Chapter 9
Breeding Strategies of Garden Pea (*Pisum sativum* L.)

Amal M. E. Abdel-Hamid and Khaled F. M. Salem

Abstract Garden pea (*Pisum sativum* L.), a member of the Fabaceae family, is one of the most important self-pollinating legume crops. Globally, the pea is an economic crop, utilized as food, feed and industrial uses. Garden pea is an annual winter-season crop grown around the world from winter to early summer depending on the country. Gene banks have conserved a large genetic resource collection of pea germplasm. *Pisum* harbors significant diversity based on biological status, geographical regions and morpho-agronomic traits. Introgression of novel alleles through crossing between various pea genetic resources, e.g. modern varieties with locally adapted varieties, enhances genetic diversity and preselection for traits of interest, which is required to ensure meaningful natural variation at the phenotypic level. Improving pea for biotic and abiotic stress tolerance traits, quality traits and yield attributes are the main objectives of breeders and geneticists. These can be achieved with genomics tools to augment traditional breeding programs. In this chapter, we will provide an overview of the origin of the pea, distribution, genetic resources, conservation, cultivation practices, recent developments in biotechnology and molecular genetics to improve traditional breeding methods.

Keywords Biodiversity · Biotechnology · Breeding · Genetic improvement · Modern pea breeding · *Pisum sativum* · Traditional breeding

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J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Vegetable Crops*, https://doi.org/10.1007/978-3-030-66969-0_9

9.1 Introduction

9.1.1 Origin and Distribution

Ben-Ze'ev and Zohary (1973) reported that pea (*Pisum sativum* L.) originated in the Mediterranean area, western and central Asia and Ethiopia. FAO designates Ethiopia and western Asia as centers of genetic diversity, with secondary centers in southern Asia and the Mediterranean Region (Singh et al. 2019). The first cultivation of pea was in western Asia and it spread to Europe, China and India (Ljuština and Mikić 2010). India is the largest vegetable pea producer worldwide (Vijay et al. 2018). Pea was already well known in Central and East Africa and was established in Uganda and Rwanda by 1860 as an important food crop. The first consumption of edible pods was recorded in the Netherlands and France during the sixteenth century (Blixt 1970).

Peas are found in most tropical countries (Mikić et al. 2007). They are grown in the highlands of East and Central Africa, Ethiopia and southern Africa but are hardly grown in West Africa. In Africa, the pea has a great deal of importance, it is found in French and English-speaking countries. Pea was grown in the United Kingdom in the Middle Ages and was introduced into the Americas after Columbus (Davies et al. 1985). Vavilov (1992) recorded the first centers of origin and diversity of crops, which are presented in Table 9.1.

Pea was the key experimental plant for the first genetic studies, performed by Gregor Mendel (Father of Genetics) in 1850 (Smýkal 2014). Mendel chose *Pisum sativum* because it has several advantages for research in genetics. Pea plants have many varieties with distinct heritable characters, grow quickly and can self-pollinate or be cross-pollinated. Mendel studied the inheritance patterns of seven traits in *P. sativum* plants. Ever since, Mendel's work has been widely analyzed and discussed (Fisher 1936) and became the foundation of the new discipline of genetics (Bateson 1902; Weldon 1902). Despite the tremendous progress in genetics and modern plant breeding in recent years, it will forever rely on the basic principles formulated by Mendel on the garden pea. Genetics has a great role in crop breeding and similarly genomic knowledge is gradually being translated to molecular breeding and genome-wide or genomic selection for the development of improved breeding lines (Smýkal et al. 2016).

About 98,000 pea accessions are preserved worldwide, only 2% are wild pea relatives, approximately 34% commercial varieties, 13% breeding lines, 38% landraces and 2% mutant stocks. In the case of true wild *Pisum* species, there are only 0.46% *P. fulvum*, 0.42% *P.* ssp. *elatius*, 1.2% *P. sativum* ssp. *sativum* (syn. *P. humile/syriacum*) and 0.36% *P. abyssinicum* of accessions Fig. 9.1.

Peas (*Pisum sativum* L., 2n = 14) are consumed as dry seeds or fresh vegetables throughout the world. According to Abbo et al. (2017), pea (*P. sativum*) varieties belong to one of the following groups: a) *P. sativum* L. ssp. *sativum* (field pea, garden pea, spring pea, English pea, common pea, green pea, b) *P. sativum* var.

Table 9.1 Centers of origin and diversity of crops around the world

Center name	Number of species	Crops
Chinese Center	138	Cereals, buckwheat, legumes
Indian Center	117	Rice, millets, legumes
Indo-Malayan Center (Indonesia, Philippines)	55	Root crops, fruit crops, sugarcane, spices
Inner Asiatic Center (Tadjikistan, Uzbekistan)	42	Wheat, rye, many herbaceous legumes, as well as seed-sown root crops, fruits
Asia Minor (Transcaucasia, Iran and Turkmenistan)	83	Wheat, rye, oats, seed, forage legumes, fruits
Mediterranean Center	84	Wheat, barley, forage plants, vegetables, fruits-especially, spices, ethereal oil plants
Abyssinian Center (Ethiopian)	38	Wheat, barley, local grains
South Mexican and Central American Centers	49	*Phaseolus*, maize, fiber plants, spices, cucurbitaceous, fruits
South America Andes region (Bolivia, Peru, Ecuador)	45	Root crops, grain, potatoes, vegetables, fruits, drugs tobacco, quinine, coca
Chilean Center	4	*Solarium tuberosum*
Brazilian-Paraguayan Center	13	*Manihot esculenta* (cassava), *Arachis hypogaea* (peanut), *Ananas comosus* (pineapple), *Hevea brasiliensis* (rubber), *Theobroma cacao* (cocoa)

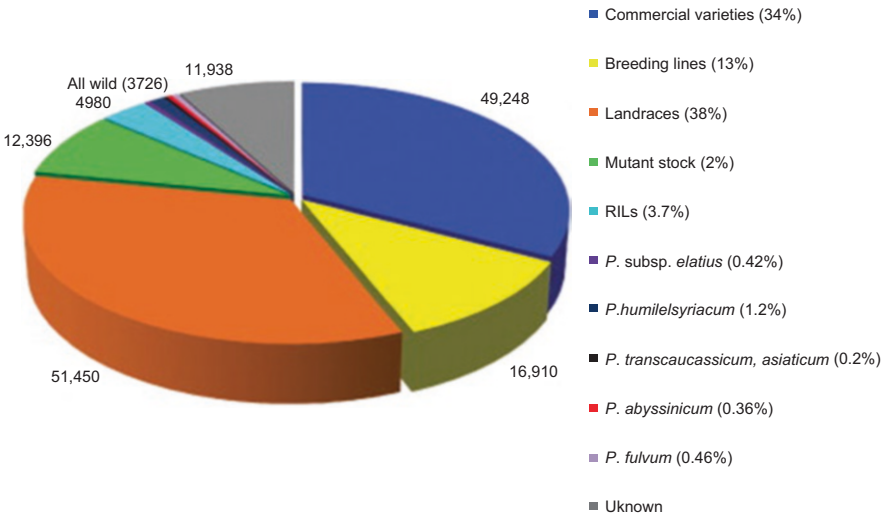

Fig. 9.1 Pea germplasm stratification. (Source: Smýkal et al. 2013)

Table 9.2 Nutritional value of garden pea

Content	Concentration (%)
Protein	21.3–32.9
Starch	36.9–49.0
Resesitant starch	2.1–6-3
Soluble sugars	5.3–8.7
Dietary fiber	14–26
Insoluble fiber	10–15
Soluble fiber	2–9
Amylose	20.7–33.7
Lipids	1.2–2.4
Ashe	2.3–3.4

Source: Dahl et al. (2012)

Fig. 9.2 Different varieties of *Pisum sativum*. (Source: www.flickr.com/photos)

saccharatum (snow pea) and c) *P. sativum* var. *macrocarpon* (snap pea or sugar snap pea) (Table 9.2; Fig. 9.2).

Holdsworth et al. (2017) assembled the USDA Pea Single Plant Plus Collection (PSPPC), which contains 431 *Pisum sativum* accessions. The collection was characterized genetically in order to maximize its value for trait mapping and genomics-assisted breeding (Fig. 9.3).

9.1.2 Economic Importance and Health Benefits

Pisum sativum plants are commonly used in several ways: fresh, canned or frozen. Peas have great nutritional value because they contain protein, carbohydrates, fiber, minerals, vitamins and antioxidant compounds (Amarakoon 2012; Hall et al. 2017; Hedley 2001; Nilsson et al. 2004; Paul and Southgate 1988). Young shoots are used

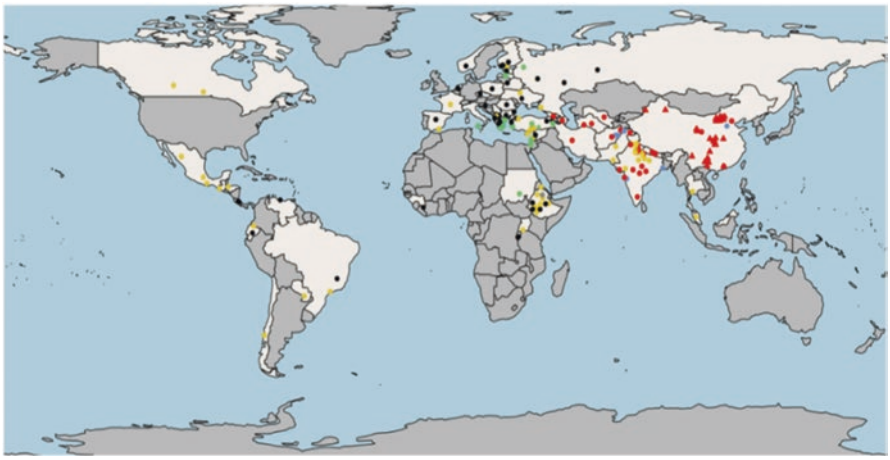

Fig. 9.3 Map of *Pisum sativum* accessions around the world. Circles indicate accessions in the original Pea Single Plant Collection (PSPPC) and triangles indicate accessions from the Chinese core collection *P. sativum* ssp. *elatius* (green); *P. sativum* ssp. *abyssinicum* (gray); *P. sativum* ssp. *sativum* – Primary (gold); *P. sativum* – Central Asia (dark blue) and *P. sativum* ssp. *sativum* -non-Mediterranean Asia (red)

as a leafy vegetable in Malawi and some Asian countries. Dry pea seeds are used for animal feed (Hedley 2001) and pea straw is used as forage, hay, silage or green manure. Importantly, peas play a key role in soil fertility by fixing atmospheric nitrogen (Messiaen et al. 2006).

Pea seeds are reputed to have beneficial effects on skin conditions in the form of face masks used to treat wrinkled skin (Aburjai and Natsheh 2003). Worldwide, peas are one of the major food legumes grown in various regions especially in Europe (Ljuština and Mikić 2010; Rana et al. 2017). Pea production has increased rapidly; production now occupies fourth place among world food legumes production after soybeans, peanuts and dry beans (Adsule and Kadam 1989). Peas are highly nutritive (Table 9.2) for both human diet (Dahl et al. 2012) and animal feed as an alternative to soybeans (Cruz-Suarez et al. 2001; Hedley 2001). Altogether, these factors position peas at a similar economic level to cereals.

Peas are of great interest as a crop in Europe, due to their capacity to produce a higher yield compared to local cultivars (Annicchiarico 2008). High yield and its stability, tolerance for biotic and abiotic stresses, in addition to high protein content, are important traits for pea development as a feed crop (Khodapanahi et al. 2012).

The increasing load of environmental pollutants, particularly heavy metal ions in soil, water and air during the last decades, due to the extensive and/or uncontrolled human activities, are reported to impose a drastic environmental stress on growth, morphogenesis and yield on higher plants, particularly those of nutritive value for humans and certain livestock (Lyanguzova 1999; Mishra and Choudhuri 1999; Nyarai-Horvath et al. 1997; Obroucheva et al. 1998). Certain vascular plants such as

legumes can respond to heavy metal ions at concentrations much lower than those required to elicit a response in animals and human beings. These plants can be utilized as indicators for pollution in the environment and to monitor their concentrations as biomonitors. In this regard, Abdel-Hamid (2000) revealed that *Pisum sativum* tends to be one of these bio-monitors. Aissani et al. (2019) found that peas can be irrigated with yeast industrial liquid effluent and give good germination and growth.

9.1.3 Domestication, Selection and Early Improvements

Harlan (1992) stated that the family Fabaceae has the greatest number of domesticated crops of any plant family. Fabaceae members have an excellent system to study as to the extent parallel variations in morphology are determined by similar mutations.

The earliest archaeological and hereditary investigation shows that the pea was domesticated in the Near East and the Mediterranean Basin (Zohary and Hopf 2000). Also, peas were found in the late Neolithic era of present-day Greece, Syria, Turkey and Jordan. In Egypt, early finds date from 4800 to 4400 BC in the Nile Delta and from 3800 to 3600 BC in Upper Egypt. Peas were present in Pakistan and western and northwestern India in 2250–1750 BC. The pea was also present in the Republic of Georgia, the Ganges Basin and southern India as a legume crop in the fifth millennium BC (Chimwamurombe and Khulbe 2011).

Pisum sativum was domesticated from the wild *P. humile* which is common in northern Iran, Iraq, Jordan, Turkey, Syria and Palestine. *Pisum sativum* arrived in India and China via the Himalayan trade routes and the Greeks. *Pisum elatius* is another wild species which is found in North Africa, southern Italy and throughout the Near East (Harlan et al. 1976; Yamashita 1980; Zeven and De Wet 1983).

The *Pisum sativum* group is cultivated around the world including in tropical Africa. Both *P. fulvum* and *P. sativum* were domesticated in the Near East about 12,000 years ago, likely from *P. humile* (otherwise called *P. sativum* ssp. *elatius*). *Pisum abyssinian* is cultivated in the northern and southeastern regions of Ethiopia; it originated from *P. sativum* independently in the Old or Middle kingdoms of Egypt around 5000 years ago. It is also grown in Yemen (Weeden 2018). Other cultivar groups, varieties or subspecies occur in southern Europe and western Asia. Subsequent breeding and developments have resulted in the production of thousands of pea genotypes today (Govorov 1937; Smýkal 2014; Vershinin et al. 2003) (Figs. 9.4 and 9.5).

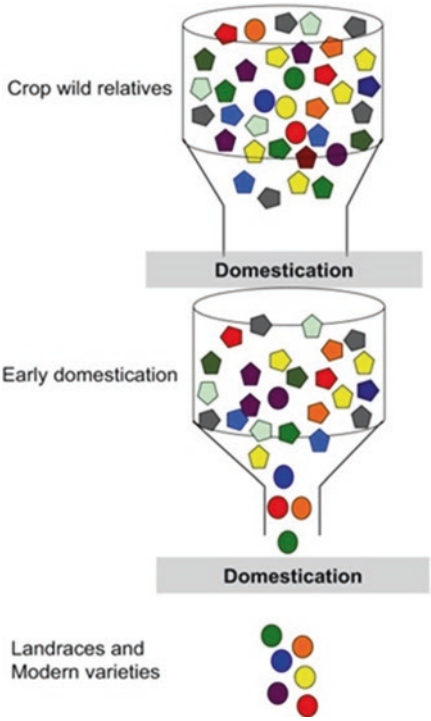

Fig. 9.4 Domestication of *Pisum sativum.* (Source: Zhang et al. 2016)

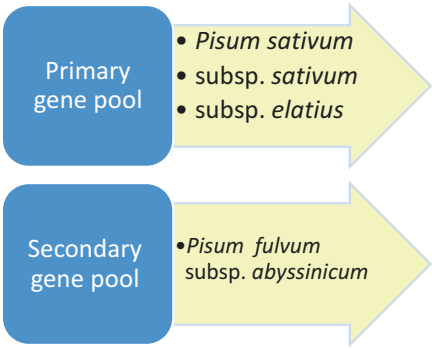

Fig. 9.5 Gene pools of *Pisum sativum*. (Prepared by Amal M.E. Abdel-Hamid)

9.2 Current Cultivation Practices and Challenges

9.2.1 Current Cultivation Practices

Garden pea (*Pisum sativum*) is one of the most common and important winter vegetable crops grown on a global scale and consumed either fresh or in processed form. It improves soil fertility due to the fixation nitrogen by *Rhizobium* bacteria (Messiaen et al. 2006; Phillips 1980). Peas are mixed with other vegetables or used

alone; they are also processed for canning and freezing to meet consumer requirements during the off-season. Important quality attributes of peas are good flavor, high chlorophyll content, the dark green color of the pods, high glucose and fructose content and good texture. There are also different quality standards required for various manufactured products. For canning, extruded peas should have a light green color and resist washing out of chlorophyll by the salty liquid in the can. However, seed freezing varieties should be dark. The color intensity of the seeds is positively associated with color pods. Peas for dryness should be large in size, wrinkled, dark green with high dry material content. Peas improve soil fertility by providing nitrogen for successive crops in rotation schemes, without the need for supplementary fertilizer (Bobille et al. 2019).

Pea cultivation requires a temperature of 18–22 °C to obtain a good germination rate. They can germinate under a starting temperature of 4–5 °C and tolerate moderate frost. As the temperature rises to 25 °C and above, the percentage of germination decreases. Pea can be grown in all soil types except heavy clays. The pea gives the best growth in acidic soils with soil pH ranging from 6.0 to 7.5. It is preferable to add organic matter or compost before planting where it serves to improve soil properties, fertility and structure. Soil service varies depending on the previous crop and plot status. If cultivated after a crop that left behind organic matter it must be tilled in and disc plowed twice perpendicular and then disked twice to create the desired soil structure. Sowing is carried out in two ways. One, by sprouting, which is double sowing if planted toward the end of September to the beginning of October, in the Northern Hemisphere. Planting seed manually or mechanically the distances between lines and other plants should be 60 cm and 25 cm, respectively, and irrigation continued until germination and the appearance of shoots above the soil surface and two, planting where there is the presence of soil moisture and irrigation of the land before planting for a sufficient period or as a result of rain, the moisture allows germination without damage to the seed shell. In the case of heavy soils, given the hard seed shell, there is less aeration and absorption of plant nutrients, which can lead to plant yellowing and death.

In fields where peas have not been grown before, seeds should be treated with nitrogen-fixing *Rhizobium* bacteria. This ensures the formation of bacterial nodes, good growth and crop quality (Messiaen et al. 2006). Manual or chemical weed control can be used. In the latter case, appropriate pesticides and specialized spraying of plants and soil must contain enough moisture to obtain high efficiency of the pesticide.

9.2.2 Current Agricultural Challenges

Vegetable crops face many abiotic and biotic stresses, which affect growth and yield due to global warming and related climate changes (Atkinson et al. 2013; Mahalingam 2015; Mittler 2006; Narsai et al. 2013; Pandey et al. 2015; Prasad et al. 2011; Prasch and Sonnewald 2013; Ramegowda and Senthil-Kumar 2015; Suzuki

et al. 2014). Salinity, drought, heat and other types of abiotic stress together are more destructive to the growth and production of vegetable crops than a stress factor occurring separately at different stages of vegetative growth of a crop (Mittler 2006; Prasad et al. 2011).

Abiotic stresses affect the spread of pathogens, insects and weeds (Coakley et al. 1999; McDonald et al. 2009; Peters et al. 2014; Scherm and Coakley 2003; Ziska et al. 2010). Also, in the future, pests may become a greater threat to the growth and production of crops (Duveiller et al. 2007). Environmental stress conditions play a direct role in plant pest interactions by altering plant organ functions and resistance (Scherm and Coakley 2003). Similarly, abiotic stress conditions such as drought increase the competitiveness of weeds for water use compared to crops (Patterson 1995; Valerio et al. 2013; Ziska et al. 2010).

9.2.3 Genetic Improvement Objectives

Early maturity and high productivity are the main objectives of pea breeding. Early-maturing crops have an important comparative advantage for farmers because of higher prices at the beginning of the production season. Also, pod attributes such as pod size and seed size are the most important qualities as they are qualities that affect the market price of peas. Reproduction for disease resistance and the development of new genotypes are the main targets of the breeding programs in some areas such as those related to *Fusarium* wilt (Shubha et al. 2019), crushed mold, rust, pea-borne mosaic virus, structural mosaic virus and yellow mosaic virus. As well is breeding for pest resistance and the development of genotypes resistant to leaf miner, weevils and aphids. Moreover, peas are frost sensitive and resistance to it is among the breeding targets to ameliorate environmental stresses. Also, peas are grown for fresh consumption, processing, canning, and freezing (Hedley 2001; Paul and Southgate 1988).

9.3 Germplasm Biodiversity and Conservation

Germplasm is the crude raw material that pea breeders use to create new genotypes. It is comprised of different types of genetic accumulations, for example, natural hybrids, primitive cultivars, wild species, obsolete varieties, breeding lines, elite lines and mutants (Haussmann et al. 2004).

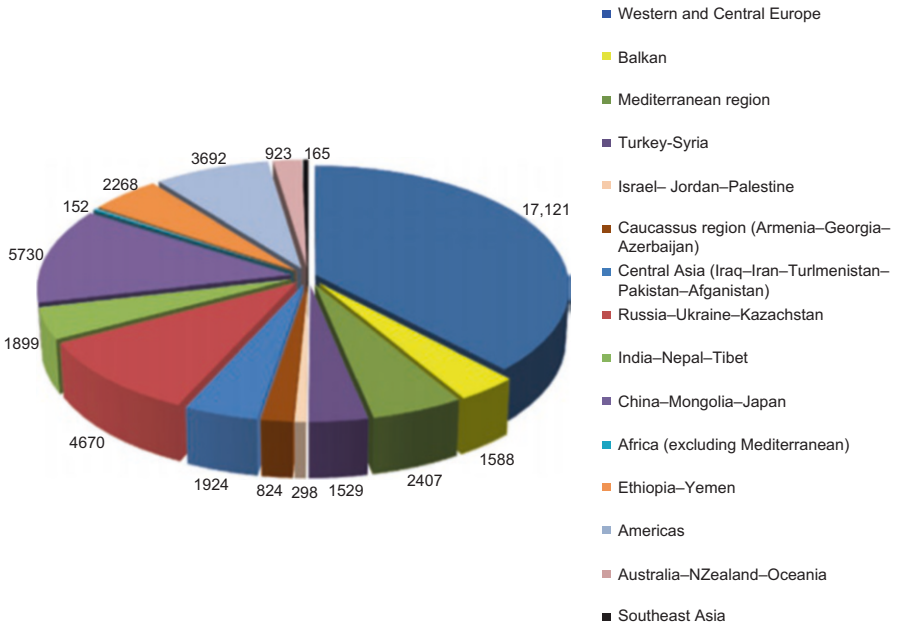

Fig. 9.6 Pea germplasm collections around the world. (Source: Smýkal et al. 2013)

9.3.1 Germplasm Diversity

A large amount of genetic diversity of *Pisum sativum* has been found in Africa and Asia. Many germplasm collections of *P. sativum* cultivars are held around the world as detailed in Fig. 9.6, and Appendix II-A. The collections contain wild and primitive varieties, cultivars with multiple disease resistance, lines carrying structural mutations, breeding lines and cultivars of specific interest (Zong et al. 2008).

Morphological and agronomical traits that are resistant to biotic and abiotic stresses, identified to individual genotypes, increases the importance of the germplasm (Ceyhan and Avci 2015; Ghafoor et al. 2005). The economic importance of a population is associated with morphology, agronomic traits, seed nutritional and quality traits. The efficient utilization of indigenous germplasm requires data on the genetic diversity of economic interest (Singh et al. 2019).

9.3.2 Cultivars Characterization and Phylogeny

Morphological traits can help breeders to develop better maintenance strategies and economic utilization of pea genetic resources. Morphological traits are influenced by environmental factors (Ceyhan and Avci 2015); therefore, breeders need stable characters to characterize different germplasm accessions. A classical method of

estimating diversity in a population is the use of molecular markers in pea (Hanci 2019).

McClendon et al. (2002) identified 8 AFLP and 15 RAPD markers associated with *Fusarium* wilt race 1 resistance in pea. These DNA markers are suitable for marker-assisted selection in pea breeding programs. Marker-assisted selection (MAS) is now being integrated into on-going conventional pea breeding. MAS is useful to speed-up selection for those traits that express lateness in plant development. Such target traits include resistance to diseases, and even lodging and seed characters. Isozyme marker alcohol dehydrogenase (*Adh1*) has been shown to be linked with resistance to pea enation virus (En.). Two new examples associated with disease resistance are the development of PCR markers designed from cDNA-AFLP fragments giving close linkage to genes (*subm-1*, *mo*) presenting resistance to pea seed-borne mosaic virus and SSR marker suitable for resistance to powdery mildew of peas, as mentioned by Ambrose (2008). QTLs for lodging resistance have been reported.

The primary example of genetic linkage in pea was described by Vilmorin and Bateson (1911) and the first genetic map was developed by Wellensiek (1925). In the twentieth century, whole genetic maps consist of 7 linkage groups (LGs) based on the pea karyotype; RAPD and RFLP markers were constructed and shown in Table 9.3 (Aubert et al. 2006; Bordat et al. 2011). Lately, the availability of pea EST databases has resulted in the design and mapping of numerous gene-based molecular markers in *Pisum sativum*. Advancement of next-generation sequencing (NGS) allowed distinguishing a great many single nucleotide polymorphism sites (SNPs) (Duarte et al. 2014; Kaur et al. 2012; Leonforte et al. 2013; Ma et al. 2017; Sindhu et al. 2014; Tayeh et al. 2015; Yang et al. 2015; Zhernakov et al. 2017). Guindon et al. 2016 used the SRAP (sequence-related amplified polymorphism) technique for linkage mapping in *P. sativum* (Fig. 9.7). Many studies (Ellis 2011; Jing et al. 2010; Smýkal et al. 2011) of *P. abyssinicum* placed it between *P. fulvum* and *P. sativum* ssp. *elatius* and additional branches were found within the cultivated pea (Fig. 9.8).

9.3.3 Genetic Resources Conservation Approaches

Conservation of *Pisum sativum* genetic resources is becoming increasingly important because of the loss of traditional varieties adapted to a specific region being substituted by foreign-origin varieties (Khoury et al. 2016), emerging new crop diseases, environmental pollution and developments in crop processing.

Table 9.3 List of different types of markers tagged for disease resistance in pea

Trait	Gene	Marker	References
Bean yellow mosaic virus resistance	*mo*	Pgm-p (isozyme), P252 (RFLP)	Weeden et al. (1984)
Pea seed borne mosaic virus	*sbm-1*	GS185 (RFLP)	Timmerman-Vaughan et al. (1993)
Ascochyta blight resistance	*QTL*	Af& I (linkage group I);p227, p105 (RFLP Linkage group IV; p236 RFLP LG VI)	Dirlewanger et al. (1994)
Fusarium wilt resistance	*Fw*	H19, Y14, Y15 (RAPD) p254, p248, p227, $p10_{\mu}$ (RFLP)	Dirlewanger et al. (1994)
Powdery mildew resistance	*er-1*	p236 (RFLP) $PD10_{650}$(RAPD to SCAR)	Dirlewanger et al. (1994)
Powdery mildew resistance	*er-2*	(SCAR) 3 AFLP primers	Tiwari et al. (1998)
Powdery mildew resistance	*er-1*	Sc-OPO-18_{1200}, Sc-OPE-16_{1600}	Frew et al. (2002)
Mycosphaerella pinodes resistance	*mp*	ccta2 (SSR), cccc1 (SSR), *acct1* (SSR)	Dita et al. (2006)
Powdery mildew resistance	*er1 er2 er3*	MAS	Ghafoor and McPhee (2012)
Pea enation mosaic virus (PEMV)	*en*	EST, MAS, RAPD, SSR, STS, TRAP,	Jain et al. (2013)
Ascochyta blight resistance	*abI-IV-2.1*	SNP	Jha et al. (2017)
Mycosphaerella blight resistance	*QTLs*	SNP	Gali et al. (2018)
Ascochyta blight resistance	*QTLs*	SNP	Carpenter et al. (2018)
Fusarium root rot resistance	*Fsp-Ps 2.1*	Ps900203	Coyne et al. (2019)

9.3.3.1 Ex Situ Conservation

The ex situ conservation of plant genetic resources began in the twentieth century as a response to the rapid loss of biodiversity and the replacement of local varieties with developed genotypes (Gepts 2006; Khoury et al. 2014; Van de Wouw et al. 2009). This replacement was done with the introduction of advanced machinery, herbicides, pesticides, fertilizers into agrarian systems that allowed the cultivation of improved varieties everywhere (Khoury et al. 2016).

A large amount of ex situ *Pisum sativum* germplasm has been collected and preserved around the world in numerous agricultural centers. These centers and the international consortium for pea genetic resources (Pea GRIC) collaborate to link key collections in Europe, the USA, Africa, Asia and Australia. In India, about 2000 pea germplasm accessions are conserved at the National Bureau of Plant Genetic Resources (NBGPGR), Indian Institute of Vegetable Research (IIVR) and Indian

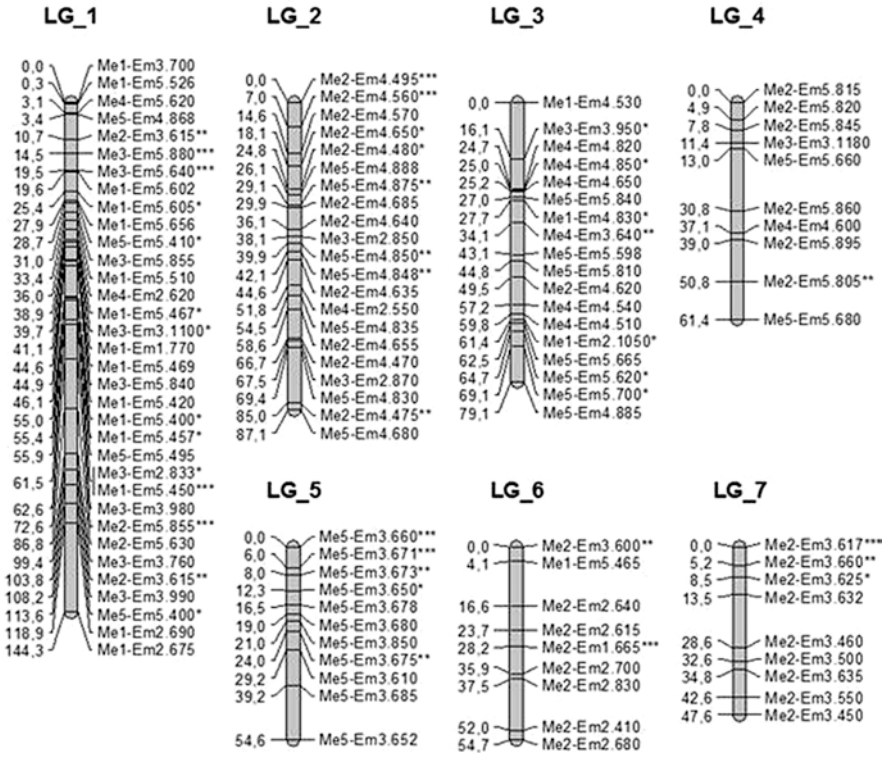

Fig. 9.7 *Pisum sativum* consensus functional map. (Source: Guindon et al. 2016)

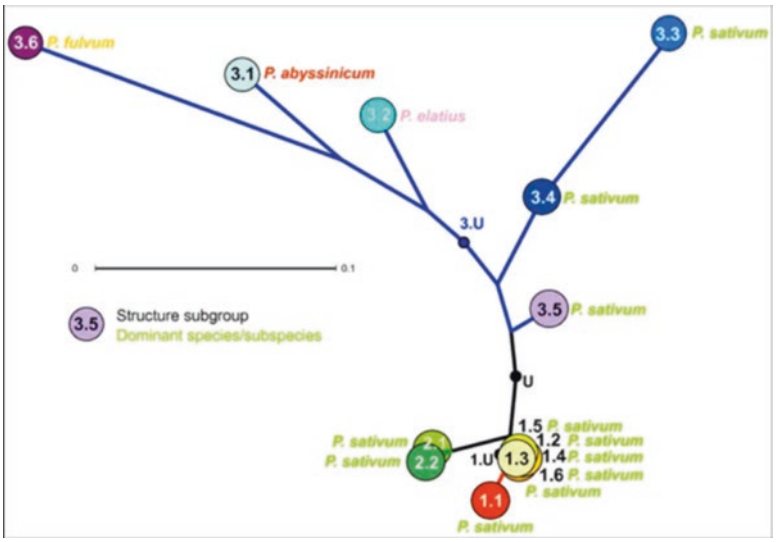

Fig. 9.8 *Pisum* genus diversity and phylogeny. (Source: Jing et al. 2010)

Table 9.4 Number of *Pisum* accessions conserved ex-situ in major collections

Species	AARI	ATFC	ICARDA	IPK	JI	NGB	W-6	VIR
P. sativum var. *sativum*	10	3683	882	2384	1680	1150	3718	6509
P. sativum var. *arvense*	15	13	ND	ND	ND	ND	58	ND
P. sativum var. *elatius*	8	17	10	15	31	8	51	3
P. sativum var. *brevipedunculatum*	1	ND	ND	ND	ND	ND	ND	ND
P. sativum var. *pumilio*	2	7	1	0	4	2	24	0
P. abyssinicum	4	16	6	41	33	4	17	4
P. fulvum	2	53	31	4	55	10	48	2
P. formosa	0	0	0	0	0	1	0	5
Total	42	3789	930	2444	1803	1175	3916	6523

AARI Aegean Agricultural Research institute, Turkey, *ATFC* Australian Temperate Field Crop, Australia, *ICARDA* International Center for Agricultural Research in the Dry Areas, Syria, *IPK* Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany, *JI* John Innes Centre, Department of Applied Genetic, UK, *NGB* Nordic Gene Bank, Sweden, *W-6* The Western Regional Plant Introduction Station, USA, *VIR* Vavilov Research Institute of Plant Industry, St. Petersburg, Russia, *ND* not differentiated

Institute of Pulses Research (IIPR), Kanpur. Besides, a few state agricultural universities are rich in vegetable pea germplasm such as Punjab Agricultural University.

Details about the status and the composition of the conserved *Pisum* species is documented by the World Information and Early Warning System on Plant Genetic Resources (WIEWS), which contains information on national PGR holdings (www.fao.org/ag/agp/pgr/'"iews/) and System-wide Information Network for Genetic Resources (SINGER) which contains information on CGIAR holdings (http://www.cgiar.org/singcr) (Maxted and Ambrose 2001) (Table 9.4).

9.3.3.2 Cryopreservation

Cryopreservation is described as preserving biological samples and storing them at especially low temperatures by using liquid nitrogen (Berjak et al. 1995; Haskins and Kartha 1980; Kartha and Engelmann 1994; McAdams et al. 1991). Low temperature preserves samples by slowing their metabolic processes and prevent deterioration of tissue (Jang et al. 2017; Kartha 1981). At present, there is no international center for pea breeding and genetic conservation (Flavell et al. 2011). No released collection is of great size or diversity. Data have been published on diverse groups of *Pisum* containing more than 2000 accessions in national gene banks of various countries (Table 9.5) (Ambrose et al. 2011; Ford-Lloyd et al. 2010; Miles et al. 2011). There is a high level of duplication among collections, which creates a deceptive impression of the true level of diversity (Ambrose et al. 2011; Miles et al. 2011). A list of important world gene banks for pea genetic resources conservation is given in Table 9.5.

Table 9.5 List of important gene banks for pea genetic resources

Gene Bank	Country	Website
Institute of Botany NAS RA, Yerevan	Armenia	http://www.botany.sci.am/
Winter Cereal Collection Gene Bank	Australia	www.dpi.nsw.gov.au/about-us/research-development/centres/
National Center for Vegetable Crops Research (CNPH) EMBRAPA	Brazil	http://www.cnph.embrapa.br
Institute for Plant Genetic Resources	Bulgaria	https://www.genesys-pgr.org/ar/wiews/BGR001
Institute of Crop Science, Chinese Academy of Agricultural Sciences	China	http://www.cgris.net/icgr/icgr_english.html
Czech Republic Gene Bank	Czech Republic	http://genbank.vurv.cz/genetic/resources/asp2/default_a.htm
Plant Gene Resources of Canada, Saskatchewan,	Canada	http://www.agr.gc.ca/pgrc-rpc
Ethiopian Biodiversity Institute (EBI)	Ethiopia	http://www.ebi.gov.et/
Centre de RessourcesGénétiques des Légumineuses, INRA-UMR LEG Dijon	France	http://www.reseau-graines.org/platform.htm
Leibniz-InstitutfürPflanzengenetik und Kulturpflanzenforschung (IPK)	Germany	https://www.ipk-gatersleben.de/en/gbisipk-gaterslebendegbis-i/
Institute for Agrobotany, Tapioszele	Hungary	http://www.nodik.org/english/
ICAR-National Bureau of Plant Genetic Resources	India	http://www.nbpgr.ernet.in
Institute of Biosciences and Bioresources	Italy	http://www.igv.cnr.it
Genetics Resources for Wheat Sciences Gene Bank	Japan	https://shigen.nig.ac.jp/wheat/komugi/
Centre for Genetic Resources, Wageningen	Netherlands	https://www.wur.nl/en/Research-Results/Statutory-research-tasks/Centre-for-Genetic-Resources-the-Netherlands-1/Expertise-areas/Plant-Genetic-Resources.htm
Plant Breeding and Acclimatization Institute Blonie Radzikow	Poland	http://www.igr.poznan.pl/
N.I. Vavilov Research Institute of Plant Industry, St. Petersburg	Russia	http://www.vir.nw.ru
Instituto Tecnológico Agrario de Castilla y León	Spain	http://www.itacyl.es

(continued)

Table 9.5 (continued)

Gene Bank	Country	Website
International Center for Agricultural Research in the Dry Areas (ICARDA), Gene Bank	Syria	http://www.icarda.org/
Nordic Genetic Resource Centre, NordGen	Sweden	http://www.nordgen.org/sesto
Yurjev Institute of Plant Breeding, Kharkov	Ukraine	http://www.bionet.nsc.ru
John Innes Centre, Norwich	UK	http://www.jic.ac.uk
USA Plant Germplasm Introduction and Testing Research Station, Pullman	USA	https://www.ars-grin.gov

9.3.3.3 In Vitro Conservation

In vitro conservation of *Pisum sativum* can be accomplished by somatic embryogenesis or organogenesis from callus cultures (Bala et al. 2010). Using organogenesis to induce shoot, root, and callus production was achieved in an Egyptian genotype of P. *sativum*. Calli were initiated from hypocotyl, leaf, root and mature embryo explants then cultured on MS medium Murashige and Skoog (1962) with some supplementation (Ghanem et al. 1996). Durieu and Ochatt (2000) tested protoplast fusion and regeneration of *P. sativum*.

9.3.3.4 DNA and Seed Banks

Gene banks were established in the mid twentieth century to preserve agricultural biodiversity when landraces began to be replaced by improved varieties (Díez et al. 2018). The major objective was to conserve biodiversity for future breeding programs (Fowler and Hodgkin 2004). Gene banks are a means of long-term preservation of genetic resources by extraction and preservation of DNA from wild and cultivated plants, and even from endangered or fossilized plant specimens (Rogers and Bendich 1985). Some important DNA gene banks for plant genetic resources conservation are listed in Table 9.6.

The most common method for storing DNA is by dissolving it in a TE buffer and storing at −80 °C or in alcohol (Mandal 1995; Mandal et al. 2000). The Svalbard global seed vault project is in an area of permafrost 1300 km north of the Arctic Circle and is the world's largest secure seed storage facility. Seeds of many of the world's legume crops are kept in this and other gene banks (Foyer et al. 2016).

9.3.3.5 Cytogenetics

All taxa of *Pisum* are diploid (2x, 2n = 14) (Smýkal et al. 2012). Samatadze et al. (2008) mention that C-banding patterns of some *P. sativum* varieties showed differences in chromosome size, the appearance of satellites and polymorphisms in heterochromatin bands located near the nucleolus-organizing regions (Fig. 9.9).

Some pea chromosomes have secondary constriction (satellites), which give valuable cytogenetic markers, facilitating differentiation between various species (Navrátilová et al. 2005; Neumann et al. 2002). The standard pea karyotype consists of seven chromosomes, five acrocentric chromosomes and two (4 and 7) with satellites (Neumann et al. 2002) (Fig. 9.10).

Samatadze et al. (2018) studied the peculiarities of meiosis, the distribution of C-heterochromatin (C-HC), and the activity of nuclei-regulating regions (NORs) of chromosomes. It was noted that meiosis analysis did not reveal any significant violations in *space* plant cells and that the total amount of C-HC did not differ significantly from control, despite the multiple-scale chromosome patterns.

Table 9.6 List of important DNA Gene banks for plant genetic resources conservation

DNA bank	Website
Australian Plant DNA Bank (APDB), Centre for Plant Conservation Genetics, Southern Cross University, Lismore, NSW, Australia	http://www.dnabank.com.au
Botanic Garden and Botanic Museum (BGBM) DNA Bank, Berlin, Germany	http://www.bgbm.org/bgbm/research/dna/
DNA Bank Brazilian Flora Species, Rio de Janeiro Botanic Garden, Brazil	http://www.jbrj.gov.br/pesquisa/div_molecular/bancodna/index.htm
DNA Bank at Kirstenbosch, South African National Biodiversity Institute, Kirstenbosch, South Africa	http://www.nbi.ac.za/research/dnabank.htm
International Rice Research Institute (IRRI), DNA Bank, Philippines	http://www.irri.org/GRC/GRChome/Home.htm
Missouri Botanic Garden DNA Bank, (MBGDB) St Louis, MO, USA	http://www.mobot.org/MOBOT/research/diversity/dna_banking.htm
National Bureau of Plant Genetic Resources (NBPGR). New Delhi, India	http://www.nbpgr.ernet.in/
National Herbarium Netherlands DNA Bank (NHNDB), The Netherlands	http://www.nationalherbarioum.nl/taskforcemolecular/dna_bank.htm
National Institute of Agrobiological Science (NIAS) DNA Bank, Tsukuba, Ibaraki, Japan	http://www.dna.affre.go.jp/
Plant DNA Bank Korea (PDBK), Graduate School of Biotechnology, Korea University, Seoul, Korea	http://www.pdbk.korea.ac.kr/index.asp
Royal Botanic Garden Edinburgh DNA Bank, Edinburgh, Scotland	http://www.rbge.org.uk/rbge/web/science/research/
Royal Botanic Garden Kew DNA bank, Richmond, England	http://www.rbgkew.org.uk/data/dnaBank/
TCD DNA Bank, Department of Botany, School of Natural Sciences, Trinity College, Ireland	http://www.dnabank.bot.ted.ie
Tropical Plant DNA Bank, Fairchild Tropical Botanical Garden and Florida International University, FL, USA	http://www.ftg.org/research

9.4 Traditional Breeding

Traditional breeding of *Pisum sativum* needs to be improved because of the high cost and effort needed for its application in terms of soil preparation, use of pesticides and fertilizer, weed control and the selection of seeds that can give good quality and yield.

9.4.1 Improvement Strategies

New pea cultivars are needed to provide lodging resistance, powdery mildew resistance, and to contend with yield quality and consistency (Warkentin et al. 2015). The dissection of these into the attributes of their component characters is needed,

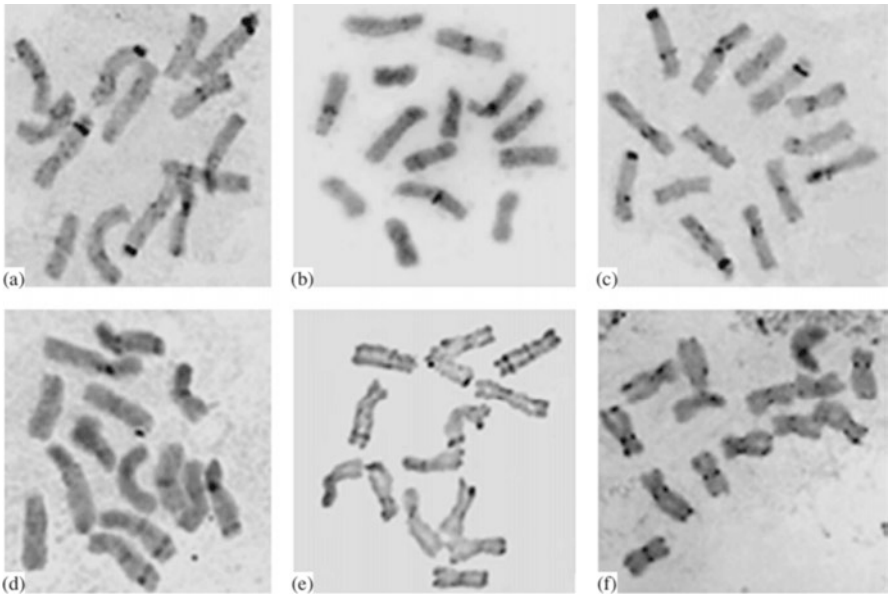

Fig. 9.9 C-metaphase banding patterns in different varieties of *Pisum sativum.* (Source: Samatadze et al. 2008)

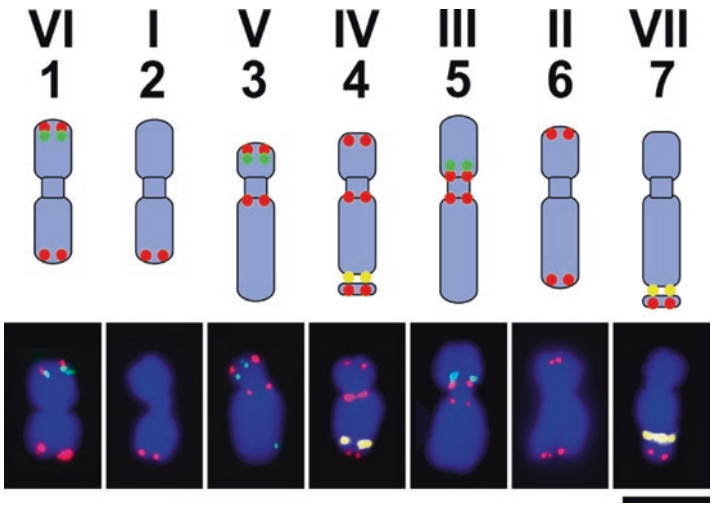

Fig. 9.10 The pea karyotype. (Source: Neumann et al. 2002)

and a continuous review of new information and resources with the point of view of their application or integration into hybridization programs. The ever-present challenges of biotic and non-biotic stresses take high precedent for action, along with changing climatic patterns in many regions of the world; together these factors increase the challenges of pea crop management (Warkentin et al. 2015).

9.4.2 Methodologies and Limitations

The most common breeding strategies used for pea improvement are selection (Mital and Verma 1991; Qasim et al. 2002; Vikas et al. 1996), pedigree, backcross (Aryamanesh et al. 2012; Clement et al. 2009) bulk selection (Kuo 1999) and single seed descent. These strategies aim to maintain desirable characters, high green pod yield, good quality attributes, sweetness, tolerance to abiotic stresses, suitability for canning and freezing and disease resistant cultivars (Gritton 1986; Kumar et al. 2015; Simakov 1989).

Genetic transformation in *Pisum* is not easy (Warkentin et al. 2015) and plant regeneration is difficult (Švábová and Griga 2008). Therefore, to discover the molecular bases underlying agriculturally evaluated characters, information is needed about the gene sequences of genomic regions which control traits of interest.

The use of next-generation sequencing technologies such as genotyping, transcriptome, and gene and genome mapping genetic resources, will contribute to pea breeding (Tayeh et al. 2015).

9.4.3 Role of Biotechnology

Biotechnology has developed rapidly in recent years and one of the benefits is the production of genetically-modified organisms (GMOs). GMO crops are tastier and healthier when grown without using pesticides or fertilizers, give high yield, have tolerance to abiotic stresses and resistance to many diseases (Kirakosyan and Kaufman 2009; Nielsen 2005) and have improved mycorrhizal and root nodule symbioses (Leppyanen et al. 2019). Metabolic engineering is another important application of biotechnology. Cells can achieve higher growth activity by a growth-based selection process (Fong et al. 2005; Jantama et al. 2008).

9.5 Molecular Breeding

9.5.1 Molecular Marker-Assisted Breeding

In recent years, pea breeding programs have begun to use PCR-based markers to reveal polymorphisms, facilitating the development of molecular maps for pea traits. Marker-assisted selection can utilize favorable gene combinations for desirable traits (Bohra et al. 2014; Collard and Mackill 2008).

Pea linkage maps containing molecular markers have been published (Dirlewanger et al. 1994; Ellis et al. 1992; Weeden et al. 1998), which help researchers characterize quantitative trait loci (QTLs) for seed weight and green seed color (Timmerman-Vaughan et al. 1997). Molecular markers have been used, including random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) (Sreedevi et al. 2009), sequence tagged sites (STS) and simple sequence repeats (SSR) (Hanci 2019).

DNA markers linked to recessive genes for resistance to *Pisum sativum* diseases have been identified like seed-borne mosaic virus (PSbMV) pathotype P-1 (Timmerman-Vaughan et al. 1993) and powdery mildew fungus (Timmerman-Vaughan et al. 1994). Current advances in molecular markers and marker-assisted selection (MAS), together with the advances in powerful new *omic* technologies, show excellent potential to develop new breeding varieties (Vignesh et al. 2011).

The rapid development of next-generation sequencing (NGS) technologies helped in the study of pea genetics. Whole-genome sequencing of *Pisum* is incomplete, but transcriptome research provides important information to create gene-based molecular markers and in building high-resolution genetic maps (Duarte et al. 2014; Gali et al. 2019; Leonforte et al. 2013; Sindhu et al. 2014; Tayeh et al. 2015).

9.5.2 Functional Genomics

Functional genomics describes the genomic and transcriptomics of an organism. It focuses on gene transcription, translation, gene expression and protein-protein interactions (Gibson and Muse 2009; Pevsner 2009). Development of NGS technologies allowed designing and mapping numerous gene-based molecular markers in pea (Aubert et al. 2006; Bordat et al. 2011) and in identifying many single nucleotide polymorphism sites (SNPs) across *Pisum* species and construction of its genetic maps (Boutet et al. 2016; Duarte et al. 2014; Kaur et al. 2012; Leonforte et al. 2013; Ma et al. 2017; Sindhu et al. 2014; Tayeh et al. 2015; Yang et al. 2015; Zhernakov et al. 2017).

ESTs (expressed sequence tags) is a valuable technique used to discover new genes and also provide a resource to develop markers in *Pisum sativum* (Davey et al. 2011; Gong et al. 2010; Zhuang et al. 2013) proving that NGS is an efficient tool to rapidly improve EST-derived SSR markers. These new EST-SSR markers will be

important tools for marker-assisted breeding, development of genetic linkage maps and the comparative mapping of *P. sativum*.

9.5.3 Bioinformatics

Gathering abundant genetics and genomics data about important crops provides a reliable source for using marker-assisted selection (MAS) and genomics-assisted breeding (GAB) for crop improvement (Bohra et al. 2014; Collard and Mackill 2008). Therefore, the convenience of genetic and genomic data is very dependent on the possibility to merge several sorts of these resource data (Appendix I-B). Despite the existence of many marker databases, they are not able of providing large quantities of data in an easy and suitable method.

The Pea Marker Database (PMD) has facilitated marker development and gene mapping by gathering data for pea gene-based markers into one database with a clear and easy-to-use interface (Kulaeva et al. 2017; Tayeh et al. 2015). It comprises two versions, (i) PMD1 contains about 2484 genetic markers, their positions in linkage groups, the sequences of corresponding pea transcripts and (ii) PMD2 an updated version including 15,944 pea markers in a similar format with numerous advanced features.

9.6 Tissue Culture

9.6.1 Micropropagation

Plant cell structure has been used in plant pathology (Braun 1974), plant morphogenesis, plant micropropagation, cytogenetics and plant breeding. Protoplast culture has been used in investigations of cell wall biosynthesis, somatic cell hybridization and genome manipulation (Power et al. 1970).

Plant biotechnology has furthered research by plant physiologists, plant breeders, botanists, agronomists, biochemists and pharmacists. The principal reasons for using biotechnology are to develop new genotypes that are resistant to biotic and abiotic stresses, have improved field-crop yield, enhanced seed germination for plant propagation and to advance the use of natural products produced by plants to satisfy human needs (Grant and Cooper 2006; Grant et al. 2003). Grant and Cooper (2006) used MS medium supplemented with 1.3 mg/L BA, 30 g/L sucrose, 8 g/L agar (Bacto), pH 5.8 and 200 mg/L timentin for pea multiplication.

9.6.2 Embryo Rescue

To create homozygous pea populations, Surma et al. (2013) examined the suitable conditions for the culture of pea embryo as the initial step to generate an in-vitro assisted single seed descent. Embryos separated from mature green seeds and cultured in vitro on modified MS media (Murashige and Skoog 1962) at 20–22 °C (day/night) to achieve shoot and root development (Fig. 9.11).

Transformation of peas was successfully achieved by Grant and Cooper (2006). An *Agrobacterium tumefaciens* strain AGL1 containing the desired construct was used. Separating immature embryos from cotyledons and cultured on B5 supplement with 1.3 mg/L 6-benzylaminopurine (BA), 30 g/L sucrose, 8 g/L agar (Bacto), pH 5.5 and 20 mg/L acetosyringone, BAP and NAA until reaching plantlet regeneration, as shown in Fig. 9.12. B5 medium supplemented with 1.3 mg/L BA, 30 g/L sucrose, 8 g/L agar (Bacto), pH 5.8, 200 mg/L timentin and 75 mg/L kanamycin sulfate were used for regeneration. B5 medium supplemented with 30 g/L sucrose, 8 g/L agar (Bacto), pH 5.8, 15 mg/L indole acetic acid and 200 mg/L timentin were used for rooting. B5 medium supplemented with 30 g/L sucrose, 8 g/L agar (Bacto), pH 5.8, 50 mg/L kanamycin sulfate, and 200 mg/L timentin were used for root selection. B5 medium supplemented with 30 g/L sucrose, 8 g/L agar (Bacto), pH 5.8, and 200 mg/L timentin were used for root elongation. However, MS medium supplemented with 1.3 mg/L BA, 30 g/L sucrose, 8 g/L agar (Bacto), pH 5.8, and

Fig. 9.11 Embryo-regenerated pea plants. (Source: Surma et al. 2013)

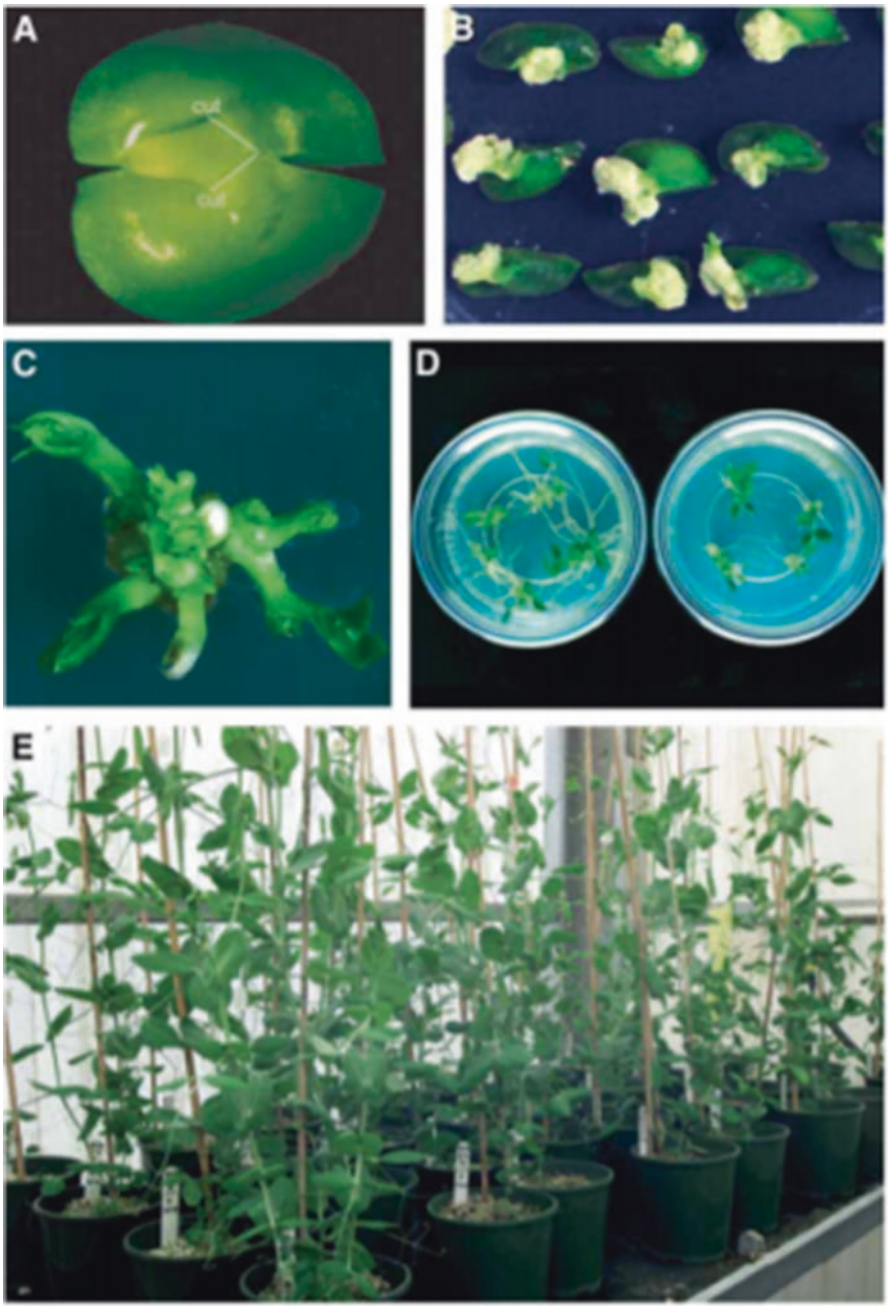

Fig. 9.12 Steps for transformation of pea. (Source: Grant and Cooper 2006)

200 mg/L timentin were used for multiplication. The frequency of regeneration depended on the genotype. Natali and Cavallini (1987a, b) demonstrated de novo origin of the shoots via organogenesis in pea, the chromosome number of regenerated plantlets showed a range of 12–16.

9.7 Genetic Engineering and Gene Editing

Genetic engineering is playing an important role in the development of plant biotechnology. Plant biotechnology aims to improve productivity, the growth of many crops and developing new crops through gene editing and metabolic engineering.

EST-derived simple sequence repeat (eSSR) markers have become an important tool for pea gene discovery and comparative mapping studies (Decarie et al. 2012). The proteome of mitochondria in mature leaves and stems of pea were analyzed by Schiltz et al. (2004).

Most genes and QTLs that account for the domestication of pea and responsible for the modifications of plant form and function have been identified (Weeden 2018). Significant contributions to the qualitative genetics of peas have been made by several scientists. Blixt (1974) has given a list of 324 qualitative genes. Vignesh et al. (2011) listed QTLs identified for different polygenic traits in pea, as shown in Table 9.7.

There is an urgent need to increase agricultural production through innovative breeding technology to increase the supply of nutritious foods worldwide. Recent advances in genome editing in CRISPR/Cas technology offer effective targeted modification in most crops (Shimatani et al. 2017), and promise to accelerate crop improvement (Chen et al. 2019). Basic editing tools that allow targeted nucleotide alternatives and describe different delivery systems, especially DNA-free methods,

Table 9.7 List of QTLs identified for different traits in *Pisum sativum*

Trait QTL	References
Green seed color	McCallum et al. (1997)
Seed weight	Timmerman-Vaughan et al. (1996)
Leaf shape	Villani and DeMason (2000)
Grain yield, seed protein and maturity	Taran et al. (2004)
Lodging resistance	Zhang et al. (2006)
Winter hardiness	Weller et al. (2012)
Frost tolerance	Dumont et al. (2009)
Ascochyta blight resistance	Hamon et al. (2011)
Salinity tolerance	Leonforte et al. (2013)
Seed mineral	Ma et al. (2017)
Agronomic traits (flowering, maturity, lodging) and seed quality traits (seed weight, grain yield)	Gali et al. (2018)

have been linked to genome editing in crop breeding (Komor et al. 2016). Genome editing applications are used to improve properties, develop gene control regulation, virus reproduction strategies and use highly productive mutant libraries (Huang et al. 2018). The future perspective is one of genome editing in plant synthetic biology and domestication, advances in delivery systems, the specificity of editing, symmetric repair and gene engines. Finally, there are challenges and opportunities for raising smaller plants and their bright future in agriculture.

9.7.1 Methodologies and Enhanced Traits

Recently, several studies have been published on pea systematics, seed quality and breeding (Arnoldi et al. 2015; Bohra et al. 2014; Dahl et al. 2012; Smýkal et al. 2015; Varshney et al. 2015). Genetics in peas develops rapidly from regular to larger methods with the help of molecular-assisted approaches to detect the molecular bases of important traits and enhance breeding. The single seed descent (SSD) technique, in combination with immature embryo culture, is applied to shorten the breeding time. Currently, SSD populations are commonly used to replace other techniques in genetic and genomic investigations (Kuchel et al. 2006; Marza et al. 2005).

9.7.2 Transgenic Cultivars

In recent years, transgenics has played an important role in improving traits that help pea plants tolerate abiotic stresses. A reproducible transgenic *Pisum sativum* plant was developed by using explants from the embryonic axis of immature seeds (Schroeder et al. 1993). Discovering genes and their functions have helped to understand the mechanisms of genes in plants under stress, which improved the productivity of pea crops under different abiotic stresses (Ali et al. 2018). Timmerman-Vaughan et al. (2001) demonstrated that transgenic pea plants can partially resist AMV (alfalfa mosaic virus). Gene editing will permit using data in gene banks more effectively and rapidly and contribute to a better explanation of their functioning (Díez et al. 2018). Transgenic *P. sativum* will need to be productive enough to justify the high costs and time involved in bringing genetically-modified peas to the market (Kahlon et al. 2018).

9.8 Mutation Breeding

Programs to increase the genetic improvement of peas using mutations were initiated in the 1940s by Gelin (1954, 1955). Methods of pea mutation are the same as those used in other annual crops (Anonymous 1980, 1982, 1983, 1984). Seeds are usually treated either with X-rays, gamma rays or chemical mutagens (Aney 2013). The highest rate of mutations was obtained using ethyl methanesulphonate (EMS), ethylene amine (EI), methyl nitroso urea (MNU), N-nitroso-N-methyl urea (NMU) and ethyl nitroso urea (ENU). Mendelian pea breeding is dedicated to inheriting seven evolutionary mutations. All the models Mendel used for crosses were available on the market; he used development anomalies to improve crops long before the concepts of genes or mutation emerged. Recently, only four of the seven Mendelian mutations have been discovered at the molecular level (Reid and Ross 2011).

9.8.1 Mutagenesis

Worldwide, there are about 3500 mutations of many morphological traits in the pea, such as stem, root, flowers and other traits, found in Sweden, Germany, Poland, Italy, India, Russia and the Netherlands (Hofer et al. 2009; Sagan et al. 1994; gan and Duc 1996; Wang et al. 2008). These mutations affect many morphological traits. Mutations have strong effects on germination, growth inhibition and infertility. Several environmental factors modulate radiosensitivity, such as seed moisture, oxygen and temperature before, during and after irradiation, as well as growth conditions, especially during the germination of treated seeds (Anonymous 1977; Badr et al. 1975; Blixt 1972; Hussein et al. 1974; Sharma and Kharkwal 1983). Seeds may be soaked in an aqueous solution for 12–16 h. Uncoated seeds are easily mutated in tissues and cells and shorten the treatment time are considered optimal. The temperature to induce mutations is 21–24 °C. The time required for radiation exposure may vary from 0.5 to 24 h, but 2 to 4 h have been shown to be enough to cause mutations. The recommended concentrations in aqueous solutions range from 0.05 to 0.3% FAO/IAEA (2018).

A clear change in the growth habit observed in mutants is age or laziness. Ageotropic or *lazy* mutations were found in peas in the late 1930s and described in the review by Howard et al. (2014).

9.8.2 *In Vitro Mutagenesis and Selection*

Sharma et al. (2009) reported that mutation induction is effective to improve the yield of pea seeds to produce the M_1 generation. A broad range of mutations in chlorophyll content and agronomic mutations were found in M_2 generations (The rate of chlorophyll mutation in generations M_2 has been increased by increased gamma radiation dose). As well as the chlorophyll mutations, the rate of xantha type (pale yellow seedlings) was greater followed by chlorina (yellowing of leaves) and albino type (exactly chlorophyll-free) mutants. In general, 0.3% EMS treatment was the most efficient in producing desired mutations at the highest rate. Over the years, desirable mutations have been separated from long and dark green pods, three or more flowers or pods on the stem, branching abundance and pod, short internal, dark green pods and male infertility of various treatments. Fatal or biotic injury was exhibited in low germination, increases with a higher dose of gamma rays and EMS. The efficiency of the mutation's effectiveness is normally increased by a rising EMS dose. Mutation breeding can play a role in improving peas to cause a positive variation of needed qualities to develop promising genotypes.

Sinjushin (2013) reported that studies on some forms in peas seem promising to reveal this unique and interesting aspect of plant development. Certainly, bridging this gap can use genomic and post genomic approaches. Targeted mutations and correct gene expression based on knowledge of the structure of some genes can serve to further improve this valuable crop culture (Sinjushin 2013).

9.8.3 *Molecular Analysis*

The development of genetically-representative collections of single or limited groups of characters is a recent activity dating back to the end of the nineteenth century. Lists were compiled of older collections of 21 pairs of pea lines cultivated for contradictory traits including plant shape, leaves, flowers and seeds that were genetically researched in a set greater than 550 genotypes (Hofer et al. 2009; Sagan and Duc 1996; Sagan et al. 1994; De Vilmorin 1911; Wang et al. 2008). Induced mutations have become widespread as a means of promoting mutation rates for developing new genetic variance for selection and the importance of using induced mutants. In legumes, development programs are still recognized (Blixt 1972; Dalmais et al. 2008; Duc and Messager 1989; Kharkwal et al. 2010; Sagan et al. 1994). The main mutant pea groups include: (i) John Innes Collection, Norwich, UK (575 accessions); (ii) IPGR group, Plovdiv, Bulgaria (122 accessions); (iii) TILLING-induced localized lesions with 4817 lines (1840 described by phenotype) and (iv) 93 symbiotic mutations for 26 genes participate in nitrogen fixation (Hofer et al. 2009; McAdam et al. 2018; Sagan and Duc 1996; Sagan et al. 1994; Wang et al. 2008).

9.8.4 Enhanced Traits and Improved Cultivars

Induced mutations were used to obtain direct mutations or in hybridization (Ahloowalia et al. 2004) to overcome yield plateaus and generate the required morphological traits. Mutation breeding programs have significantly increased plant development, leading to the release of at least 2250 genotypes of various crops. For example, in India, at least 300 genotypes have been improved in at least 55 plant species (Kharkwal et al. 2004). The effectiveness of mutagenesis used in mutagenesis programs needs to be explained. The success of the mutation breeding program depends on improved testing procedures to isolate desired mutations, which appear at very low rates, among many other mutations of small reproductive importance (Solanki and Sharma 2002). Analysis of induced variance of chlorophyll and potential morphological mutations in the M_2 generation was the most reliable tool for using valuable mutations for effective crop development (Kumar et al. 2007). The number of known and characterized mutations is strongly disproportional for different categories. For example, 66 mutations influencing the leaf development are listed in the PGene database (Zelenov et al. 2008).

Over the past 20 years, traditional breeding programs have made important contributions in developing pea varieties. A yield increase of approximately 2% was achieved annually (Warkentin et al. 2015). Lodging resistance was improved by selection for stem strength (Banniza et al. 2005). Varieties adapted to winter sowing have been developed and disseminated in Europe and the northwestern USA, providing the possibility to achieve better yields due to the length of the growing season, higher biomass production and early maturation, to avoid late-season drought and heat stress (Hanocq et al. 2009). Quantitative inheritance, transgressive segregation and heritability have been moderately high for seed color, shape and dimpling (Ubayasena et al. 2011) allowing good progress in breeding programs. For example, the seed protein concentration was maintained in pea varieties (Jha et al. 2013).

9.9 Hybridization

9.9.1 Conventional Hybridization

In general, landrace collections are protectors of genetic variability and sources of many valuable genes, especially those for adaptation (Chahal and Gosal 2002). They are used either for release after selection for high yield and wide or specific adaptation or crossed with exotic materials. In the absence of the desired variability from existing materials, hybridization is the best method to create variability (Lakić et al. 2019). In most cases, the exotic materials, with desirable characters (large seed size, white/green seed color, erect plant stature) but not adaptable, will be crossed with the local adapted materials, but lack some useful characters. Based on the

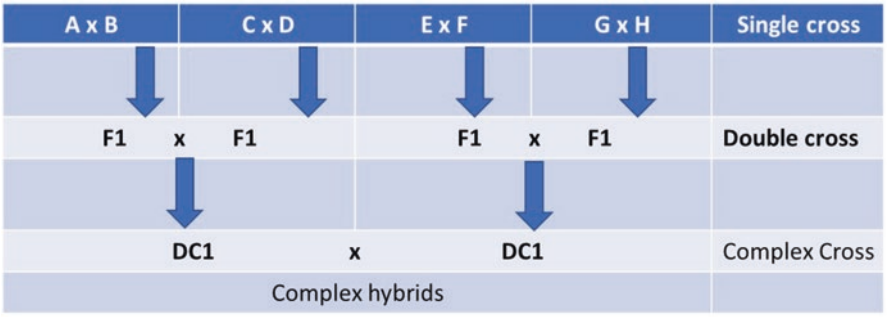

Fig. 9.13 Type of hybrids in pea breeding program for high yield and its component traits (A, B, C, D, E, F, G, H are different pure line parents). (Prepared by Khaled F.M. Salem)

taxonomic relationship between the two parents, hybridization may be intraspecific or interspecific. When the parents involved belong to the same species, it is referred to as intraspecific or intervarietal hybridization. Contrarily, when the parents involved belong to different species within a genus, it is referred to as interspecific or distant hybridization. Crosses can be single (A x B), when only two parents are involved, or multiple, when more than two parents are involved (Fig. 9.13). If three parents are involved, it is referred to as a three-way cross [(A × B) × C]. Similarly, if four parents are involved, then it is referred to as a double-cross [(A × B) × (C × D)], and so on. When the desirable characters are distributed among several parents, to bring all the desired traits into a single genotype, multiple crossing needs to be employed (Fig. 9.13). For instance, a series of crosses are required to bring the desired traits distributed among eight separate parents into a single genotype (Fig. 9.13). Trials to produce F_1 hybrids have been successful. Thus, hybridization relies on three principles: (i) pedigree method, (ii) backcross (Aryamanesh et al. 2012; Clement et al. 2009) and (iii) single-plant or mass selection (Mital and Verma 1991; Qasim et al. 2002; Vikas et al. 1996). To ensure the flowering of all plants, different parents are staggered (Acquaah 2012).

On sunny and warm days, flowers open in the morning; on cloudy and cool days it is delayed. Crossing can be carried out according to Jarso et al. (2009). F_2 seeds can be produced by the selfing of a single F_1 plant. F_2 seeds are sown and 200 seedlings are randomly selected, transplanted and genotyped. The plants are self-harvesting and the resulting F_3 seeds are harvested for each F_2 plant.

9.9.2 Somatic Cell Hybridization

Since methods have been established to incorporate protoplasts and assimilate DNA and external organelles by protoplasts, emphasis has been on physical hybridization in higher plants. Polyethylene glycol (PEG) is an effective catalyst for fusion (Constabel 1984). Protoplast treatment with PEG produces especially

heterogeneous fusion products of 5–30%. Protoplasts of various species, races, and even families were matching at fusion. A number of protoplast sets (soybeans/corn, soybeans/peas, soybeans/tobacco, carrots/barley) initiated fusion products that exhibit constant cell division and callus formation. Fusion products showed heterogeneity at first. In heterogeneous cell division, it was observed that the random distribution of the mitotic nucleus is accompanied by a multi-wall formation and is caused by cerebral callus. The nucleus of juxtaposition proposed the division of nuclear fusion and hybrid formation (Arcioni et al. 1997). The fusion of heterogeneous interphase nuclei is manifested in soybeans and peas and carrots and heteromalt bristles. Provided that parental protoplasts carry appropriate markings, fusion products can be identified.

For successful isolation and cloning of hybrid cells, integration must be completed with the selection system. Complementing two non-allelic genes that stop or inhibit growth under special cultural conditions is a principle based on the selection of somatic hybrids. Since some species are initiated to regenerate entire plants, the improvement of hybrid plants from primitive fusion products is now possible and has already been introduced into tobacco.

In the achievement of transgenic development, the pea genotype regeneration system independency and replicability are the primary prerequisites. In peas, a protocol for regeneration has been described by several researchers. Embryogenesis or organogenesis has been described for different pea explants such as immature leaflets (Fujioka et al. 2000; Mroginski and Kartha 1981; Rubluo et al. 1984), cotyledonary nodes (Grant et al. 1995; Jackson and Hobbs 1990; Rajput and Singh 2010), hypocotyl regions (Nielsen et al. 1991), embryos (Kysely et al. 1987; Natali and Cavallini 1987b; Sanchez and Mosquera 2006; Surma et al. 2013; Tetu et al. 1990), various organs of seedlings (Aslam et al. 2006; Ezhova et al. 1985; Hussey and Gunn 1984; Malmberg 1979; Pniewski and Kapusta 2005; Sharma and Kaushal 2004; Tzitzikas et al. 2004), mature seeds (Zhihui et al. 2009), cotyledons (Pniewsky et al. 2003) and protoplast cultures (Jacobsen and Kysely 1984; Lehminger-Mertens and Jacobsen 1989; Puonti-Kaerlas et al. 1990; Tapingkae et al. 2012).

9.9.3 Hybrid Cultivars

The most expensive seed genotypes in agricultural markets are often classified as F_1 hybrid seeds. In the hybridization of two pure lines with each other, the result is known as an F_1 hybrid. The following year, hybrid seeds are planted. As a result of this mutual fertilization, genetic development occurs. There are difficulties, of course, where it takes 7–8 years to develop a pure line through conventional breeding. Sometimes, a pure line consists of several previous crossings to begin creating and developing the required correct features before they are used in hybridization.

The F_1 hybrid is the result of crossing two pure lines to achieve the desired traits. Strict scientific breeding programs have helped not only to focus the outstanding qualities of native plants, but in most cases, these qualities have been improved and

new desirable traits added to hybrids. In addition to qualities such as vigor, quality, high yield and components and the standardization of hybrid plants, other characteristics such as resistance to diseases, tolerance to drought, salt and early maturing are integrated into most F_1 hybrids.

The uniformity and maturity of plants, along with the uniformity in maturity, shape or size, make hybrids in general very suitable for mechanical harvesting. Since the development of pure lines to produce F_1 hybrids requires several years, these pure lines must be maintained continuously, so that the F_1 hybrid seed can be obtained every year. Hybrid seed is expensive because of the high cost of production annually by manual methods. Sagar and Chandra (1977) reported that the appearance of heterosis in legume crops may be applied to the selection of possible hybrids in legumes for their genetic improvement. Also, Singh et al. (2017) reported that the appearance of heterosis in pea may be applied to the selection of possible hybrids for yield and its related traits. The difficulty is exacerbated because to certify that self-pollination does not occur, sometimes all hybridization of the two pure lines must be performed manually. The seeds are often collected manually to make sure that every plant is as productive as possible. Not only do growers benefit, but there are benefits for breeders as well. With regular genotypes, anybody can plant them and harvest seeds that can be replanted in the field or sold. Therefore, plant breeders who do a lot of work in creating a non-F_1 genotype can find someone else who sells seed and gets a share of the financial reward. But the seeds collected from F_1 hybrids will not produce plants like those they are collected from (yield decreases by 50% at least in F_2 generation). Only by crossing pure lines can the variety be made, and only the original breeder has the pure lines necessary.

9.10 Conclusion and Prospects

In view of the great economic importance of peas due to their uses as food, feed, seed, and industrial uses, considerable research has already been carried worldwide. Since classical breeding methods are laborious and time-consuming, the introgression of novel alleles through crossing plants from various plant genetic resources e.g. modern varieties with locally adapted varieties enhances the pea genetic diversity and pre-selection for traits of interest which is required to ensure that meaningful natural variation at the phenotype level. Although new pea biotechnology approaches that use DNA sequences and molecular methods have attracted pea breeders and geneticists, traditional pea breeding methods are still the key and initial point to develop new pea cultivars with desirable traits. Many promising varieties adapted to climate change and biotic and abiotic stress conditions have been developed. For that, breeding approaches to develop new varieties are needed in pea for high yield and resistance to biotic and abiotic stresses.

Overpopulation and associated increased food demands, along with biotic and abiotic stresses, are the most important challenges in pea breeding. Pre- and post-flowering stresses are key obstacles affecting pea growing. Global climate change

such as frost or high temperature are the major important climatic changes attracting considerable pea breeder attention worldwide. Also, diseases such as seed-borne mosaic virus (SbmV), bean yellow mosaic virus (BYMV), pea enation mosaic virus (PEMV) and pests such as pea aphids, spiny pod borer, which are present in some regions, are predicted to spread fast and affect food security in the involved countries and worldwide. Therefore, there is a need for more investment in breeding programs and training of new pea pathologists and breeders. Also, more efforts must be made to breed new varieties with wide adaptability to extend pea cultivation under abiotic stress such as drought and saline soils, to reduce the effect of global warming. Recent biotechnology tools have been applied to develop promising new pea cultivars with desirable traits. Also, the new pea genome has been sequenced and molecular methods have attracted breeders and geneticists to develop new pea cultivars with desirable agronomic traits and tolerance to biotic and abiotic stresses.

Because there are no genetically distinct pure lines in peas of the most economically-important crop traits, it is necessary to obtain pure lines through the production of haploids and doubled haploids (DH) lines which can be exploited in breeding programs. As well, DNA markers closely linked to important biotic and abiotic stresses, physiological, yield and related traits must be developed. Genes or QTLs should be identified for qualitative and quantitative attributes to improve these traits. Furthermore, germplasm and biotechnology should be improved to speed up and facilitate the improvement of promising new lines with high yield and quality. Optimization of pea interactions with microorganisms like mycorrhiza and rhizobia to give crops the ability to better tolerate stresses. Additionally, there is the need for adaptation of seed composition and plant morphology and phenology into novel breeding efforts.

Appendices

Appendix I-A: Major World Institutions Holding Pisum Germplasm

Country/ Continent	FAO Inst. code	Institute	Number of accessions
Africa	IBCR	Institute of Biodiversity Conservation, Addis Ababa, Ethiopia	1600
Australia	AFTC	Australian Temperate Field Crop Collection, Horsham, England	6567
Bulgaria	SAD	Institute of Plant Introduction and Genetic Resources, Sadovo, Bulgaria	2787
China	ICAR-CAAS	Institute of Crop Sciences, CAAS, China	3837

(continued)

Country/ Continent	FAO Inst. code	Institute	Number of accessions
Czech Republic	CZE	AGRITEC, Research, Breeding and Services Ltd., Sumperk, Czech Republic	1284
France	INRA	INRA CRG Légumineuse à grosses graines, Dijon, France	1891
Germany	GAT	Leibniz Institute of Plant Genetics and Crop Plant Research, Gaterleben, Germany	5336
Hungary	HUN	Institute for Agrobotany, Tapioszel, Hungary	1188
Italy	BAR	Istituto del Germoplasma, Bari, CNR – Istituto di GeneticaVegetale, Italy	4297
Netherlands	CGN	Centre for Genetic Resources, Wageningen, Netherlands	1008
Poland	WTD	Plant Breeding and Acclimatization Institute Blonie, Radzikow, Poland	2899
Russia	VIR	N.I. Vavilov Research Institute of Plant Industry, St. Petersburg, Russia	6790
Sweden	NGB	Nordic Gene Bank, Nordic Genetic Resource Centre, Alnarp, Sweden	2724
Syria	ICARDA	International Center for Agricultural Research in the Dry Areas, Aleppo, Syria	6105
Ukraine	UKR	Yurjev Institute of Plant Breeding, Kharkov, Ukraine	1671
United Kingdom	JIC	John Innes Centre, Norwich, UK	3557
United States	USDA; NYSAES	Plant Germplasm Introduction and Testing Research Station, Pullman; NY State Agricultural Experiment Station, USA	5400; 2500

Source: Smýkal et al. (2012)

Appendix I-B: List of Web Databases Providing Links to Pea Related Information

Database	Website
Bioinformatics gateway towards integrative legume biology	http://www.legoo.org/
Cool Season Food Legume Genome Database	http://www.gabcsfl.org/
INRA Dijon Legume genetic and genomic resources	http://www.thelegumeportal.net
INRA Legume Base	http://195.220.91.17/legumbase/index.php?mode=0&id=
International Legume Database & Information Service (ILDIS)	http://www.ildis.org/
Know Pulse	http://knowpulse2.usask.ca

(continued)

Database	Website
Legume Information System (LIS)	http://www.comparative-legumes.org/
Legume IP	http://plantgrn.noble.org/LegumeIP
Legume phylo-informatics database	http://www.public.asu.edu/~mfwojci/legumephylo_dBase.html
Legume proteomes	http://iant.toulouse.inra.fr/plants/legumes/cgi/legumes.cgi
Medicago truncatula HapMap Project	http://www.medicagohapmap.org/cgi-bin/gbrowse/mthapmap/
Phytozome -Soybean Gbrowser	http://www.phytozome.net/cgi-bin/gbrowse/soybean/
UTILLdb: URGV TILLING pea database	http://urgv.evry.inra.fr/UTILLdb

Source: Smýkal et al. (2012)

Appendix II-A: List of Recommended Varieties of Peas in India

State	Recommended varieties
Bihar	DDR-23 (Pusa Prabhat), V L Matar -42
Chhattisgarth	Shubhra (IM-9101), Vikas (IPFD-99-13), Paras
Gujarat	JP-885, IPFD 10–12, Indra, Prakash
Haryana	Uttra (HFP-8909), DDR-27 (Pusa panna), Hariyal (HFP-9907 B), HFP-9426, Alankar, Jayanti (HFP-8712), Aman(IPF5-19)
Jharkhand	PL Matar-42, V L Matar -42
Madya Pradesh	Prakash (IPFD 1–10), Vikas (IPFD-99-13)
Maharashtra	JP-885, Ambika, Indra (KPMR-400), Adarsh (IPF 99-25), IPFD 10–12
Punjab	Jay (KPMR-522), Pant pea-42, KFP-103 (Shikha), Uttra (HFP8909), Aman (IPF5-19)
Rajasthan	DMR-7 (Alankar), Pant Pea-42
Uttar Pradesh	Swati (KFPD-24), Malviya Matar-15 (HUDP-15), Vikas, Sapna (KPMR-1441), IPF 4-9
Uttarakhand	Pant Pea-14, Pant Pea-25, V L Matar -47

Source: Seednet GOI, Min of Agri & FW & ICAR-IIPR, Kanpur, Dhall (2017)

Appendix II-B: World List of Recommended Varieties of Pisum sativum in some Producing Countries

Country	Recommended varieties
Czech Republic	Adept, Alan, Baryton, Bohatyr, CanisCarrera, Catania, Garde, Gotik, Grana, Hardy, Harnas, Herold, Jackpot, Janus, Kamelot, Komet, Lantra, Madonna, Menhir, Merkur, Olivin, Pegas, Power, Primus, Profi, Romeo, Sonet, Sponzor, Tempra, Terno, Tyrkys, Zekon
Egypt	Master B, Little Marvel, Lincoln, Luxer, Sugary, Sohag 1, Sohag 2, Ambassador, Hurst Greenshaft, Senator, Sugar Snap, Delikett, Victory Freezer
Ethiopia	Burkitu, Adet-1, Sefinesh, Gume, Tegegnech, Wolmera, Hassabe
Pakistan	Climax, Matar, Meteor, Climax, Greenfeast and Rondo
UK	Manager, Cascade, Capulet, Deity, Croft, Pastoral Swift, Venture, Madras, Salamanca

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