Rendition-3508/F-7663web.pdf

Daskaloff S (1973) Investigation of induced mutants in Capsicum annuum L. III. Mutants in the variety Zlaten Medal. Genet Plant Breed 6:419–429

Daskaloff S (1974) Investigation of induced mutants in sweet pepper (Capsicum annuum L.). In: Proceedings of the 1st meeting of the capsicum breeding and genetics, Budapest, 1–4 July 1974, pp 81–90

Daubeze AM, Palloix A, Pochard E (1990) Resistance of androgenetic autodiploid lines of pepper to Phytophthora capsici and tobacco mosaic virus under high temperature. Capsicum Newsl 8:47–48

Daubèze AM, Hennart JW, Palloix A (1995) Resistance to *Leveillula taurica* in pepper (*Capsicum annuum*) is oligogenically controlled and stable in Mediterranean regions. Plant Breed 114:327–332. https://doi.org/10.1111/j.1439-0523.1995.tb01243.x

de Oliveira CD, Braz LT, dos Santos JM, Banzatto DA, de Oliveira PR (2009) Hot peppers resistance to root-knot-nematodes and stump/rootstock compatibility among hot peppers and red pepper hybrids. Hortic Bras 27:520–526. (in Portuguese with English abstract)

Dhaliwal MS, Jindal SK (2014) Induction and exploitation of nuclear and cytoplasmic male sterility in pepper (Capsicum spp.): a review, The Journal of Horticultural Science and Biotechnology, 89:5, 471–479

De Souza VL, Café-Filho AC (2003) Resistance to *Leveillula taurica* in the genus *Capsicum*. Plant Pathol 52:613–619. https://doi.org/10.1046/j.1365-3059.2003.00920.x

Delis M, Garbaczewska G, Niemirowica Szczytt K (2005) Differential of adventitious buds from Capsicum annuum L. hypocotyls after co-culture with Agrobacterium tumefaciens. Acta Biol Cracov Ser Bot 47:193–198

Deshpande AA, Anand N, Pathak CS, Sridhar TS (1985) New sources of powdery mildew resistance in *Capsicum* species. Capsicum Eggplant Newsl 4:75–76

Di Vito M, Zacheo G, Catalano F, Oreste G (1995) Effect of temperature on stability of resistance to root-knot nematode (Meloidogyne spp.). In: Proceedings of EUCARPIA IXth meeting on genetics and breeding on capsicum and eggplant, Budapest, 21–25 Aug, pp 230–232

Diaz I, Moreno R, Power JB (1988) Plant regeneration from protoplasts of Capsicum annuum. Plant Cell Rep 7:210–212

Dolcet-Sanjuan R, Claveria E, Huerta A (1997) Androgenesis in Capsicum annuum L.-effects of carbohydrate and carbon dioxide enrichment. J Am Soc Hortic Sci 122:468–475

Doñas-Uclés F, Jiménez-Luna M, Góngora-Corral JA, Pérez-Madrid D, Verde-Fernández D, Camacho-Ferre F (2014) Influence of three rootstocks on yield and commercial quality of Italian sweet pepper. Ciência e Agroteccnologia 38:538–545

Dumas de Vaulx R, Chambonnet D, Pochard E (1981) Culture in vitro d'anthères de piment (Capsicum annuum L.): amelioration des taux d'obtention de plantes chez différents génotypes par des traitements à+ 35 C. Agronomie 1:859–864. (in French)

Dunn AR, Lange HW, Smart CD (2014) Evaluation of commercial bell pepper cultivars for resistance to Phytophthora blight (*Phytophthora capsici*). Plant Health Research 15:19–24

Fari M, Czako M (1981) Relationship between position and Plant Health Research morphogenetic response of pepper hypocotyl ex-plant culture in vitro. Sci Hortic 15:207–213

Esin A, Hilal B, Selcen Y, Tolga Y. 2016. Androgenic responses of 64 ornamental pepper (Capsicum nnuum L.) genotypes to shed-microspore culture in the autumn season. Turkish Journal of Biology 40(3): 706-717

Geboloğlu N, Yanar Y, Yanar D, Akyazi F, Çakmak P (2011) Role of different rootstocks on yield and resistance for Fusarium oxysporum, Verticillium dahliae and Meloidogyne incognita in grafted peppers. Eur J Hortic Sci 76:41–44

Gemes Juhasz A, Kristof Z, Vagi P, Lantos C, Pauk J (2009) In vitro anther and isolated microspore culture as tools in sweet and spice pepper breeding. Acta Hortic 829:61–64

George L, Narayanaswamy S (1973) Haploid Capsicum through experimental androgenesis. Protoplasma 78:467–470

Gisbert C, Sánchez-Torres P, Raigon MD, Nuez F (2010) Phytophthora capsici resistance evaluation in pepper hybrids: agronomic performance and fruit quality of pepper grafted plants. J Food Agric Environ 8:116–121

Gisbert C, Trujillo-Moya C, Sánchez-Torres P, Sifres A, Sánchez-Castro E, Nuez F (2013) Resistance of pepper germplasm to Meloidogyne incognita. Ann Appl Biol 162:110–118

Guerrero-Moreno A, Laborde JA (1980) Current status of pepper breeding for resistance to *Phytophthora capsici* in Mexico. In: Proceedings of the IVth Eucarpia Meeting of the Capsicum working group, Wageningen

Gunay AL, Rao PS (1978) In vitro plant regeneration from hypocotyl and cotyledon explants of red pepper. Plant Sci Lett 11:365–372

Gyulai G, Gemesne JA, Sagi Z, Venczel G, Pinter P, Kristof Z, Torjek O, Heszky L, Bottka S, Kiss J et al (2000) Doubled haploid development and PCR-analysis of F1 hybrid derived DH-R2 paprika (Capsicum annuum L.) lines. J Plant Physiol 156:168

Han K, Jeong HJ, Sung J, Keum YS, Cho MC, Kim JH, Kwon JK, Kim BD, Kang BC (2013) Biosynthesis of capsinoid is controlled by the Pun1 locus in pepper. Mol Breed 31:537–548

Harini I, Lakshmi SG (1993) Direct somatic embryogenesis and plant regeneration from immature embryos of chilli (Capsicum annuum L.). Plant Sci 89:107–112

Harp TL, Pernezny K, Lewis Ivey ML, Miller SA, Kuhn PJ, Datnoff L (2008) The etiology of recent pepper anthracnose outbreaks in Florida. Crop Prot 27:1380–1384. https://doi.org/10.1016/j.cropro.2008.05.006

Hendy H, Pochard E, Dalmasso A, Bongiovanni M (1985) Transmission héréditaire de la résistance aux nematodes Meloidogyne Chitwood (Tylenchida) portée par 2 lignées de Capsicum

annuum L.: étude de descendances homozygotes issues d'androgenèse. Agronomie 5:93–100. (in French)

He Y, Zhu Z, Yang J, Ni X, Zhu B (2009) Grafting increases the salt tolerance of tomato by improvement of photosynthesis and enhancement of antioxidant enzymes activity Environ Expt Bot 66(2):270–278

Hennart JW (2014) Capsicum rootstock. Patent number US20140096289 A1

Hirata Y, Ogata S, Kurita S, Nozawa GT, Zhou J, Wu S (2003) Molecular mechanism of graft transformation in Capsicum annuum. Acta Hortic 625:125–130

Hospital F, Charcosset A (1997) Marker-assisted introgression of quantitative trait loci. Genetics 147:1469–1485

Husain S, Jain A, Kothari SL (1999) Phenylacetic acid improves bud elongation and in vitro plant regeneration efficiency in Capsicum annuum L. Plant Cell Rep 19:64–68

Iqbal J, Yousaf U (2018) Anther culturing a unique methodology in achieving homozygosity. Asian J Adv Agric Res 8(2):1–9

Jang Y, Moon JH, Lee JW, Lee SG, Kim SY, Chun C (2013) Effects of different rootstocks on fruit quality of grafted pepper (Capsicum annuum L.). Korean J Hortic Sci Technol 31:687–699

Jin A, Yongsheng H, Boaxi Z (2007) Genetic linkage map construction and QTL analysis for *Phytophthora capsici* L. in pepper. 10:003

Jindal SK, Dhaliwal MS, Meena OP (2019) Molecular advancements in male sterility systems of Capsicum: a review. Plant Breed China Vegetables. https://doi.org/10.1111/pbr.12757

Joshi, S. and Singh, B. (1980). A note on hybrid vigour in sweet pepper (Capsicum annuum L.). Haryana J. Hort. Sci 9:90–92

Joshi S, Thakur PC, Verma TS (1995) Hybrid vigour in bell shaped paprika (Capsicum annuum L.). Veg Sci 22(2):105–108

Keles D, Pınar H, Ata A, Taskın H, Yıldız S, Buyukalaca S (2015) Effect of pepper types on obtaining spontaneous doubled haploid plants via anther culture. HortScience 50(11):1671–1676

Kim B, Hur J (1990) Inheritance of resistance to bacterial spot and to Phytophthora blight in pepper. J. Kor. Soc. Hort. Sci. 31:350–357

Kim CH, Park KS (1988) A predictive model of disease progression of red pepper anthracnose. Korean J Pathol J. Kor. Soc. Hort. Sci. 4:325–331

Kim YS, Kuk YL, Kim KM (2007) Inheritance and expression of transgenes through anther culture of transgenic hot pepper. J Biosci 62:743–746

Kim HJ, Naham SH, Lee HR, Yoon GB, Kim KT, Kang BC et al (2008a) BAC-derived markers converted from RFLP linked to *Phytophthora capsici* resistance in pepper (*Capsicum annuum* L.). 118:15–27. https://doi.org/10.1007/s00122-008-0873-5

Kim M, Jang IC, Kim JA, Park EJ, Yoon M, Lee Y (2008b) Embryogenesis and plant regeneration of hot pepper (Capsicum annuum L.) through isolated microspore culture. Plant Cell Rep 27:425–434

Ko MK, Soh H (2007) Stable production of transgenic pepper plants mediated by Agrobacterium tumefaciens. Hortic Sci 42:1317–1501

Kristiansen K, Andersen SB (1993) Effects of donor plant temperature, photoperiod, and age on anther culture response of Capsicum annuum L. Euphytica 67:105–109

Kumar AM, Reddy KN, Sreevathsa R, Ganeshan G, Udayakumar M (2009) Towards crop improvement in bell pepper (Capsicum annuum L.): Transgenics (uid A::hpt II) by a tissue-culture-independent Agrobacterium-mediated in planta approach. Sci Hortic 119:362–370

Kumar S, Singh V, Singh M, Rai S, Kumar S, Rai S K and Rai M (2007) Genetics and distribution of fertility restoration associated RAPD markers in pepper (Capsicum annuum L.)Scientia Horticulturae 111:197–202

Kuo JS, Wang YY, Chien NF, Ku SJ, Kung ML, Hsu HC (1973) Investigations on the anther culture in vitro of Nicotiana tabacum L. and Capsicum annuum L. Acta Bot Sin 15:47–52

Lacasa A, Marsal JI, Tello JC (1993) Epistasis in the resistance of pepper to Phytophthora stem blight (Phytophthora capsici L.) and its significance in the prediction of double cross performances. 72:149–151. https://doi.org/10.1007/BF00023784

Lee JM, Oda M (2003) Grafting of herbaceous vegetable and ornamental crops. Hortic Rev 28:61–124

Lee YH, Kim HS, Kim JY, Jung M, Park YS, Lee JS, Choi SH, Her NH, Lee JH, Hyung NI, Lee CH, Yang SG, Harn CH (2004) A new selection method for pepper transformation: callus-mediated shoot formation. Plant Cell Rep 23:50–58

Lee CJ, Yoo EY, Shin JH, Lee J, Hwang HS, Kim BD (2005) Non-pungent Capsicum contains a deletion in the capsaicinoid synthetase gene, which allows early detection of pungency with SCAR markers. Mol Cell 19:262–267

Lee J, Hong J, Do J, Yoon J, Lee JD, Hong JH, Do JW, Yoon JB (2010a) Identification of QTLs for resistance to anthracnose to two Colletotrichum species in pepper. J Crop Sci Biotechnol 13:227–233. https://doi.org/10.1007/s12892-010-0081-0

Lee J, Yoon JB, Park HG (2008) A CAPS marker associated with the partial restoration of cyltoplasmic male sterility in chilli pepper. Mol Breeding 21:95–104

Lee JM, Kubota C, Tsao SJ, Bie Z, Hoyos Echevarria P, Morra L, Oda M (2010b) Current status of vegetable grafting: diffusion, grafting techniques, automation. Sci Hortic 127:93–105

Lee J, Han JH, An CG, Lee WP, Jae B, Yoon JB (2010c) A CAPS marker linked to a genic male-sterile gene in the colored sweet pepper, 'Paprika' (Capsicum annuum L.). Breed Sci 60:93–98

Lee J, Yoon JB, Han JH, Lee WP, Do JW, Ryu H, Kim SH, Park HG (2010d) A codominant SCAR marker linked to the genic male sterility gene (ms1) in chili pepper (Capsicum annuum). Plant Breed 129:35–38

Lefebvre V (2004) Molecular markers for genetics and breeding: development and use in pepper (Capsicumspp.). In: Lörz H, Wenzel G (eds) Biotechnology in agriculture and forestry, vol 55. Springer, Berlin/Heidelberg, pp 189–214

Lefebvre V, Palloix A (1996) Both epistatic and additive effects of QTLs are involved in polygenic induced resistance to disease: a case study, the interaction pepper-*Phytophthora capsici* Leonian. Theor Appl Genet 93:503–511. https://doi.org/10.1007/BF00417941

Lefebvre V, Goffinet B, Chauvet J-C, Caromel B, Signoret P, Brand R, Palloix A (2001) Evaluation of genetic distances between pepper inbred lines for cultivar protection purposes: comparison of AFLP, RFLPand phenotypic data. Theor Appl Genet 102:741–750

Lefebvre V, Pflieger S, Thabuis A, Caranta C, Blattes A, Chauvert JC, Daubèze A, Palloix A (2002) Towards the saturation of the pepper linkage map by alignment of three intraspecific maps including known-function genes. Genome 45:839–845. https://doi.org/10.1139/g02-053

Lefebvre V, Daubèze AM, Rouppe van der Voort J, Peleman J, Bardin M, Palloix A (2003) QTLs for resistance to powdery mildew in pepper under natural and artificial infections. Theor Appl Genet 107:661–666. https://doi.org/10.1007/s00122-003-1307-z

Li D, Xie B, Zhang B, Zhao K, Luo K (2000) The current problems and the solution for pepper disease-resistant gene engineering. Acta Hortic Sin 27:509–514. (in Chinese)

Li D, Zhao K, Xie B, Zhang B, Luo K (2003) Establishment of a highly efficient transformation system for pepper (Capsicum annuum L.). Plant Cell Rep 21:785–788

Liu W-Y, Kang J-H, Jeong H-S, Choi H-J, Yang H-B, Kim K-T et al (2014) Combined used of bulk-segregated analysis of microarrays reveals SNP markers pinpointing a major QTL for resistance to *Phytophthora capsici* in pepper. 127:2503–2513. https://doi.org/10.1007/s00122-014-2394-8

Louws FJ, L Cary, CL Rivard, C Kubota (2010) Grafting fruiting vegetables to manage soilborne pathogens, foliar pathogens, arthro-pods and weeds. Sci Hort 127:127–146

Mahasuk P, Khumpeng N, Wasee S, Taylor PWJ, Mongkolporn O (2009) Inheritance of resistance to anthracnose (Colletotrichum capsici) at seedling and fruiting stages in chili pepper (Capsicum spp.). Plant Breed 128:701–706. https://doi.org/10.1111/j.1439-0523

Manoharan M, Sree Vidya CS, Lakshmi Sita G (1998) Agrobacterium-mediated genetic transformation in hot chilli (Capsicum annuum L. var Pusa jwala). Plant Sci 131:77–83

Martin JA, Crawford JH (1951) Several types of sterility in C. frutescens. Proc. Am. Soc. Hortic. Sci 57:335–338

Milerue N, Nikorpun M (2006) Studies on heterosis of chili (Capsicum annuum L.) Kasetsart J. Nat. Sci. 34:190–196

Minamiyama Y, Tsuro M, Kubo T, Hirai M (2007) QTL analysis for resistance to *Phytophthora capsici* in pepper using a high density SSR-based map Breeding Science 57:129–134

Mityko J, Andrasfalvy A, Csillery G, Fári M (1995) Anther-culture response in different genotypes and F1 hybrids of pepper (Capsicum annuum L.). Plant Breed 114:78–80

Montri P (2009) Pathotypes of *Colletotrichum capsici*, the causal agent of chili anthracnose in Thailand. Plant Dis 93:17–20. https://doi.org/10.1094/PDIS-93-1-0017

Murthy HMK, Deshpande AA (1997) Studies on genetics of powdery mildew [*Leveillula taurica* (Lev.) Arn.] resistance in chilli (*Capsicum annuum* L.). Veg Sci 24:127–131

Naresh P, Lin S-W, Lin C-Y, Wang Y-W, Schafleitner R, Kilian A, Kumar S (2018) Molecular markers associated to two non-allelic genic MALE sterility genes in peppers (Capsicum annuum L.). Front Plant Sci 9:1343. https://doi.org/10.3389/fpls.2018.01343

Nianiou I, Karavangeli M, Zambounis A, Tsaftaris A (2002) Development of Pepper Transgenic Plants via Agrobacterium and Biolistic Transformation. Acta Hortic 579:83–87

Nikolova V, Todorova V, Stefanova Y, Tomlekova N (2010) Cytological particularities in nuclear and nuclear-cytoplasmic male sterile pepper lines. Caryologia 63:262–268

Nogueira DW, Nogueira DG, Maluf WR, Maciel GM, dos Reis Figueira A, de Menezes CB (2012) Marker-assisted selection for resistance to potyvirus in sweet pepper. Pesq agropec bras Brasília 47(7):955–963

Nowaczyk P, Olszewska D, Kisiala A (2009) Individual reaction of Capsicum F2 hybrid genotypes in anther cultures. Euphytica 168:225–233

Ochoa-Alejo N, Ireta-Moreno L (1990) Culture differences in shoot forming capacity of hypocotyls tissue of chilli pepper (Capsicum annuum L.) cultured in vitro. Sci Hortic 42:21–28

Ogundiwin EA, Berke TF, Massoudi M, Black LL, Huestis G, Choi D et al (2005) Construction of 2 intraspecific linkage maps and identification of resistance QTLs for *Phytophthora capsici* root-rot and foliar-blight diseases of pepper (*Capsicum annuum* L.). 48:698–711. https://doi.org/10.1139/g05-028

Ohta Y (1991) Graft-transformation, the mechanism for graft-induced genetic changes in higher plants. Euphytica 51:91–99

Oka Y, Offenbach R, Pivonia S (2004) Pepper rootstock graft compatibility and response to Meloidogyne javanica and M. incognita. J Nematol 36:137–141

Ortega RG, Espanol CP, Zueco JC (1991) Genetics of resistance to Phytophthora capsici in the pepper line 'SCM-334'. Plant Breed 107:50–55. https://doi.org/10.1111/j.1439-0523.1991.tb00527.x

Ortega RG, Espanol CP, Zueco JC (1992) Genetic relationships among four pepper genotypes resistant to Phytophthora capsici. 108:118–125. https://doi.org/10.1111/j.1439-0523.1992.tb00110.x

Ozsan T, Onus AN (2018) Does glutamine promote the development of pepper (Capsicum annuum L.) Anthers in vitro? J Sci Eng Res 5(11):228–236

Palada MC, Wu DL (2008a) Evaluation of chili rootstocks for grafted sweet pepper production during the hot-wet and hot-dry seasons in Taiwan. Acta Hortic 767:167–174

Palada MC, Wu DL (2008b) Evaluation of chili rootstocks for grafted sweet pepper production during the hot-dry seasons in Taiwan. Acta Hortic 767:151–156

Palloix A, Daubeze AM, Pochard E (1988) *Phytophthora* root rot of pepper influence of host genotype and pathogen strain on the inoculum density-disease severity relationships. 123:25–33. https://doi.org/10.1111/j.1439-0434.1988.tb01033.x

Palloix A, Daubeze AM, Phaly T, Pochard E (1990) Breeding transgressive lines of pepper for resistance to *Phytophthora capsici* in a recurrent selection system. Euphytica 51:141–150

Park S, Kim SH, Park HG, Yoon JB (2009) *Capsicum* germplasm resistant to pepper anthracnose differentially interact with *Colletotrichum* isolates. Hortic Environ Biotechnol 50:17–23

Parra-Vega V, Renau-Morata B, Sifres A, Segui-Simarro JM (2013) Stress treatments and in vitro culture conditions influence microspore embryogenesis and growth of callus from anther walls of sweet pepper (Capsicum annuum L.). Plant Cell Tissue Org 112:353–360

Penella C, Nebauer SG, Lopéz-Galarza S, SanBautista A, Gorbe E, Calatayud A (2013) Evaluation for salt stress tolerance of pepper genotypes to be used as rootstocks. J Food Agric Environ 11:1101–1107

Penella C, Nebauer SG, Bautista AS, López-Galarza S, Calatayud T (2014) Rootstock alleviates PEG-induced water stress in grafted pepper seedlings: physiological responses. J Plant Physiol 171:842–851

Penella C, Nebauer SG, Quiñones A, Bautista AS, López-Galarza S, Calatayud A (2015) Some rootstocks improve pepper tolerance to mild salinity through ionic regulation. Plant Sci 230:12–22

Peterson PA (1958) Cytoplasmically inherited male sterility in Capsicum. Am Nat 92:111–119

Pflieger S, Palloix A, Caranta C, Blattes A, Lefebvre V (2001) Defense response genes co-localize with the quantitative disease resistance loci in pepper. 103:920–929. https://doi.org/10.1007/s001220100726

Piedra-Buena A, García-Alvarez A, Díez-Rojo MA, Ros C, Fernández P, Lacasa A, Bello A (2007) Use of pepper crop residues for the control of rootknot nematodes. Bioresour Technol 98:2846–2851

Pinheiro JB, Boiteux LS, Almeida MRA, Pereira RB, Galhardo LCS, Carneiro RMDG (2015) First report of Meloidogyne enterolobii in Capsicum rootstocks carrying the Me1 and Me3/Me7 genes in Central Brazil. Nematropica 45:184–188

Pochard ED, Daubeze AM (1980) Study and evaluation of components of a case of polygenic resistance: the resistance of red pepper to *Phytophthora capsici*. Annales de l'Amelioration des Plantes 30:377–398

Pochard E, Palloix A, Daubèze AM (1986) The use of androgenetic autodiploid lines for the analysis of complex resistance systems in the pepper. In: VIth Eucarpia meeting on genetics and breeding on capsicum and eggplant, Saragossa, Annales de l'Amelioration des Plantes pp 105–109

Poonpolgul S, Kumphai S (2007) Chilli pepper anthracnose in Thailand. In: Oh DG, Kim KT (eds) Abstracts of the first international symposium on chilli anthracnose. National Horticultural Research Institute, Rural Development of Administration, Jeollabukdo

Poulos MJ (1994) Pepper breeding (Capsicumspp.): achievements, challenges and possibilities. Plant Breed Abst 64(2):143–154

Qin X, Rotino GL (1993) Anther culture of several sweet and hot pepper genotypes. Capsicum Eggplant Newsl 12:59–62

Rao MK, Devi KU, Arundhati A (1990) Applications of genic male sterility in plant breeding. Plant Breed 105:1–25

Reifschneider FJB, Boiteux LS, Dellavecchia PT, Poulos JM, Kuroda N (1992) Inheritance of adult plant resistance to *Phytophthora capsici* in pepper. 62:45–49. https://doi.org/10.1007/BF00036086

Rodeva V, Irikova T, Todorova V (2004) Anther culture of pepper (Capsicum annuum L.): comparative study on effect of the genotype. Biotechnol Biotechnol Equip 3:34–38

Rodeva V, Grozeva S, Todorova V (2006) In vitro answer of Bulgarian pepper (Capsicum annuum L.) varieties. Genetica (Serbia) 38:129–136

Ros C, Gueffero MM, Gutrao P, Lacasa A, Martinex MA, Torres J, Barcelo N, Gonzalez A (2002) Response of pepper stock to Meloidogyne incognita in glasshouses in the southeast of Spain. Nematology 4:237. (Abstract)

Ros-Ibáñez C, Robertson L, Martínez-Lluch MC, Cano-García A, Lacasa-Plasencia A (2014) Development of virulence to Meloidogyne incognita on resistant pepper rootstocks. Span J Agric Res 12:225–232

Rouphael Y, Cardarelli M, Rea E, Colla G (2008) Grafting of cucumber as a means to minimize copper toxicity. Environ Expt Bot 63:49–58

Saadoun M, Allagui MB (2013) Management of chili pepper root rot and wilt (caused by Phytophthora nicotianae) by grafting onto resistant rootstock. Phytopathol Mediterr 52:141–147

Saini SS, Sharma PP (1978) Inheritance of resistance to fruit rot (*Phytophthora capsici* Leon) and induction resistance in bell pepper (*Capsicum annuum* L.). 27:721–723. https://doi.org/10.1007/BF00023707

Sariah M (1994) Incidence of *Colletotrichum* spp. on chili in Malaysia and pathogenicity of *C. gloeosporioides*. Biotrop Spec Publ 54:103–120

Sastrosumarjo S (2003) Formation of Antracnose-Resistant Chili Varieties with a Conventional Method and Biotechnology Approach. Research Report RUT VIII, Ministry of Research and Technology RI LIPI, Jakarta

Saxena A, Raghuvanshi R, Singh HB (2014) Molecular, phenotypic and pathogenic variability in Colletotrichum isolates of subtropical region in North Eastern India, causing fruit rot of chillies. J Appl Microbiol 117:1422–1434. https://doi.org/10.1111/jam.12607

Scott P, Lyne RL, Ap Rees T (1995) Metabolism of maltose and sucrose by microspores isolated from barley (Hordeum vulgare L.). Planta 197:435–441

Schwarz D, Öztekin GB, Tüzel Y, Brückner B., Krumbein A (2013) Rootstocks can enhance tomato growth and quality characteristics at low potassium supply Sci Hort 149:70–79

Segui-Simarro JM, Corral-Martinez P, Parra-Vega V, Gonzalez-Garcia B (2011) Androgenesis in recalcitrant solanaceous crops. Plant Cell Rep 30:765–778

Sharma PN, Kaur M, Sharma OP, Sharma P, Pathania A (2005) Morphological, pathological and molecular variability in Colletotrichum capsici, the cause of fruit rot of peppers in the subtropical region of north western India. J Phytopathol 153:232–237. https://doi.org/10.1111/j.1439-0434.2005.00959.x

Sharma VK, Punetha S, Sharma BB (2008) Heterosis studies for earliness, fruit yield and yield attributing traits in bell pepper. Afr J Agric Res 8(29):4088–4098. 1 August, 2013

Shifriss C (1997) Male sterility in pepper (Capsicum annuum L.). Euphytica 93:83–88

Shifriss C, Guri A (1979) Variation in stability of cytoplasmic genic male sterility in Capsicum frutescens L. J Am Soc Hortic Sci 1044:94–96

Shifriss C, Pilovsky M (1993) Digenic nature of male sterility in pepper (Capsicum annuum L.). Euphytica 67:111–112

Shifriss C, Pilovsky M, Zack JM (1992) Resistance to *Leveillula taurica* mildew (*Oidiopsis taurica*) in *Capsicum annuum* L. In: VIIIth Eucarpia meeting on genetics and breeding on capsicum and eggplant, Rome, pp 172–177

Sibi M, Dumas de Vaulx R, Chambonnet D (1979) Obtention de plantes haploïdes par androgenèse in vitro chez le piment (Capsicum annuum L.). Ann Amélior Plantes 29:583–606. (French)

Sudha A, Lakshmanan P (2009) Integrated disease management of powdery mildew (*Leveillula taurica* (Lev.) Arn.) of chilli (*Capsicum annuum* L.). Arch Phytopathol Plant Protect 42:299–317. https://doi.org/10.1080/03235400601037198

Sood S, Kumar N (2010) Heterosis for fruit yield and related horticultural traits in bell pepper. Intern J. Veg. Sci., 16:361–373

Sugita T, Yamaguchi K, Kinoshita T, Yuji K, Sugimura Y, Nagata R et al (2006) QTL analysis for resistance to Phytophthora blight (*Phytophthora capsici* Leon.) using an intraspecific doubled-haploid population of *Capsicum annuum*. 56:137–145. https://doi.org/10.1270/jsbbs.56.13

Sun CY, Mao SL, Zhang ZH, Palloix A, Wang LH, Zhang BX (2015) Resistances to anthracnose (Colletotrichum acutatum) of Capsicum mature green and ripe fruit are controlled by a major dominant cluster of QTLs on chromosome P5. Sci Hortic 181:81–88. https://doi.org/10.1016/j.scienta.2014.10.033

Sujiprihati S, Yunianti R, Syukur M, Undang (2007). Pendugaan nilai heterosis dan daya gabung beberapa komponen hasil pada persilangan dialel penuh enam genotipe cabai (Capsicum annuum L.). Bul Agron. 35:28–35.

Supena EDJ, Custers JBM (2011) Refinement of shed-microspore culture protocol to increase normal embryos production in hot pepper (Capsicum annuum L.). Sci Hortic 130:769–774

Supena EDJ, Muswita W, Suharsono S, Custers JBM (2006a) Evaluation of crucial factors for implementing shedmicrospore culture of Indonesian hot pepper (Capsicum annuum L.) cultivars. Sci Hortic 107(3):226–232

Supena EDJ, Suharsono S, Jacobsen E, Custers JBM (2006b) Successful development of a shed-microspore culture protocol for doubled haploid production in Indonesian hot pepper (Capsicum annuum L.). Plant Cell Rep 25:1–10

Suwor P, Thummabenjapone P, Sanitchon J, Kumar S, Techawongstien S (2015) Phenotypic and genotypic responses of chili (Capsicum annuum L.) progressive lines with different resistant genes against anthracnose pathogen (Colletotrichum spp.). Eur J Plant Pathol 143:725–736. https://doi.org/10.1007/s10658-015-0723-7

Sy O, Steiner R, Bosland PW (2005) Inheritance of Phytophthora stem blight resistance as compared to Phytophthora root rot and Phytophthora foliar blight resistance in *Capsicum annuum* L. 130:75–78

Sztangret J (1998) Male sterility in hybrid seed production of sweet pepper (Capsicum annuum L.). MSc Thesis, Journal of the American Society for Horticultural Science Warsaw University of Life Sciences, Warsaw (in Polish), Journal of the American Society for Horticultural Science

Tai SF, Huang HY, Lin CH, Tseng MJ, Chang WN (2004) Studies on the graft compatibility of pepper scions grafted onto eggplant rootstocks. Research Bulletin of KDARES 15:40.

Taller J, Hirata Y, Yagishita N, Kita M, Ogata S (1998) Graft-induced genetic changes and the inheritance of several characteristics in pepper (Capsicum annuum L.). Theor Appl Genet 97:705–713

Taller J, Yagishita N, Hirata Y (1999) Graft-induced variants as a source of novel characteristics in the breeding of pepper (Capsicum annuum L.). Euphytica 108:73–78. 476

Tanaka Y, Yoneda H, Hosokawa M, Miwa T, Yazawa S (2014) Application of marker-assisted selection in breeding of a new fresh pepper cultivar (Capsicum annuum) containing capsinoids, low-pungent capsaicinoid analogs. Sci Hortic 165:242–245

Thabuis A, Palloix A, Pflieger S, Daubeze AM, Caranta C, Legebvre V (2003) Comparative mapping of Phytophthora resistance loci in pepper germplasm: evidence for conserved resistance loci across Solanaceae and for a large genetic diversity. Theor Appl Genet 106:1473–1485. https://doi.org/10.1007/s00122-003-1206-3

Thabuis A, Palloix A, Servin B, Daubèze AM, Signoret P, Hospital F, Lefebvre V (2004a) Marker-assisted introgression of 4 *Phytophthora capsici* resistance QTL alleles into a bell pepper line: validation of additive and epistatic effects. Mol Breed 14:9–20. https://doi.org/10.1023/B:MOLB.0000037991.38278.82

Thabuis A, Lefebvre V, Bernard G, Daubeze AM, Phaly T, Pochard E et al (2004b) Phenotypic and molecular evaluation of a recurrent selection program from a polygenic resistance to *Phytophthora capsici* in pepper. Theoret Appl Genet 109:342–351

Than PP, Prihastuti H, Phoulivong S, Taylor PWJ, Hyde KD (2008) Chili anthracnose disease caused by Colletotrichum species. J Zhejiang Univ Sci B 9:764–778. https://doi.org/10.1631/jzus.B0860007

Thies JA, Fery RL (2000) Heat stability of resistance to Meloidogyne incognita in Scotch Bonnet peppers (Capsicum chinense Jacq.). J Nematol 32:356–361

Thorup TA, Tanyolac B, Livingstone KD, Popovsky S, Paran I, Jahn M (2000) Candidate gene analysis of organ pigmentation loci in the Solanaceae. Proc Natl Acad Sci U S A 97:11192–11197

Truong HTH, Kim KT, Kim DW, Kim S, Chase Y, Park JH et al (2012) Identification of isolate-specific resistance QTLs to Phytophthora root rot using an intraspecific recombinant inbred line population of pepper (*Capsicum annuum*). Plant Pathol 61:48–56. https://doi.org/10.1111/j.1365-3059.2011.02483.x

Tsaballa A, Athanasiadis C, Pasentsis K, Ganopoulos I, Nianiou-Obeidat I, Tsaftaris A (2013) Molecular studies of inheritable grafting induced change in pepper (*Capsicum annuum*) fruit shape. Scientia Hort 149:2–8

Ullassa BA, Rawal RD, Sohi HS, Sing DP (1981) Reaction of sweet pepper genotypes to anthracnose leaf spot and powdery mildew. Plant Dis 65:600–601. https://doi.org/10.1094/PD-65-600

Valera-Montero LL, Ochoa-Alejo N (1991) A novel approach for chilli pepper (Capsicum annuum L.) plant regeneration: shoot induction in rooted hypocotyls. Plant Sci 84:215–219

Voorrips RE, Finkers R, Sanjaya L, Groenwold R (2004) QTL mapping of anthracnose (Colletotrichum spp.) resistance in a cross between *Capsicum annum* and *C. chinense*. Theor Appl Genet 109:1275–1282. https://doi.org/10.1007/s00122-004-1738-1

Vižintin L, Bohanec B (2004) In vitro manipulation of cucumber (Cucumis sativus L.). Acta Biol Cracoviensia Ser Bot 46:177–183

Vinod KS, Shailaja P, Sharma BB (2013) Heterosis studies for earliness, fruit yield and yield attributing traits in bell pepper.African Journal of Agricultural Research 8(29): 4088–4098

Walker SJ, Bosland PW (1999) Inheritance of Phytophthora root rot and foliar blight resistance in pepper. 124:14–18. https://doi.org/10.1094/PHYTO-09-12-0242-R
Wang D, Bosland PW (2006) The genes of Capsicum. HortScience 41:1169–1187
Wang YY, Sun CS, Wang CC, Chien NJ (1973) The induction of pollen plantlets of Triticale and Capsicum annuum anther culture. Sci Sinica 16:147–151
Wang Y, Yang M, Pan N, Chen Z (1991) Plant regeneration and transformation of sweet pepper (Capsicum frutescens). Acta Bot Sin 33:780–786
Wang LH, Zhang BX, Lefebvre V, Huang SW, Daubèze AM, Palloix A (2004) Qtl analysis of fertility restoration in cytoplasmic male sterile pepper. Theor Appl Genet 109:1058–1063
Wu FN, Mueller LA, Crouzillat D, Petiard V, Tanksley SD (2006) Combining bioinformatics and phylogenetics to identify large sets of single-copy orthologous genes (COSII) for comparative, evolutionary and systematic studies: a test case in the euasterid plant clade. Genetics 174:1407–1420
Wu FN, Eannetta NT, Xu YM, Durrett R, Mazourek M, Jahn MM, Tanksley SD (2009) A COSII genetic map of the pepper genome provides a detailed picture of synteny with tomato and new insights into recent chromosome evolution in the genus Capsicum. Theor Appl Genet 118:1279–1293
Yagishita N, Hirata Y (1987) Graft-induced changes in fruit shape in Capsicum annuum L. 1. Genetic analysis by crossing. Euphytica 36:809–814
Yagishita N, Hirata Y, Okochi K, Mimura K, Mizukami H, Ohashi H (1985) Characterization of graft-induced change in capsaicin content of Capsicum annuum L. Euphytica 34:297–301
Yagishita N, Hirata Y, Mizukami H, Ohashi H, Yamashita K (1990) Genetic nature of low capsaicin content in the variant strains induced by grafting in Capsicum annuum L. Euphytica 46:249–252
Yazawa S, Kenmi T, Uemura N, Adachi K, Takashima S (1980) Use of interspecific hybrids of Capsicum as rootstocks for green pepper growing. Sci Rep Kyoto Prefecture 32:25–29
Zecevic B (1997). Heterosis effect on some cultivar hybrids of pepper (Capsicum annuum L.). Review of research work at Faculty of Agriculture (Yugoslavia) 42(1):169-181-64
Zhang BX, Huang SW, Yang GM, Guo JZ (2000) Two RAPD markers linked to a major fertility restorer gene in pepper. Euphytica 113:155–161
Zhang X, Henriques R, Lin SS, Niu QW, Chua NH (2006) Agrobacterium-mediated transformation of Arabidopsis thaliana using the floral dip method. Nat Protoc 1:641–646
Zhu Y-X, Ou-Yang W-J, Zhang Y-F, Chen Z-L (1996) Transgenic sweet pepper plants from Agrobacterium mediated transformation. Plant Cell Rep 16:71–75
Zygier S, Chaim AB, Efrati A, Kaluzky G, Borovsky Y, Paran I (2005) QTL mapping for fruit size and shape in chromosomes 2 and 4 in pepper and a comparison of the pepper QTL map with that of tomato. Theor Appl Genet 111:437–445

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