Capsicum Breeding: History and Development

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Abstract

Capsicum or chili peppers were predominantly domesticated first in America and was introduced from there to rest of the world by Columbus. Capsicum breeding initially started as selection from wild species for different purposes and further improvement was based on the art of selection. With time, the breeding for crop improvement became more scientific and classical methods like mass selection, pedigree method, single-seed descent method, backcross, and hybridization are currently being utilized for capsicum improvement. Genetic diversity of capsicum is large, allowing alternatives to several new gene rearrangements. Capsicum fruits have high nutritional value, bringing benefits to consumer's health. This fact has contributed to increase the market and consumption of capsicum in the world. Search for capsicum genotypes with increased yield, disease and abiotic stress resistance and improved quality is the goal in capsicum breeding programs. Lately, new strategies for improvement like mutation breeding, polyploidy, haploid breeding, embryo rescue, and utilization of molecular markers have been used in capsicum breeding. With continuous advancement in molecular technologies, it is becoming an essential tool which when combined with traditional selection and crossing techniques can result in significant progress in already established capsicum genetic breeding program.

3.1 Introduction

Capsicum belonging to Solanaceae family are cultivated worldwide which are being utilized for different purposes with different quality and trait requirements. There is a huge amount of diversity in *capsicum* genus and so is the diversity in its usage. Capsicums contain all the important nutrients for which it has been considered as a food and used in fresh or dried form for many years. Capsicum fruits are known for its high vitamin C content which is reported to be twice that of citrus fruits. Contrary to this, dried red chilies are very high in vitamin A and are a great source of β-carotene (Shetty et al. 2013). They promote health benefits such as reducing obesity and diabetes (Kwon et al. 2007). Chilies have antibacterial qualities and also contain bioflavonoid along with antioxidants most commonly present in apple juice. It is also reported to be effective in protecting against cancer

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(Pramanick and Srivastava 2013). Capsaicin cream is used to relieve the sensation of pain in such conditions as arthritis and other painful chronic conditions (Bhattacharya et al. 2010). Capsicum extracts are used in cosmetics as well as pharmaceuticals. Nowadays, growing capsicum in pots or gardens for ornamental purpose is also gaining importance (Bosland and Votava 2012).

World spice trade is dominated by hot pepper while sweet pepper has become a popular vegetable in the tropics. Based on fruit shape and size and utilities, capsicum market can be grouped into five broad categories: (i) fresh market which produces green, red, or multicolor whole fruits; (ii) fresh processing market for sauce, paste, canning, pickles; (iii) dried spice market for whole fruits and pepper powder; (iv) industrial extracts like oleoresin, capsaicinoids, and carotenoids; and (v) ornamental types (plants and/or fruits) (Poulos 1994). Based on the end user's demand the focus of capsicum breeding programs will change. Capsicum breeding programs also focus to reduce the stresses imposed by pests as well as extreme environmental conditions.

3.2 History of Capsicum Breeding

Western hemisphere is the place of origin of capsicums, and they were known and used as food since 7500 BC. They are native of South America and from there they spread to Central America. Columbus is credited for introducing capsicum to Europe from where it spread to Africa and Asia. Classical studies in capsicum in early times focussed mainly on genetic inheritance of important horticultural traits, mutant forms, disease resistance traits, male sterility, and quality traits. There are various reports stating these traits to be governed by single genes having dominant or recessive mode of action and some traits by quantitative trait loci (Deshpande 1933; Daskalov 1973; Shuh and Fontenot 1990). Studies of this type have been summarized in Table 3.1.

Capsicum annuum is the most important species of the genus *capsicum* as it is cultivated widely on a commercial scale. Initially, plant breeding was mostly based on the art of selecting individuals which was rather slow and casual process. However, with the introduction of Mendelian principles on genetics and heredity, plant breeding which was considered as art became "science". Currently, different methods of selection are used in breeding plants (including chili peppers), and the choice of method depends mainly on objective(s) of the breeding program (Greenleaf 1986; Singh et al. Singh et al. 2014a, b). Basically, the strategy of capsicum breeders is to develop a single genotype with higher genetic potential as productivity, disease resistance, and content of bioactive compounds.

3.3 Current Breeding Objectives for Capsicum Improvement

Capsicum breeding objectives for both hot and bell pepper differ with the country of cultivation, purpose of cultivation, cultivation condition, end user as well as customer preference of the region. Some countries prefer hot- and pungent-type pepper while some prefer sweet types. The diseases affecting the crop also vary with climate prevalent in the respective countries. Broadly, the objectives of capsicum breeding have been summarized in Fig. 3.1. However, disease resistance breeding is one of the foremost objectives in capsicum breeding (Paran et al. 2004). Pohronezny (2003) has provided a complete illustration of various diseases affecting capsicum. Disease resistance breeding basically starts with the identification of resistant sources, understanding its genetics followed by introgression in promising genotypes. In capsicum, substantial utilization of disease and pest resistance from wild species introgressed into elite cultivars to improve disease resistance has been documented. Interspecific hybridization program for resistant gene introgression also involves understanding the level of crossability between species.

Table 3.1 Genes of major horticultural traits and disease resistance in *capsicum*

Plant character	Character type and its gene symbol	Reference
Anthocyanin coloration of leaves, stem, flower, and immature fruits	*A* (incomplete dominance) along with modifier gene *MoA*	Lippert et al. (1965), Odland (1960)
Plant height	Dwarf nature controlled by nine recessive genes *dw1* to *dw9*	Daskalov (1973a), Restaino (1989), Yazawa et al. (1991), Aniel et al. (2001)
Branchless	*Bl* (recessive gene)	Bergh and Lippert (1964)
Branching	*ct* (for plant habit), *fa* (clustered fruit habit) and *dt* (for determinate growth) along with modifiers Dominant genes Dt and Ct (indeterminate growth habit)	Mc Cammon and Honma (1984)
Leaf shape	Nl (narrow leaf), broad leaf (bl), small leaf (sl-1 & sl2), curved leaf (cl), folded leaf (fl), round tip leaf (rl-1, rl-2, rl-3),	Daskalov (1973b), Aniel et al. (2001)
Leaf pubescence	Two dominant genes *H* & *Sm* (HHSmSm: presence of pubescence, hhsmsm: glabrous leaves)	Shuh and Fontenot (1990)
Flowers	Multiple flowers by three dominant genes (Mf-1, Mf-2 and Mf-3) *ef*: early flowering *lf*: late flowering *nf*:n o flowering	Shuh and Fontenot (1990), Pathak et al. (1985)
Fruit shapes	*P*: Pointed fruit shape *fb*: Non bulging fruit shape *ce*: fruit base with enclosed calyx *O*: round fruit shape with modifiers *up-1* & *up-2*: Erect fruit *pf*: parthenocarpy	Deshpande (1933), Daskalov and Poulos (1994), Peterson (1959), Gopalkrishnan et al. (1989), Lippert et al. (1965), Pathak et al. (1983)
Immature fruit color	Three alleles of a recessive gene: *sw1, sw2 & sw3* *sw1*: sulphur white *sw2*: yellowish green *sw3*: cedar green sw1 > sw2 > sw3	Odland and Porter (1938)
Mature fruit color	y^+: red colour *y*: yellow colour *cl* & *y* + : brown colour *Ccs:* capsanthin-capsorubin synthase enzyme that synthesizes red carotenoid pigment *Psy:* locus responsible for development of fruit colour *Psy/C2*: rate limting factor in carotenoid production	Boswell (1937), Smith (1950), Hurtado-Hernandez and Smith (1985), Papovsky and Paran (2000), Lefebvre et al. (1998), Thorup et al. (2000), Huh et al. (2001)
Pungency	*Pun*: Controls acyl transferase responsible for capsaicin synthesis *lov*: non pungency due to loss of vesicles on the placental walls	Deshpande, (1935), Greenleaf (1952), Daskalov and Poulos (1994), Votava and Bosland (2002)
Beta carotene	*B, t and bc*: Confer high beta carotene contents	Chalukova et al. (1993), Daskalov et al. (1995)

(continued)

Table 3.1 (continued)

Plant character	Character type and its gene symbol	Reference
Male sterility	Genetic male sterility: Total 20 genes have been identified, *ms-1* to *ms-20* Cytoplasmic male sterility: Major gene *ms* in interaction with *S* cytoplasm *Rf*: Restorer of fertility locus	Shifriss and Frankel (1969), Shifriss and Rylski (1972), Daskalov (1973a), Daskalov and Poulos (1994), Shifriss (1973), Meshram and Narkhade (1982), Pathak et al. (1983), Peterson (1958), Novac et al. (1971) Daskalov (1973a)
Tobacco mosaic virus	L^3, L^2, L^1, L^+: series of multiple alleles L^2: localization of TMV L^1: imperfect localization of TMV L^+: mottling $L^3 > L^2 > L^1 > L^+$	Homes (1937), Boukema et al. (1980), Boukema, (1980)
Cucumber mosaic virus	*cm*: recessive geneand 4 QTLs	Singh and Thakur (1977), Gil-Ortega and Arteaga (1988), Ben Chaim et al. (2001)
Tomato spotted wilt virus	*Tsw*: Hypersensitive resistance to TSWV	Moury et al. (Moury et al. 1997a, b)
Bacterial leaf spot	*Bs1, Bs2, Bs3, Bs4*: Hypersensitive resistance *bs5* and *bs6*: nonhypersensitive recessive resistance *gds*: general defense system	Cook and Guevara (1984), Cook and Stall (1963), Hibberd et al. (1987), Kim and Hartmann (1985), Sahin and Miller, (1997), Csillery et al. (2004), Szarka and Csillery (1995), Jones et al. (2002)
Phytophthora disease	*Psr:* Stem resistance to *Phytophthora* *Pfo*: Foliar resistance *Pfr*: Fruit rot resistance	Sy et al. (2005), Walker and Bosland (1999), Saini and Sharma (1978)
Anthracnose resistance	*Anr1*: Resistance to *Colletotrichum dematium* *Anr2, Anr3, Anr4*: Resistance to *Colletotrichum gleosporoides* *Anr5*: Resistance to *Colletotrichum capsici*	Park et al. (1990), Fernandes and Ribeiro (1998), Lin et al. (2002)
Bacterial Wilt	Two genes with incomplete dominance	Matsunaga et al. (1998)
Powdery Mildew	Three genes: *lmr-1, lmr-2, lmr-3*	Shifriss et al. (1992)
Root-knot nematodes	*N:* Resistance to *M. incognita acritaMe1, Me2, Me3, Me4, Me5*: Resistance to Meloidogynae spp. *Me6*: *M. arenaria* and *M. javanica* *Mech1* and *Mech2*: Suppresses nematode resistance	Fery and Harrison (1990), Hendy et al. (1995), Pegard et al. (2005), Djian-Caporalino et al. (2004)
Bentazon herbicide resistance	*Bzt*: Tolerance	Fery and Harrison (1990)

Important diseases of attention in present-day scenario worldwide are viral diseases like Potyviruses [*Potato virus Y* (PVY), *Potato virus X* (PVX), *Pepper mottle virus* (PepMoV), *Pepper veinal mottal virus* (PVMY), *Tobacco etch virus* (TEV), *Chilli veinal mottle virus* (CVMV), *Pepper severe mosaic* (PSMV), *Pepper yellow mosaic* (PYMV)], Tospoviruses [*tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV), *Groundnut ringspot virus* (GRSV), *Groundnut bud-necrosis virus* (GBNV)], Cucumovirus [*Cucumber mosaic virus* (CMV)], Tobamoviruses [*Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), *Pepper mild-mosaic virus* (PMMV)], fungal diseases like powdery mildew, phytophthora root rot, anthracnose,

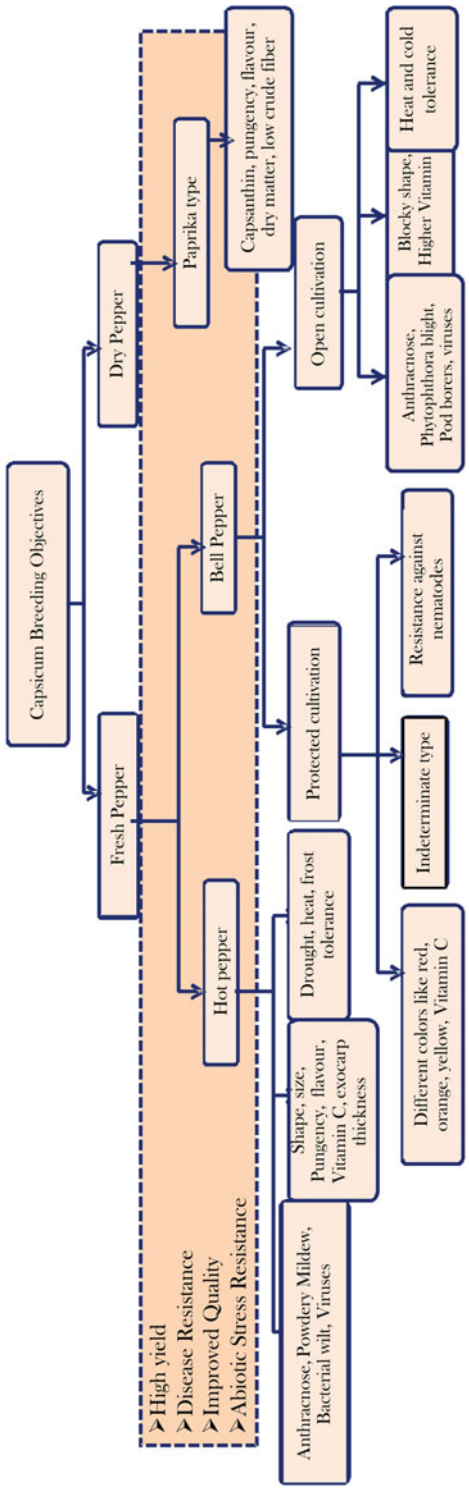

Fig. 3.1 Breeding objectives for capsicum improvement

bacterial diseases like bacterial wilt, and bacterial spot and pests like whiteflies, thrips, mites and root-knot nematodes. List of wild species utilized as disease-resistant sources is summarized in Table 3.2. Utilizing wild germplasm for introgression of disease resistance genes into promising genotypes has contributed significantly to crop improvement, particularly in terms of yield and quality improvement as well as stability in capsicum production. Introgression efforts to transfer disease resistance genes into superior genotypes have often been difficult, especially when resistance traits are under polygenic control and linked to undesirable horticultural and economic traits. With continuous evolution and emergence of new pathogen races and strains against available resistant genotypes necessitates regular search and use of new resistant sources.

The second objective for which capsicum breeders throughout the world are striving is increasing yield, thereby increasing overall productivity. In this respect, heterosis breeding program is gaining importance. Emphasis on development of hybrids based on male sterility systems is desired as it saves time and labor required for hybrid seed production. Both genetic (GMS) and cytoplasmic male sterility (CMS) systems have been utilized to produce hybrids, but CMS system is more widely exploited. With the identification of new CMS sources, their maintainers and diversification of CMS systems also become an important objective. Further for development of good hybrid, identification of restorers with good general and specific combining ability, and incorporation of resistant genes in these CMS lines and restorers should also be an area of focus.

Breeding objectives of capsicum also depend on the market demand and end utility. This includes breeding for horticultural and biochemical traits. Fresh market breeders look for traits like fruit color at unripe stage usually green (light, medium, or dark), fruit length and its width and pericarp thickness. Apart from this, the level of pungency is an important and unique aspect of capsicum breeding. Understanding people's preferences for pungency in a particular

Table 3.2 List of wild and cultivated species as source of disease resistance

Capsicum species	Resistant source	Diseases and resistant genes	References
C. baccatum	PBC 80	*Colletotrichum* spp. (anthracnose)	Montri et al. (2009), Mongkolporn and Taylor (2011)
	PBC 81	*Colletotrichum* spp. (anthracnose)	Montri et al. (2009), Mongkolporn and Taylor (2011)
	C-153	TSWV	Rosellol et al. (1996)
C. chacoense	PI260435	*Bs2* (*Xanthomonas campestris pv. vesicatoria*)	Cook and Guevara (1984)
C. chinense	7204	*Tsw* (tomato spotted wilt tospovirus)	Moury et al. (1997a, b)
	CNPH725	*Tsw* (tomato spotted wilt tospovirus)	Boiteux and de Avila (1994), Boiteux (1995)
	ECU-973	*Tsw* (tomato spotted wilt tospovirus)	Cebolla-Cornejo et al. (2003)
	PI152225	L^3(tobacco mosaic virus)*Pvr1* (TEV-C, TEV-F, PepMoV, PVY) *Tsw* (tomato spotted wilt tospovirus)	Boukema (1980, 1982, 1984), Boukema et al. (1980), Kyle and Palloix (1997), Black et al. (1991), Boiteux (1995), Jahn et al. (2000)
	PI159236	L^3 (tobacco mosaic virus) *Pvr1* (TEV-C, TEV-F, PepMoV, PVY) *Pvr7* (PepMoV) *Tsw* (tomato apotted wilt tospovirus)	Boukema (1980, 1982, 1984), Kyle and Palloix (1997), Grube et al. (2000) Black et al. (1991), Boiteux (1995), Jahn et al. (2000)
	PI315008	L^3 (tobacco mosaic virus)	Boukema (1980)
	PI315023	L^3 (tobacco mosaic virus)	Boukema (1980)
	PI315024	L^3(tobacco mosaic virus)	Boukema (1980)
	PBC932	*co1, co2, co3* (*Colletotrichum capsici*)	Pakdeevaraporn et al. (2005), Mahasuk et al. (2009a, b)
C. chacoense		*Tswv*	Boukema (1982)
C. pubescens	PI235047	Bacterial spot (*Xanthomonas campestris pv. vesicatoria*)	Sahin and Miller (1998)
C. annuum	CM334 (Serrano Criolle de Morelos-334)	*Phytophthora capsici* *Pfo* (*Phytophthora* foliar rot) *Pfr*(*Phytophthora* fruit rot) *Psr* (*Phytophthora* stem rot) *Pvr4* (PVY pathotypes 0, 1 and 2 and PepMoV) *Pvr5* (common PVY strains) *Me* (*Meloidogyne* sp.)	Saini and Sharma (1978), Gil-Ortega et al. (1991), Walker and Bosland (1999), Sy et al. (2005, 2008)
	PI264281	*Pvr2* (PVY pathotypes 0 and 1; TEV)	Kyle and Palloix (1997)
	SC46252	*pvr2* (PVY pathotypes 0 and 1; TEV)	Kyle and Palloix (1997)
	PM687 (inbred PI322719-a local Indian population)	Bacterial wilt (*Ralstonia solanacearum*) *Meloidogyne* spp.	Lafortune et al. (2005)
	PM217 (PI201234)	*Meloidogyne* spp.	Djian-Caporalino et al. (1999)
	AC2258 (PI201234)	*Phytophthora capsici*	Djian-Caporalino et al. (1999)

region is a very significant aspect. Pungency, an important attribute of capsicum commercially, is due to the presence of chemical complex or alkaloids known as capsaicinoids (Perucka et al. 2001). Capsaicin and dihydrocapsaicin are the two most abundant capsaicinoids in capsicum constituting about 90%, where capsaicin alone accounts for ~71% of the total capsaicinoids in most of the pungent varieties (Kosuge 1970). Capsaicin content of capsicum is one of the

major quality parameters that capsicum breeders look into while developing commercial varieties (Ohnuki et al. 2001; Kawabata et al. 2006; Hachiya et al. 2007). Capsaicinoid content is, nowadays, determined by high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC–MS), and liquid chromatorgraphy–mass spectrometry (LC–MS) techniques. HPLC analytical technique is also used to estimate capsanthin content in capsicum. *Capsicum* genus uniquely has capsanthin–capsorubin synthase (CCS) which is an enzyme that synthesizes two red pigments—capsanthin and capsorubin (Guzman et al. 2011). Breeding for higher capsanthin is targeted in red capsicum to be used as dried spice (whole fruits and powder), and for industrial extracts (paprika oleoresin, capsaicinoids, and carotenoids). The red color in chili which is due to capsanthin and capsorubin, and yellow color due to β-carotene and violaxanthin is measured in American Spice Trade Association (ASTA) units (Englewood 1985). Generally, the higher the ASTA color value, the deeper is the red color of the genotype on ripening. The range of capsanthin content is 70 ASTA units (low), 71–100 ASTA units (medium), and 101–150 ASTA units (high). ASTA color affects the brightness of a product, while the surface color has an impact on the hue of product.

Hence, the development of paprika varieties to meet high demand of nonpungent pods with high color value for oleoresin extraction for industries is another important objective in capsicum breeding. Besides conventional nutritional uses in food, the other uses of capsicum such as in defense, spiritual, and ethnobotanical are also known (Kumar et al. 2006; Meghvansi et al. 2010). Accordingly, the breeding goals also vary.

For dry capsicum, dry matter content is an important quality character to be bred for making dry powder and whole dry fruit purpose. These are also the major characters desired for export purpose. A high dry-matter content of red chili fruit is important from commercial viewpoint in spice industry, but there is no positive relation between the dry matter content and its capsaicin content (Dhall 2008). Thin pericarp is necessary for dry capsicum as drying can be more easily accomplished. On drying, fruits with thick pericarp show wrinkled surface and dull appearance.

Increasing industrialization, risks of crop failure due to changing climate and demand (domestic and export) for more nutritious and safer foods emphasis are also being laid on breeding for genotypes with increased tolerance to high temperature, drought and wide adaptability.

3.3.1 Specific Objectives for Sweet Pepper Breeding

The main objectives of sweet pepper genetic improvement are developing varieties with blocky shape and different colors like medium or dark green at unripe stage and red, yellow or orange at ripe stage. The main objective is to select and develop new breeding lines and/or cultivars of capsicum with high levels of antioxidants and vitamins. These include: ascorbic acid (vitamin C), flavonoids (phenolics), red and yellow/orange carotenoids (including vitamin A-precursors like α- and β-carotene, β-cryptoxanthin) (Tomlekova et al. 2009a, b). Breeding efforts also include selection for high fruit set and yield under a range of growing conditions like open and protected, including the study of abiotic stresses like low temperatures, water stress, and saline stress. (Hein 2017; Negi et al. 2018). Activities also include breeding for stability of carotenoid extracts under long-term storage conditions and against photo-oxidation. Sweet pepper and hot pepper are affected by many common pathogens, but disease of importance in sweet pepper is Phytophthora fruit rot, anthracnose, viruses, powdery mildew, and bacterial wilt under open cultivation. 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 (Ferrara et al. 2011). 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 goals (Parker et al. 1995; de Swart 2007).

3.4 Breeding Methods for Capsicums

Conventional breeding methods like mass selection, pureline selection, pedigree breeding, single-seed descent method, backcross breeding, and heterosis breeding have been used for the crop improvement in capsicum. Other breeding methods like mutation breeding and polyploidy breeding have also been attempted to create variation and subsequently utilize in capsicum improvement programs. Mass selection, pureline selection, pedigree breeding, single-seed descent method, and backcross breeding were strategies utilized earlier for capsicum improvement when systematic plant breeding started. Mass selection is one of the simplest techniques which has been used for capsicum improvement (Table 3.3). Improvement for multiple traits of simple inheritance can be done simultaneously without any concerns about pedigree. Initially, it was used to improve landraces or open-pollinated cultivars of capsicum. In this approach, characters with high heritabilities are easily fixed and a reasonable level of variability is also maintained. Pure line selection was basically applicable to landraces/local cultivars which were being grown by farmers. In this method, superior plants are selected, then harvested separately and evaluated next year to observe plant progeny performance. Progeny showing superior performance and devoid of genetic variability, is bulk harvested and evaluated further with check cultivar(s) in replicated trials. This method has been extensively used to develop several varieties for commercial cultivation in chili capsicum (Table 3.3).

Pedigree selection is a breeding scheme where selection is affected among and within family, and the selected individuals are given a pedigree number so that any progeny in any generation can be traced back to the original plant which was first selected in F_2 generation. This has been one of the most commonly followed approaches for cultivar development in capsicum (Table 3.3). Selection of superior parental cultivars is crucial step in this method. This method is often utilized in conjunction with backcrossing to introgress important genes into advanced inbreds.

In single-seed descent (SSD) method, one seed from a single fruit is harvested from each plant in a segregating generation without applying any selection. The segregating generation are grown under greenhouse facilities to advance more generations in a year, to generate large number of inbred lines to be used in test crosses for development of hybrids and to generate recombinant inbred line populations to be utilized in mapping studies. Backcross method is the most widely used strategy in disease resistance breeding program of capsicum. This is normally used to transfer single gene/few genes from primitive cultivars/wild forms to leading cultivars. In some cases, even BC_2 families may be routed through pedigree method of breeding (modified backcross) instead of following a routine backcrossing program which needs 5–6 backcrosses with the recurrent parent.

Heterosis breeding has been advantageous for increased hot pepper or bell pepper production although open-pollinated varieties are still commonly available. Several hybrids have been developed in capsicum; however, the hybrid development program should be continuous so as to make the seeds available to the growers at affordable cost. F_1 hybrids of capsicum are gaining popularity after the initiation of research and seed production work in vegetables by a large number of private sector seed companies. To make seed production, more economic male sterility is extensively utilized in chili for hybrid seed development. The discovery of some male-sterile mutants which help to eliminate more laborious operations of emasculation combined with various marker genes further facilitate identification of undesirable types at seedling stage itself. Presently in chili, genetic male sterility (GMS) and cytoplasmic-genetic male sterility (CGMS) are being commercially exploited for the development of hybrids. Of the

Table 3.3 Achievements made by different breeding methods in capsicum improvement

Breeding approach	Significant achievements/varieties released	References
Introduction	• NuMex Centennial (Mexico), • CO 4 (introduced from Srilanka in India), • CO 3 (introduced from Srilanka in India),	TNAU Portal: http://agritech.tnau.ac.in/horticulture/horti_vegetables_chilli.html Vidhi J: http://www.biologydiscussion.com/vegetable-breeding/top-7-breeding-methods-of-capsicum-india/68448
Mass Selection	• It is still used in Mexico to select seed for Poblano, guajillo and other traditional capsicum landraces. • Heritage New Mexico 6-4: Selection made from NuMex Big Ji	Dewitt 2014; Vidhi J
Pureline Selection	• G 1, G 2, G 3, G 4, NP 46A, K 1, Co 1,CO.2, Musalwadi, Sindhur, Patna Red, Pant C 1, PLR1, KI	Gopalakrishnan (2007), Ramachandran (2013); Vidhi J
Single plant Selection	• New Mexico No. 6 • NuMex Conquistador: single plant selection from 'New Mexico 6-4 • NUMEX JOE E. PARKER: single plant selection from population of 'New Mexico 6-4 • NuMex Sweet: single plant selection from population of 'New Mexico 6-4	Dewitt 2014
Pedigree Method	• Andhra Jyoti, Pusa Jwala, Pusa Sadabahar, X 235, K2, Punjab Lal and Jawahar 218 (India) • New Mexico No. 9 Sandia: Cross of New Mexico No. 9 and California Anaheim Rio • Grande 21: Cross of New Mexico No. 6 and Anaheim • Española Improved: hybridization between 'Sandia' and a Northern New Mexico line of chilli • Numex Sunrise, Numex Sunset and Numex Eclipse: All three cultivars originated from a hybridization between 'Permagreen,' a green bell pepper, and 'New Mexico 6-4 • Numex RNaky: cross of 'Rio Grande 21,' 'New Mexico 6-4,' Bulgarian paprika, and an early-maturing native type • Numex Sunburst, Numex Sunflare and Numex Sunglo: derived by pedigree breeding from a seed source from India in Mexico • Numex Primavera, NuMex Memorial Day' and 'NuMex Thanks, Numex Garnet, NuMex Primavera' • AVPP0506, Berke's Joy, AVPP0105, AVPP0206, AVPP0303, AVPP0409, AVPP0411, AVPP0512, AVPP0514 (from World Vegetable Centre) • Bell Pepper: Spartan Garnet – California Wonder × Dwarf Pimiento Selection from the variety Santanka • Spartan Emerald – Morgold × California Wonder • Sonnette-An F_2 derived line originating from the cross (Morgold × California Wonder) × Keystone Resistant Giant	Ramachandran (2013), Dewitt 2014; Vidhi J: http://www.biologydiscussion.com/vegetable-breeding/top-7-breeding-methods-of-capsicum-india/68448; AVRDC: https://avrdc.org/seed/improved-lines/chili-capsicum/

(continued)

Table 3.3 (continued)

Breeding approach	Significant achievements/varieties released	References
Backcross method	• Pyramiding of genes conferring resistance to PMMoV, PVY and TSWV in sweet Charleston capsicum lines (BC: Backcrossing; L4: The gene conferring resistance to PMMoV pathotype 1, 2, 3. c Tsw: The gene conferring resistance to TSWV; dPVY: Potato Virus Y pathotype 1–2) • Introgression of heat shock protein (Hsp70 and sHsp) genes into the Malaysian elite chilli variety Kulai (*capsicum annuum* L.) • Resistance has been successfully introgressed into commercial capsicum cultivars, including resistance to tobamoviruses from *capsicum chacoense*and*C. Chinense* • Resistance to tomato spotted wilt virus (TSWV) from *C. chinense* and *C. baccatum* • Resistance to anthracnose fruit rot from *C. chinense* • Resistance to *Phytophthora capsici* from *C. Annuum* cv. CM334 and resistance to bacterial leaf spot disease from *C. annuum* and *C. chacoense*. • *p-AMT* and*Pun1* markers were used to develop a new fresh cultivar containing capsinoids, named 'Maru Salad'.	Vidhi J: http://www.biologydiscussion.com/vegetable-breeding/top-7-breeding-methods-of-capsicum-india/68448; Boukema (1980), Cook and Guevara (1984), Kim and Hartmann (1985), Hibberd et al. (1987), Boiteux et al. (1994), Berzal-Herranz et al. (1995), de la Cruz et al. (1997), Voorrips et al. (2004), Vallejos et al. (2010), Mallard et al. (2013), Hoang et al. (2013), Soler et al. (2017), Liu et al. (2014), Usman et al. (2008)
Heterosis breeding	• No. 1 Zao Fong: First F1 hybrid of China • Based on CGMS system: Kashi Surkh, Kashi Early, (IIVR, India) • Arka Meghana, Arka Sweta, Arka Harita (IIHR, India) • YU JING NO 2, (China) • F1 Hybrid Coral & F1 Hybrid Dara, Clover Seeds, Hong Kong China • VNR38, VNR108, VNR174, VNR Seeds, India • VNR200, VNR332 • F1 Forever Tropicasem, Senegal Many SSA countries • Remington, F1 Alpha Seeds, South Africa Many SSA countries • F1 TSS AVRDC No.4 Suntech Seeds, Taiwan Taiwan • F1 TSS AVRDC No.2 Yung Shan Seeds, Taiwan Taiwan • F1 Hsing AVRDC No.3 Suntech Seeds, Taiwan Taiwan • (sweet pepper) Yun Pepper No.2 Horticulture Research Institute, YAAS, China China • Yun High Pungency No.1 Horticulture Research Institute, YAAS, China China • Ulka F1, Masaya 315, East-West Seeds, India India • Yuvraj IN	Tong (1998), Gopalkrishnan (2007), Lin et al. (2013), Dhaliwal et al. (2015); IIHR Website: https://www.iihr.res.in/division-varities/786; IIVR website: https://www.iivr.org.in/iivr-varieties/by-crop

(continued)

Table 3.3 (continued)

Breeding approach	Significant achievements/varieties released	References
	• Super F1, Muria F1 East-West Seeds, Thailand Sri Lanka • Hybrid Indus Seeds, India • Based on GMS system: • CH-1, CH-3, CH-27 multiple disease resistant hybrid PAU, Ludhiana, India	
Mutation Breeding	• Horgoskaslatka-X-3–resistant to CMV; Yugoslavia Karasz • Albena: Attractive fruits with better flavor, Bulgaria S. Daskalov, Institute of Genetics, Sofia 113 • Krischimski ran: Hybrid variety with high yield, earliness and improved fruit quality, Bulgaria S. Daskalov and L. Milkova Institute Institute of Genetics, Sofia 113 • MDU 1: Compact plant type with higher yield and capsaicin content, Tamil Nadu Agriculture University, India • Lyulin: Hybrid variety based on induced male sterility, very early and high yield, Institute of Genetics, Sofia 113	Daskalov (1986)

two types of male sterility, CGMS has been preferred over GMS for hybrid seed production because maintenance of GMS shows segregation of male sterility and male fertility (Table 3.3).

In capsicum, CGMS was first reported by Peterson (1958) in the USDA accession PI164835. Till date, no other CMS sources have been reported. In capsicum CMS system, male sterility is caused by two abnormal mitochondrial genes—"*orf507*" and "*atp6-2*" (Kim et al. 2001a, b; Kim and Kim 2005; Gulyas et al. 2006). As the genes are present in the mitochondria, these are maternally inherited. Expression of male sterility also requires the absence of a nuclear gene for the restoration of fertility. For successful hybrid seed development, a restorer line is needed where the restoration of fertility is governed by a single dominant gene. Male sterility maintenance requires a maintainer line with a fertile cytoplasm but the absence of nuclear gene for fertility restoration. As the CGMS system of hybrid seed production requires three lines, i.e., CMS line, maintainer of male-sterile line, and a restorer of fertility in hybrids; it is called three-line system of hybrid seed production in capsicum.

GMS system has also been used for hybrid capsicum production but to a limited extent. In the GMS system, expression of male sterility is controlled by homozygous recessive genes (*ms/ms*) while homozygous dominant or heterozygous plants (*Ms/MS* or *Ms/ms*) exhibit male fertility. Maintenance of male sterility in GMS requires isogenic line with difference only at *Ms* locus is required, i.e., *MsMs* and *Msms*. Crossing between these two lines produces progeny with a mixture of male fertile (Ms/ms) and male sterile (ms/ms) in equal proportions. Male fertile plants are identified in field visually and discarded while the male-sterile lines are used for hybrid seed production (Shifriss 1997, Table 3.3).

3.5 Other Strategies Utilized for Capsicum Improvement and Achievements

Success in capsicum cultivar development initially relied considerably on the breeder's experience, his discretion to isolate promising genotypes as well as luck. Even today with the

availability of advanced breeding techniques, the breeder's experience and judgement are important factors for success in cultivar development. Therefore, plant breeding is still regarded as a combination of science and art. Apart from the established breeding methods for improvement of capsicum, many other techniques have also been attempted and success has been registered to some extent in these strategies which include mutation breeding, polyploid and haploid development, transgenics and marker-assisted breeding.

3.5.1 Mutation Breeding

Mutations are the ultimate source for creating genetic variations. Mutation breeding involves generation of new variability through chemical and physical mutagenesis followed by the development of new varieties utilizing this variability. It is now a pillar of modern plant breeding. Mutation breeding has been found to be effective and efficient breeding tool in capsicum. Daskalov (1986) has written an exhaustive review on this subject. Seeds are the most desirable parts to be treated with mutagen in capsicum. It is recommended to use seeds of uniform size, possessing 96–100% germinability and moisture content (about 13%) to obtain good reproducibility of results. When ionizing radiations are used as mutagen lethal dose should assure survival of 40–60% (Raghavan and Venkatasubban 1940) while it should be 70–80% when chemical mutagens are used (Paran et al. 2007; Hwang et al. 2014; Arisha et al. 2015, Jo et al. 2016).

Bell peppers are in general more radiosensitive than the hot peppers. Pollen grains have also been treated with gamma rays or X-rays and used for the pollination of emasculated nonirradiated flowers immediately after irradiation (Daskalov 1986). The M_1 generation (first generation after mutagen treatment) plants must be raised on isolated plots (at least 700 m apart from other capsicum plants) to prevent cross-pollination followed by bagging of the M_1 flowers to avoid outcrossing. At least 3000–5000 M_1 plants must be raised per experiment. 20–25 M_2 plants per M_1 plant or 10–15 M_2 plants per M_1 fruit (with 2–3 fruits per M_1 plant) are grown in the next generation. The size of the M_2 field population is about 70,000–100,000 plants, but it depends on the kind of selection to be performed and the number of observations to be made. The selection of desirable mutants is carried out mainly in the M_2 generation. To allow progeny testing all discovered mutants must be selfed, usually by bagging the flowers.

Mutation breeding approach has often been used in capsicum for functional gene annotation and also to create novel variability to be utilized in breeding. Sweet pepper cultivar "Maor" has been used to generate mutation population which was later utilized for isolation and characterization of genes controlling plant architecture and flowering (Daskalov 1974; Elitzur et al. 2009; Jeifetz et al. 2011; Cohen et al. 2014). Similar mutation populations have been generated in chili peppers using the cultivar "Yuwol-cho" (Hwang et al. 2014). Jeong et al. (2012) attempted targeted induced local lesions in genome (TILLING) approach in the same cultivar "Yuwol-cho" and successfully isolated a line that exhibited resistance to the *tobacco etch virus* (TEV) from this population.

Daskalov (1968, 1973b) had developed large mutation populations in capsicum using X-rays and gamma irradiation. Novel male-sterile lines were isolated from these populations and then characterized for utilization in breeding. These populations were also utilized to develop capsicum cultivars with useful characteristics such as resistance to *cucumber mosaic virus* (CMV), improved flavor, higher yield, and compact plant stature (Daskalov 1986). Honda et al. (2006) used heavy ion beams (^{12}C and ^{20}Ne ion beams) to develop a mutat population, but the screening of mutants was mainly performed in the M_1 generation. Ultraviolet irradiation has been used in capsicum to create mutants with increased level of vitamin C and E (alpha tocopherol) (Daskalov 1986).

Three male-sterile lines were isolated from capsicum mutant population created by gamma irradiation by Daskalov and Mihailov (1988) and

subsequently utilized in hybrid development. Recently, mutants with changed shoot architecture in hot pepper (Paran et al. 2007), some induced mutants in sweet pepper (Honda et al. 2006) and capsicum with increased β-carotene and orange color on maturity (Tomlecova et al. 2009a, b) have been recovered. After gamma irradiation of the dry seeds of capsicum (*C. annuum* L.), many promising mutants were obtained, the most interesting of which were induced male-sterile mutations. Male sterility is governed by single recessive genes, denoted by *ms-3, ms-4, ms-6, ms-7,* and *ms-8.* The male-sterile lines *Pazardjishka kapia* ms-3 and *Zlaten medal* ms-8 recovered after mutagen treatment were used to test their combining ability against original male-sterile line used for hybrid production. The results obtained indicate that there is no significant difference in the combining ability for early and total yields. Three male-sterile lines were crossed with a large number of lines in order to obtain hybrid combinations for different purposes. Most of the hybrid combinations exceeded the check with regard to early yield. Some hybrids were also characterized by an increase in total yield. Two hybrid combinations, named Krichimski ran and Lyulin, were released as cultivars utilizing the male-sterile lines recovered from mutat population.

3.5.2 Polyploidy Breeding

Polyploidization events are often associated with increase in vigor followed by adaptation of the newly formed polyploid to novel conditions. According to Van de Peer et al. (2009), superiority of polyploids over their diploid counterparts has been attributed to the phenomenon of transgressive segregation, i.e., formation of extreme phenotypes. Malhova's 1977 work suggests that *capsicum* may respond to changes in ploidy in the same way as *Solanum.* It is relatively easy to increase or decrease ploidy levels artificially in *capsicum*. Somatic doubling can be achieved by treating wounded leaf axils with colchicine. However, synthetic autotetraploids seem to have no agronomic or breeding advantages over diploids. Polyploid capsicum usually expresses morphological characters like stunted growth and the presence of larger, thicker, and dark green leaves (Tapadar 1963; Bose and Panigrahy 1969; Biswas and Bhattacharyya 1971; Indira and Susan 1977). The deep green color of the leaves in polyploids has been attributed to the presence of more numerous and larger chloroplasts (Raghuvanshi and Joshi 1964). Tetraploid capsicum exhibit increased dry weight in leaf, stem and root, leaf area and thickness when compared with the diploid one. The tetraploids have reported increased ability to absorb water, NO_3–N and K with a consequent increase in the photosynthetic ability; and bear smaller but uniform-size fruits, independent of fruit loading (Takizawa et al. 2008).

The tetraploid capsicum has been found to flower about one month later than the diploids. The total number of flowers produced was less; this decrease being primarily due to the non-branching nature of the polyploidy (Tapadar 1963; Biswas and Bhattacharyya 1971; Indira and Susan 1977). Raghuvanshi and Sheila (1964) observed delayed and extended flowering with larger and varied number of floral parts in the colchiploids of *capsicum frutescens*. Larger flowers with increased size of pollen grains are also characteristic of polyploids (Watts 1980). Colchicine treatment of seeds has produced tetraploid plants of *C. annuum* variety "Chigusa" (Nihon Horticultural Production Institute) (Ishikawa et al. 1997). Flow cytometric analyses of these seeds showed that ≈20% of the seeds treated with colchicine were tetraploid. In comparison with diploid flowers which typically had six petals and stamens tetraploid flowers had seven petals and stamens, 20% larger ovaries, and 25% larger diameter pollen grains (Ishikawa 2001). Polyploids have also been reported to have sterility which may be attributed to abnormalities observed in meiosis (Pal et al. 1941). Following colchicine treatment, a plant of chili pepper cv. CO-2 was found to have chromosome numbers ranging from $2n = 38$ to 96. It had 4.95% pollen fertility and set no seed, and its growth was stunted (Rao 1987). Haploids

produced through anther culture have been doubled using colchicines, but the homozygotes thus produced have not as yet been exploited to produce commercial F_1 hybrids with exhibiting heterosis but have been used to study the genetic mechanism of resistance to pests (Hendy et al. 1985) and diseases (Daubeze et al. 1990; Palloix 1992).

Malhova (1977) produced interspecific hybrid which otherwise was difficult between *capsicum. pubescens* and *C. annuum* by pollinating *C. pubescens* with pollen of autotetraploid *C. annuum*. This result gives a direction that induced autotetraploidy may be used to overcome post-fertilization barriers in other interspecific crosses of *capsicum* genus. Pochard (1970, 1977) has produced a set of trisomies for *C. annuum.* These trisomies can be utilized to identify genes present on particular chromosomes either because of distorted segregation ratios which occur in the progeny of F_1 hybrids trisomic for that chromosome (Pochard 1977) or because of dosage effects which can be detected when trisomics are compared to normal diploid individuals (Tanksley 1984). Location of gene *c* (controlling pungency) on acrocentric chromosome number '*XI*' (Pochard 1977) and its presence on long arm (Pickersgill 1977) were confirmed using these trisomics as the trait pungency segregated independently of the markers present one short arms of the acrocentric chromosomes.

3.5.3 Haploid Breeding

First haploids in the *genus capsicum* were developed through in vitro anther culture of *C. annuum* and *C. frutescens* (George and Narayanaswamy 1973; Kuo et al. 1973; Wang et al. 1973; Novak 1974). Lower recovery of haploid plants from androgenic cultures in earlier studies encouraged to design experiments which aimed to identify the factors influencing induction of androgenesis. From the various experiments conducted on haploid induction, it was concluded that androgenic response depended on growing conditions, age, and genotype of the donor plant (Ercan et al. 2006; Niklas-Nowak et al. 2012; Grozeva et al. 2013; Koleva-Gudeva et al. 2013; Alremi et al. 2014), developmental stage of microspores in the anther (Nowaczyk and Kisiała 2006; Parra-Vega et al. 2013; Barroso et al. 2015), culture medium composition, concentration and combination of growth regulators, organic and inorganic additives (Büyükalaca et al. 2004; Zhao et al. 2010; Taşkin et al. 2011; Roshany et al. 2013; Olszewska et al. 2014), and pretreatment of flower buds and/or anthers (Koleva-Gudeva 2007; Özkum and Tıpırdamaz 2007; Irikova et al. 2011; Nowaczyk et al. 2015).

Technology for development of doubled haploids is one of the fastest technique to achieve complete homozygosity in any crop species, but its application in capsicum improvement is still limited because of recalcitrant nature of capsicum (Grozeva et al. 2009; Ercan and Sensoy 2011; Olszewska et al. 2014). Capsicum breeding requires genetically stable and homozygous plants to understand genetics as well as mapping and identification of genes for various morphological traits and biotic and abiotic stress-related traits. Despite low frequency of results, several studies concerning the practical aspect of the haploid breeding in different *capsicum* species is being undertaken (Olszewska et al. 2010, 2011; Shrestha et al. 2010; Luitel et al. 2012; Luitel and Kang 2013a, b; Shmykova et al. 2014; Trajkova and Koleva-Gudeva 2014). There have been reports on development of varieties and F_1 hybrids based on parental lines developed from doubled haploid (DH) technology (Chunling and Baojun 1995; Pauk et al. 2010). DH capsicum lines with improved yield characteristics and dry matter content in fruits were also obtained (Kisiała et al. 2011). Superior DH lines with considerable variation in plant and fruit traits (Shrestha et al. 2011) and androgenic capsicum lines with positive traits have also been isolated (Koleva-Gudeva and Trajkova 2012). Capsicum DHs with improved quality aspect like fruit shape, taste, fruit firmness, dry matter content, total soluble content, phenolic content, and antioxidant activity like CUPRAC and FRAP have been developed (Luitel and Kang 2013b).

Nowaczyk et al. (2014) used DH technology for stabilization of soft-flesh *capsicum* spp. recombinants.

The DH lines obtained from anther culture of capsicum in vitro exhibited different levels of resistance to *Xanthomonas campestris* pv. *vesicatoria* (Hwang et al. 1998) and *Phytophthora capsici* (Nervo et al. 2007). These resistant DH lines can be used to develop new multiple-disease–resistant genotypes. Resistant lines to PVY and lines with important qualitative and quantitative traits have also been isolated through anther culture (Arnedo Andrés et al. 2002; Mitykó and Gémes Juhász 2006). Todorova et al. (2013) recovered capsicum lines with high productivity, improved fruit traits, and low susceptibility to Verticillium wilt were produced through haploid culture. Application of microspore embryogenesis has been used to create genotypes with improved productivity, resistance to *Verticillium dahliae* Kleb (Grozeva et al. 2009; Koleva-Gudeva and Trajkova 2012; Todorova et al. 2013; Trajkova and Koleva-Gudeva 2014) and *Tobacco mosaic virus* (TMV).

3.5.4 Embryo Rescue

Embryo rescue has most often been used to overcome the post-zygotic hybridization barriers in interspecific crosses. Incompatibility during hybridization is more common among *capsicum* species belonging to different gene pools, but incompatility has been reported within same gene pool also as between *C. annuum* and *capsicum chinense* or *C. frutescens*. Many interspecific crosses in *capsicum* spp. produce fruits with shriveled seeds which are incapable of germinating normally because endosperm and/or embryo have not developed properly. There have been reports on successful recovery of hybrid embryo of interspecific crosses in *capsicum* genus. The first attempt of embryo rescue in *capsicum* spp. was done by Fari et al. (1983) where embryo was recovered from the cross of *C. annuum* and *C. baccatum*. Another example for wide hybridization is between *C. annuum* and *C. baccatum* where immature interspecific embryos or embryo were/was rescued before abortion occurs (Shivanna and Bahadur 2015). Embryo excision and in vitro embryo culture is a technically complex process. Also, the stage at which embryo abortion occurs after hybridization may depend on the specific genotypes involved in the cross. Within *capsicum* genus, some authors could rescue interspecific embryos at the latest immature stages (Yoon et al. 2006) while there are also examples in which embryos had to be rescued at the earliest stages (Hossain et al. 2003; Manzur et al. 2015). However, embryo rescue at earlier stage is more difficult with lower efficiency of recovering interspecific hybrids (Shen et al. 2011). Anthracnose resistance found in *C. baccatum* lines has been introgressed into *C. annuum* via the rescue followed by culture of embryo obtained from interspecific crosses between these two species (Yoon et al. 2006). Genetic bridge which is the based on the use of phylogenetically closer species to the two species affected by crossability barriers is an alternative approach to overcome the above problem. In this method, the bridge species is used to which has the ability to cross with both the target species. The bridge species is crossed first with one target species, and the hybrid so obtained is then crossed with the second target species (Shivanna and Bahadur 2015). *C. chinensis* has been found to be an ideal bridge species to perform the wide hybridization between *C. annuum* and *C. baccatum* (Pickersgill 1988).

3.5.5 Transgenic Development

Genetic transformation has provided an alternative approach for capsicum improvement program. The major advantage which transgenics technology offers is that it overcomes interspecific or intergeneric barriers and enables transfer of useful genes or novel traits into capsicum. The first capsicum transformation work has been reported in 1990 (Liu et al. 1990). However, poor reproducibility of capsicum is the major limiting factor for capsicum transformation studies. Table 3.4 summarizes the major efforts

Table 3.4 Transgenic studies in capsicum using Agrobacterium mediated transformation with CAMV 35S promoters

Genotype	Explant	Agrobacterium strain	Selection marker	Gene of interest/Reporter gene	Vector	Reference
Yolo Wonder, California Wonder, NVH3051, Jupiter, Liberty Bell, Guatemalan wild accession	Leaves, cotyledons, hypocotyls	A281/C58	Kanamycin (Neomycin phosphotransferase II (*nptII*)	B-glucuronidase (GUS)	p_3-1-GUS	Liu et al. (1990)
Golden tower	Cotyledons	LBA 4404	Kanamycin, carbenicillin	Cucumber Mosaic Virs I_{17}N-satellite RNA	pRok/105	Lee et al. (1993)
Zhong Hua No. 2	Leaves, cotyledons, hypocotyls	GV3111-SE	Kanamycin	CMV-CP	pHCM40	Zhu et al. (1996)
Pusa Jwala	Cotyledons	EHA 105	Kanamycin	GUS	pBI 121	Manoharan et al. (1998)
No. 40017	Cotyledons	C58CIRifR, LBA 4404, EHA 101, A281	Kanamycin, geneticin, hygromycin (*hpt*), methorexate (*dhfr*), phosphinothricin (*bar*)	GUS	pRGG plasmid set	Mihalka et al. (2000)
Nockkwang	Hypocotyls	LBA 4404	Kanamycin	*OsMADS1*	pGA1209	Kim et al. (2001a, b)
'Pusa Jwala'	Cotyledons	C58	Kanamycin	*GUS*	pGV1040	Shivegowda et al. (2002)
VS300-1	Young embryonic tissue, Cotyedons	LBA 4404	Chlorsulfuron (*sur B*)	*CaCel1*	pWTT2132	Harpester et al. (2002)
Golden tower	Hypocotyls, Cotyledons	LBA 4404	Kanamycin	TsiI	pMBP2	Shin et al. (2002b)
Nockkwang	Hypocotyls, Cotyledon		Kanamycin	GUS	pMBP2	Shin et al. (2002a)
F1 Xiangyan 10, Zhongjiao, Zhongjiao 5, Zhongjiao 6	Cotyledons	LBA 4404	Kanamycin	GUS	pBI121	Li et al. (2003)

(continued)

Table 3.4 (continued)

Genotype	Explant	Agrobacterium strain	Selection marker	Gene of interest/Reporter gene	Vector	Reference
Feherozon	Cotyledons	ShooterGRif^{R}, GV3170Rif^{R}	Hygromycin, Kanamycin	GUS	pRGG*hpt*, pRGG*neo*	Mihalka et al. (2003)
P915, P4090, P410, P101	Hypocotyls, Cotyledon	EHA 105, LBA 4404	Kanamycin	*TMV-CP, PPII*	pCAMBIA	Lee et al. (2004)
'K1', 'K2', 'PLR1'	Shoot tips	EHA 105	Cefotaxime and Kanamycin	*GUS*	pIG121 Hm	Sobhakumari and Lalithakumari (2005)
Byadagi Kaddi	Cotyledon, Hypocotyl, Cotyledonary nodal region/shoot tip	EHA105	Kanamycin	*cryI(Ac)*	pBinBt3	Channappagoudar (2007)
Nockkwang	Hypocotyls, Cotyledon	GV3101	Hygromycin	GUS	pCAMBIA1301	Ko et al. (2007)
C. frutescens	Pollen transformation	EHA 101	Hygromycin	*GUS*	pCAMBIA 1301	Sharma et al. (2008)
Habanero Pepper 'Naranja'	Leaf	LBA 4404	Rifampicin, Streptomycin, Kanamycin	GUS	pCAMBIA2301/pCAMex, pCAMex::PR10/pCAMex::Esterase	Arcos-Ortega et al. (2010)
F1 hybrids Fiesta, Ferrari and Spirit	Cotyledons	GV3101	Kanamycin, Cefotaxime, thidiazuron (TDZ)	BnBBM	pMP90 Ti plasmid	Heidmann et al. (2011)
'Arka Gaurav', 'Arka Mohini'	Hypocotyl	EHA 105	Kanamycin	*GUS*	pBinARβC1	Kumar et al. (2012)
California Wonder		LBA 4404	Kanamycin and rifampicin	GUS	pBI121	Verma et al. (2013)
Pusa Sadabahar, Pusa Jwala	Cotyledons, Hyotcotyls	LBA 4404	Kanamycin	*GUS*	pCAMBIA2301	Mahto et al. (2018)

made in developing transgenic capsicum. Major capsicum transformation work has been done for disease resistance particularly against viruses, viz, tobacco mosaic virus (TMV), pepper mild mottle virus (PMMV) (Lee et al. 2004), tomato mosaic virus (ToMV) (Shin et al. 2002a), and cucumber mosic virus (CMV) (Shin et al. 2002b). Such transgenic virus resistance mechanism utilizing viral coat protein regions and satellite RNA is currently known as RNA silencing (Voinnet 2001). Transformation and overexpression of *TsiI*, a tobacco pathogenesis-related (PR) gene in capsicum displayed broad spectrum resistance against different pathogens like PMMV, CMV, bacterial pathogen *Xanthomonas campestris* pv. *vesicatoria,* and a fungal pathogen *P. capsici* (Shin et al. 2002a). Besides disease resistance, transformation studies in capsicum on other aspects are limited. Suppression of ripening-related endo-1.4-β-glucanase in transgenic capsicum was demonstrated by Harpester et al. (2002). Studies on transformation of foreign genes identified from other plants or organisms into capsicum are quite rare. A dwarf transgenic capsicum has been produced upon transformation with *OsMADS1* gene from rice (Kim et al. 2001a, b). RNA silencing approach has been used to identify a new gene ketoacyl-ACP reductase (*CaKR1*) in capsicum responsible for producing nonpungent fruits (Koeda et al. 2019). Capsicum transformation studies has most commonly used GUS gene (β-glucuronidase) as reporter gene, CaMV 35S as promoter, and nopaline synthase (NOS) as terminator gene (Liu et al. 1990; Zhu et al. 1996; Manoharan et al. 1998; Li et al. 2003; Mihalka et al. 2003; Kim et al. 2001a, b; Shin et al. 2002a; Lee et al. 2004). Capsicum transgenics have been most commonly developed employing agrobacterium mediated transformation using cotyledons and/or hypocotyls as explants in most studies (Manoharan et al. 1998; Pozueta-Romero et al. 2001; Kim et al. 2001a, b; Shin et al. 2002a, b; Lee et al. 2004). Direct transformation using gene gun has been attempted recently in *C. frutescens* (Chee et al. 2018).

3.5.6 Marker-Assisted Breeding

Molecular marker-assisted breeding (MAB), also called molecular-assited breeding has been now being widely utilized in improvement of capsicum. Different types of molecular markers have been developed for capsicum like isozyme markers, amplified fragment length polymorphism (AFLPs), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLPs), simple sequence repeat (SSR), and single nucleotide polymorphism (SNP and conserved ortholog set (COS) II markers. These markers have been extensively utilized to understand inheritance of important traits as well as mapping of horticultural and disease resistance genes and quantitative trait loci (QTLs).

3.6 Limitations of Traditional Breeding

Conventional plant breeding is the principle approach to crop improvement where plant genomes are manipulated within the primary gene pool of the genus. It involves methods such as hybridization followed by selection, introgression by backcross breeding, creation of new variants through induced mutagenesis and somatic hybridization to create newer combination of different genomes. Identification of commercially important new traits is based on phenotypic assessments of segregating progenies.

There are a number of issues where the applicability of conventional plant breeding in enhancement of quality and yield beyond a certain point becomes very difficult. The major distinction between modern and traditional breeding is the separation between phenotype and genotype. Genes are inherited while the phenotype is an expression of those genes within an environment. Genetic variation is induced at the DNA level, but screening and selection of genotypes is done based on phenotypic

expression. As a result, new cultivars not only contain traits of breeder's interest but also display a number of undesirable features not considered during the selection process and through conventional breeding this transfer of undesirable traits from existing to new varieties is generally inevitable.

A second issue is encountered when breeders try to capture the genetic diversity available within sexually incompatibile groups. The new traits are introgressed into cultivated varieties through wide hybridization and extensive backcrossing of generated hybrids with recipient parent. However, the targeted traits of interest do not come alone but come along with larger segments of wild chromosomes or in other words are associated with linkage drag and this linkage drag might contain genes some of which are undesirable. The third limitation results from the inability of traditional breeding to control expression of target genes in a new genetic background. The use of modern breeding strategies such as marker-assisted selection not only accelerates the introgression process but also aim to reduce linkage drag.

In short, it can be said that traditional methods in plant breeding will continue to develop new and improved varieties. However, these methods are, by themselves, not sufficient to allow complete expression of genetic potential of a genotype, where molecular breeding plays its role being precise, rapid and cost effective in comparison with conventional phenotypic selection.

3.7 Need of Molecular Breeding

Direct selection which is based on the performance of the genotype or phenotypic values of target traits is effective for qualitative traits while selection for quantitatively inherited complex character is often elusive due to environmental influence. Under such condition, indirect selection is said to be a better alternative. Conventional breeding has met with limited success due to polygenic control of resistance traits, wide range of pathogen strains distributed in different environments, complexity of host–pathogen interaction and wide variability in pathogenicity. Indirect selection can be based on some other traits which is easily measured but tightly linked to the other traits of interest which is difficult to measure or is influenced by environment. Indirect selection for yield is limited via other traits. Due to the limitation of equipment, facilities, and resources, selection for target genes often becomes impractical. Advent of molecular (DNA) markers has created a powerful and practicable tool to perform gene selection in plant breeding. Although marker-assisted gene selection is not a real gene selection, it provides the best indirect selection tool for target genes at the DNA level. So, marker-assisted selection (MAS) is an effective and reliable approach.

Kole and Gupta (2004) and Collard and Mackill (2008) have illustrated the advantages of MAS when compared with conventional phenotypic selection. Selection using molecular markers is simpler compared to phenotypic breeding. Apart from this, selection may be carried out at any stage of the plants and single plant may be selected with high reliability. Molecular markers have offered large opportunities ranging from the localization of a gene to development of newer genotype combinations having good yield with stress-resistant genes. This saves a lot of time in the breeding process. They have aided in discovering more information about the function of the genes of interest. Apart from gene location and its selection, molecular markers assist in genetic diversity assessment, quality control, and marker-assisted breeding. To meet the growing demand for increasing capsicum production as well as disease-resistant genotypes, use of molecular markers is important to hasten the pace of improvement program. Molecular markers in capsicum have been utilized for DNA fingerprinting as well as genetic diversity analysis of capsicum (Bahrami et al. 2009; Hossain et al. 2014; Rego et al. 2011; Costa et al. 2016), QTL analysis of important biotic stresses (Lee et al. 2011; Lu et al. 2012; Dwivedi et al. 2013; Han et al. 2018) and MAS (Grube et al. 1996; Tanaka et al. 2014; Jeong et al. 2015; Suwor et al. 2017). Estimating the genetic diversity among capsicum genotypes helps in reliable differentiation of

genotypes. Genetic diversity analysis and varietal identification in capsicum have been carried out using different types of marker system like isozymes (Litoriya et al. 2010); RAPD (Bhadragoudar and Patil 2011, Thul et al. 2012); AFLP (Lafebvre et al. 2001; Ibiza et al. 2012); SSR (Ibiza et al. 2012) and ISSR markers (Thul et al. 2012). Genetic diversity assessment using molecular markers is useful in selecting diverse parental combinations for hybrid development, understanding evolutionary relationship between different capsicum species and for exact varietal identification. Molecular characterization of germplasm is important for the conservation and utilization of plant genetic resources (Thul et al. 2012). MAS is a molecular breeding technique that helps to avoid the difficulties concerned with conventional plant breeding. Advances in the science of genomics has led to the identification of thousands of DNA markers in capsicum which include mapped micro-satellite markers and more recently, single nucleotide polymorphisms (SNPs) (Huang et al. 2001; Lee et al. 2013; Buso et al. 2016; Cheng et al. 2016; Taranto et al. 2016).

With the SSRs and SNPs, some genes controlling biotic and abiotic stress resistances, quality characters and various aspects of plant development have been cloned and characterized in capsicum, which are excellent assets for molecular-assisted breeding (Sect. 3.5.5). At present, SSRs are the most widely used markers by capsicum researchers due to their availability in large numbers in the public domain including their simplicity and effectiveness (Cheng et al. 2016; https://solgenomics.net/). Genes/QTLs for several important traits of capsicum such as pungency (Lee et al. 2005; Stewart et al. 2005, 2007), fertility restoration (Zhang et al. 2000; Min et al. 2008; Jo et al. 2010), soft flesh and deciduous fruits (Rao and Paran 2003), capsanthin content (Lefebvre et al. 1998), fruit size and shape (Ben Chaim et al. 2001; Rao et al. 2003), male sterility (Chen et al. 2012), parthenocarpy (Tiwari et al. 2011), resistance to CMV (Kang et al. 2010), potyviruses (Murphy et al. 1998; Kang et al. 2005), chili veinal mottle virus (Ruffel et al. 2006; Hwang et al. 2009), tobamoviruses (Berzal-Herranz et al. 1995; Tomita et al. 2011), bacterial spot (Tai et al. 1999; Mazourek et al. 2009; Pierre et al. 2000; Jordan et al. 2006), anthracnose (Voorrips et al. 2004), Phytophthora rot (Thabuis et al. 2003; Ogundiwin et al. 2005; Kim et al. 2008), powdery mildew (Lefebvre et al. 2003), and root-knot nematodes (Djian-Caporalino et al. 2001, 2007) have been mapped, and some of them have been utilized for MAS (Grube et al. 1996; Tanaka et al. 2014; Jeong et al. 2015; Suwor et al. 2017) which otherwise would have been very difficult using conventional breeding.

3.8 Future Prospects

Achievements made in capsicum breeding illustrate its possibilities for further improvement. There is considerable opportunity for further improvement of capsicum. With the availability of whole genome sequence of capsicum and single nucleotide polymorphism (SNP) discovery through genotyping by sequencing method, the genetic diversity in capsicum has been unraveled. With this genomic information in hand, we can believe that the genetic makeup of capsicum may be modified to a much greater extent than we normally appreciate. However, studies reporting association between this vast genetic diversity and the observed phenotypic variability is still poor. Finding new associations between the generated genomic resources and important traits of importance in capsicum such as fruit size, fruit production, pungency, abiotic stress tolerance, nutritional content, and disease resistance is an important research area. The exploitation of transgenic technology in capsicum is also slow as capsicum is highly recalcitrant to transformation and regeneration process. Further with the availability of capsicum genome sequence, latest genome-editing technologies and their potential applications in the genetic improvement of capsicum can be explored. However, lack of well-characterized target gene information is a major limiting factor that restricts the broad application of gene/genome-editing technologies to capsicum. Utilization of new upcoming

technologies will continue to advance which in combination with traditional techniques of selections and crosses already established in capsicum genetic breeding will become an essential tool.

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