

# Chapter 10

## Genetic Advancement in Dry Pea (*Pisum sativum* L.): Retrospect and Prospect



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### 10.1 Introduction

Field pea or dry pea (*Pisum sativum* L.) is one of the important, highly productive cool season food legume crops grown around the world to consume as food, feed and fodder (Dahl et al. 2012; Warkentin et al. 2015; Holdsworth et al. 2017; Rubiales et al. 2019). It has yellow, green and orange cotyledon varieties which are consumed by human being in various forms such as *soup*, *chat*, *chhola*, *dal*, *stew*, *snacks*, vegetables and flour, whereas the whole seed is used as animal feed (Dahl et al. 2012; Parihar et al. 2016; Singh et al. 2018). Since it is an excellent source of protein, starch and fibre, therefore, it is being widely used as an ingredient in many food industries around the world (Dixit et al. 2014; Gupta and Parihar 2015; Parihar et al. 2016). It is a good source of proteins (21.2–32.9%) and carbohydrates (56–74%) along with vitamins, essential amino acids and micronutrients. It is considered as one of the cheapest sources of easily digestible protein for human and livestock consumption owing to the absence of major anti-nutritional factors. The seed coat and cotyledon are the dietary fibre-rich part of seed, i.e. water-insoluble and water-soluble fibre, respectively (Reichert and MacKenzie 1982; Guillon and Champ 2002; Tosh and Yada 2010; Parihar et al. 2016). In case of micronutrients, the potassium is the most prevailing element followed by phosphorus, magnesium and calcium. The dry pea is also a good source of other micronutrients such as Fe (97 ppm), Se (42 ppm), Zn (41 ppm) and Mo (12 ppm) (Reichert and MacKenzie 1982). In addition, dry pea also has sizeable amount of folate (101 µg per 100 g) with many vitamins (Dang et al. 2000; Hedges and Lister 2006). It has many health benefits

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such as helps in prevention and management of type 2 diabetes (Marinangeli et al. 2009; Marinangeli and Jones 2011), reduces and stabilizes bold cholesterol (Daveby et al. 1998; Ekvall et al. 2006), improves cardiovascular health (Slavin 2008; Singh et al. 2013) and also has cancer combating and antioxidant properties (Kalt 2001; Kleijn et al. 2001; Boker et al. 2002; Steer 2006). Besides, it helps in weight management and betterment of gastrointestinal function (Fernando et al. 2010; Tosh and Yada 2010; Lunde et al. 2011). Given nutritional quality makes dry peas as important international food commodities, which cater the dietary requirement of resource poor undernourished individuals of developing countries (FAOSTAT 2011). The production of dry pea has been unstable during recent past decades due to many prevalent biotic and abiotic stresses. Of them, biotic stresses are powdery mildew, rust, ascochyta blight, *Fusarium* root rot, common root rot and *Fusarium* wilt, while abiotic stresses are high temperature, drought and cold. Since majority of the pulses including dry pea are cultivated under low-input agriculture around the world. Dry peas produced under these conditions by resource-poor farmers are more vulnerable to attack by biotic and abiotic stresses. The high-input farmers have more resources to stride against these stresses through the use of recently developed technologies (fertilizer, irrigation, pesticides and management strategies). However the application of such inputs and management can increase cost of cultivation which ultimately reduces profit of farmers and also has negative impact on environment, and even many pests are not effectively controlled with chemical treatment. Hence, incorporating host plant resistance mechanism in the crop through conventional, molecular and genomic-assisted breeding strategies is the most economically efficient way of tackling these stresses. Therefore, in this chapter, we have covered the present scenario of dry pea cultivation, present status of trait-specific genetic improvement happened in dry pea over the years and their future perspectives towards sustainable dry pea production for nutritional security of resource poor farmers.

## 10.2 Dry Pea Area, Production, and Productivity Scenario at Worldwide

Dry pea is being cultivated around the world about in 94 countries (Smýkal et al. 2012), and the total production and area of dry peas at worldwide is at present approximated to be 16.20 mt and 8.14 mha, respectively, increasing trend observed during 2007–2017 (Fig. 10.1). In addition, the production and area of green peas is 20.69 mt and 2.66 mha, respectively. The top 10 major share-holding countries in production are Canada (21.85%), Russian Federation (10.31%), China (7.53%), India (4.32%), the USA (4.26%), France (3.77%), Ukraine (2.90%), Australia (1.91%), Ethiopia (1.85%) and Germany (1.17%) (Fig. 10.2). Region-wise production situation at global level witnessed that the Americas (38.6%) are accountable for highest share in total production followed by Europe (34.4%) and Asia (19.0%)

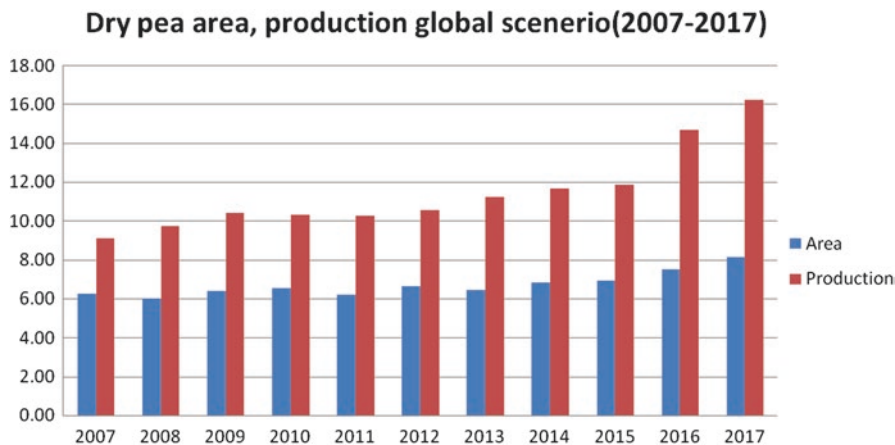


Fig. 10.1 Dry pea area and production trend during 2007–2017 at worldwide

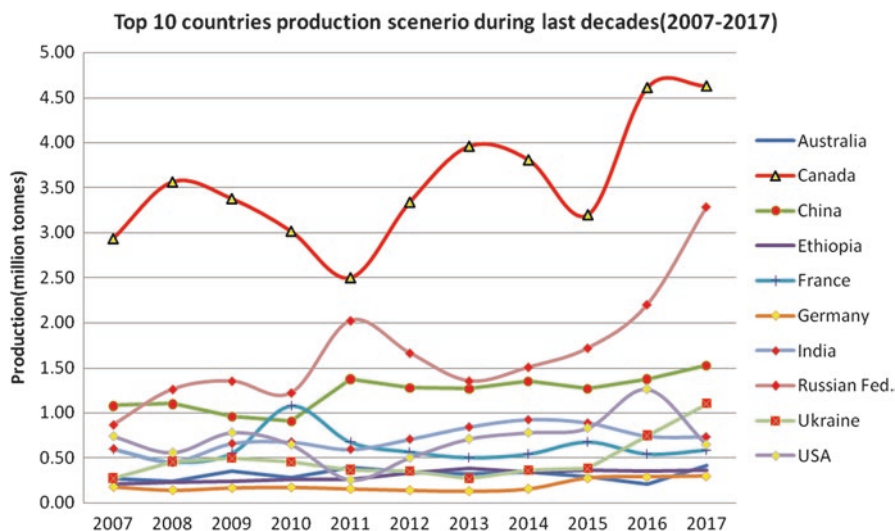


Fig. 10.2 Top 10 countries dry pea production trend during 2007–2017

(FAOSTAT 2019). During the past five decades, the yield gain is just 15.3 kg/ha/year in dry pea at global level, much lower than other crops, which demonstrating that least concentration is invested on dry pea improvement programme. In addition, the yield gain in Canada is 2.0% which is greater than the yield gain in most of the crops at global level witnessed large investment has been made in pea research programme over the years (Rubiales et al. 2019). However, the global dry pea productivity has been increased more than 36% during 2007–2017 and currently in tune of 1.9 tonnes/ha. The countries having highest productivity are the Netherlands (4877 kg/ha), Denmark (4463 kg/ha), Belgium (3824 kg/ha), Ireland (3571 kg/ha),

Germany (3487 kg/ha) and France (3222 kg/ha). On the contrary, in other dry pea-growing countries like India, China, Australia and Myanmar, productivity is low as compared to above-mentioned countries varied between 1000 and 2000 kg/ha. Some of the countries like the USA, Finland, Brazil, Ireland, Belgium, Pakistan and the Netherlands portrayed negative tendency in production during 2007–2017, while the opposite trend has been recorded for the Russian Federation, Ukraine, Germany, Canada, Denmark, India, Australia, China and Myanmar where production showed increase. The highest increase in production and productivity has been recorded in Russian Federation, Ukraine, Germany, Australia, China, Ethiopia, Canada, Belgium and Denmark, while the decrease recorded in Finland, the USA, Italy, Ireland and Pakistan. Interestingly, the Netherlands is the only country where production decreased in spite of substantial increase in productivity.

### 10.3 Systematic, Origin, and Domestication

The pea is a self-pollinated diploid ( $2n = 14, x = 7$ ) annual crop and its a member of third largest flowering plant family Leguminosae, largest subfamily Papilionoideae and the tribe Fabeae (Doyle et al. 1997; Lavin et al. 2005; Lewis et al. 2005). The tribe Fabeae comprised of five genera such as *Lathyrus*, *Lens*, *Vicia*, *Pisum* and *Vaviloviaformosa* (Smýkal et al. 2011; Mikič et al. 2013; Rubiales et al. 2019). The genus *Pisum* L. mainly have three species such as cultivated pea (*P. sativum* subsp. *sativum*) with its five subspecies (*elatius*, *sativum*, *humile*, *arvense* and *hortense*), Ethiopian pea (*P. abyssinicum*) and *P. fulvum* (Maxted and Ambrose 2001; Warkentin et al. 2015; Trněný et al. 2018). These species are cross-compatible and produce hybrids; however, the fertility level may be subsidized owing to karyological and nuclear – cytoplasmic incompatibility (Ben-Ze'ev and Zohary 1973; Bogdanova et al. 2015). In addition, based on crossing ability, the genus *Pisum sativum* contains the following subspecies which are considered as varieties, namely, *P. sativum* L. var. *hortense* (garden pea), *Pisum sativum* L. var. *arvense* (field pea), *Pisum sativum* L. var. *macrocarpum* (whole pod edible pea) and *Pisum sativum* L. var. *syriacum* (wild form) (Nasiri et al. 2009; Mohan et al. 2013).

The Near East and Mediterranean region is considered as the primary centre of origin/diversity for pea where two wild species, i.e. *P. fulvum* and *P. sativum* subsp. *elatius* are cultivated today also. The distribution of *P. fulvum* is restricted to the Middle East (Ladizinsky and Abbo 2015), while wild pea (*P. sativum* subsp. *elatius*) is noticed all over the Mediterranean basin and the maximum diversity available in the Near East, which is accounted as the centre of pea diversity (Smýkal et al. 2017). The secondary centres of diversity are the upland Asiatic region of the Hindu Kush with the long-vined Afghan types, and the upland regions of Ethiopia and Yemen, with *P. abyssinicum* (Rubiales et al. 2019). Further, the cultivation of pea expands from the Fertile Crescent to today's Russia, North and West Europe, Greece and Rome. Simultaneously, pea cultivation has extended eastward to Persia, India and China (Makasheva 1979; Chimwamurombe and Khulbe 2011). Most recently,

*P. humile* has been incorporated as extra taxa and at present exist only in secondary habitats (Abbo et al. 2013).

The archaeological facts witnessed that the pea is the world oldest grain legume and domesticated about 10,000 years ago in the Near East and Central Asia (Baldev 1988; Zohary and Hopf 2000). During early civilization in the Middle East and Mediterranean, it was consumed with cereals as important dietary components (Abbo et al. 2010; Rubiales et al. 2019). In Europe, it has been grown since the Stone and Bronze Ages and in India from 200 BC (De Candolle 2007). Over the years due to domestication, several changes happened in plant type such as from indeterminate, tall, slender, bushy or climbing types with small and coloured seeds to short, determinate mechanical harvested crop with large seeds without tannins (Smýkal et al. 2018). Cultivated pea is described by characters resulted from domestication, like non-dehiscing pods and lack of seed dormancy (Abbo et al. 2013; Smýkal et al. 2014; Trněný et al. 2018). Similarly, based on uses, peas have been classified in many groups such as the mature round seed with yellow, green, red cotyledon varieties typically used in the dehulled/split form in foods which is known as field pea or dry pea. The large seeds, blocky shape, green and yellow cotyledons are different from wrinkled type called as marrowfat field pea used for snacks and mushy pea. The mottled seed coat (maple) and high biomass (forage) types are consumed as feed and fodder for birds and animals (Warkentin et al. 2015; Rubiales et al. 2019).

## 10.4 Available Genetic Resources at Global Level

Genetic resources and their judicious utilization is the quintessential step towards development of high-yielding varieties with targeted traits. In pea approximately 98,000 pea accessions comprising commercial varieties, breeding lines, landraces, mutant stock and wild species are existing in different gene banks at global level, of them 59,000 are unique (Smýkal et al. 2013; Warkentin et al. 2015; Rubiales et al. 2019) The five largest active *Pisum* germplasm-holding institutions include National Institute for Agricultural Research (INRA) of France held at Dijon (8839 accessions); Australian Temperate Field Crop Collection, Horsham, having 7432 accessions; N.I. Vavilov Research Institute of Plant Industry (VIR), St. Petersburg, Russia, holds 6790 accessions; the US Department of Agriculture (USDA) (6827 accessions); and International Center for Agricultural Research in the Dry Areas (ICARDA) holds 6105 accessions (Table 10.1). There are other national collection centres of pea germplasm in different countries' national gene banks such as in Germany (5343 accessions), Italy (4558 accessions), China (3837 accessions), India (3609 accessions), the UK (3567 accessions), Poland (2896 accessions), Sweden (2849 accessions) and Bulgaria (2100 accessions). Furthermore, the national gene banks maintain more than 1000 germplasm accessions of *Pisum* available in at least nine other countries also. Among all the countries, Australia has the least duplicative and most diverse ex situ collection so far for *Pisum*. The busiest

**Table 10.1** List of major dry pea germplasm collections (>3000 accessions) institutions at global level

S.N.	Name of institutions/ organization	Number of accessions	Share (%) of total accessions		
			Commercial varieties	Wild species	Others (breeding lines, landraces and mutant stock)
1	N.I. Vavilov Research Institute of Plant Industry, St. Petersburg, Russia	6790	98.0	–	2.0
2	INRA CRG Légumineuse à grosses graines, Dijon, France	8839	14.9	0.7	62.1
3	Australian Temperate Field Crop Collection, Horsham, Australia	7432	15.7	2.8	81.0
4	Plant Germplasm Introduction and Testing Research Station, Pullman, USA	6827	22.0	1.2	59.1
5	International Center for Agricultural Research in the Dry Areas, Aleppo, Syria	6105	19.4	3.7	74.4
6	Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany	5343	56.3	0.9	35.6
7	Istituto del Germoplasma, Bari, Italy	4558	–	–	100.0
8	Institute of Crop Sciences, CAAS, China	3837	13.9	–	86.3
9	ICAR-National Bureau of Plant Genetic Resources, New Delhi, India	3609	5.8	–	61.8
10	John Innes Centre, Norwich, UK	3567	30.0	10.3	34.9

Source: Warkentin et al. (2015)

websites for supplying germplasm are the JI Centre (JIC; <http://www.jic.ac.uk/germplasm/>) and the USDA (<http://www.ars-grin.gov/npgs/>). Both the portals have the highest proceeds of international requisition of readily available *Pisum* accessions. In addition, there are other exciting national collections of pea germplasm, for example, in Israel the gene bank having a collection of wild relative's *P. fulvum* and *P. sativum* subsp. *elatius* var. *pumilio* collected in the Middle East. The land races are contributed highest in total germplasm available at international level. Interestingly, the tiny share (about 2%) of conserved germplasm accessions represents wild pea (Smýkal et al. 2013; Warkentin et al. 2015). Of them, 706 accessions belongs to *P. fulvum*, 624 to *P. s.* subsp. *elatius*, 1562 to *P. s.* subsp. *sativum* (syn. *P. humile/syriacum*) and 540 to *P. abyssinicum* (Smýkal et al. 2013). Wild *Pisum*

species and subspecies are reservoir of many useful traits, for instance, pea seed weevil resistance (Clement et al. 2002; Byrne et al. 2008; Clement et al. 2009), rust (Barilli et al. 2010), powdery mildew resistance (Fondevilla et al. 2007b) and many other yield components (Mikič et al. 2013). The commercially least favoured germ-plasm such as pigmented flower and pigmented seed coat have been confirmed as an outstanding sources of *Aphanomyces* root rot resistance (Hamon et al. 2011) and *Fusarium* root rots (Weeden and Porter 2007; Grunwald et al. 2003). There are several international collection databases, which having important information of pea, such as European Cooperative Programme for Plant Genetic Resources (ECPGR), Germplasm Resources Information Network (GRIN), System-wide Information Network for Genetic Resources (SINGER) and GRIN-Global. Most recently, numerous databases, namely, Cool Season Food Legume Database (<https://coolseasonfoodlegume.org>; Washington State University) and KnowPulse (<https://knowpulse.usask.ca>; University of Saskatchewan), have been developed to store and share information related to phenotypic and genotypic data sets. To speed up germ-plasm evaluation and their judicious utilization, eight core collections have been made in Australia, China, the Czech Republic, France, Poland, Spain, the UK and the USA (Warkentin et al. 2015; Rubiales et al. 2019).

## 10.5 Genetic Improvement of Important Agronomic Traits (Retrospect)

Genetic improvement in grain yield with stability is a major objective of plant breeders across the crops. Grain yield is an intricate attribute influenced by many traits directly or indirectly. In dry pea breeding program, the improvement in overall productivity has been mainly approached through breeding for tailoring plant type (especially lodging resistance and plant height), resistances to key biotic (powdery mildew, rust, ascochyta blight, etc.) and abiotic (heat, drought and cold) stresses.

### 10.5.1 Breeding for Lodging Resistance

Earlier plant type in pea was used to be tall type with bulky vegetative growth. Over the years dramatic development has been embraced by researcher in pea plant type by reducing plant height from 1–2 m to 0.3–0.6 m. In spite of considerable dwarfing of pea plant, the lodging earlier remains major problems due to high biomass (Davies 1977a, b; Donald and Hamblin 1983; Amelin et al. 1991). Therefore, the alternative strategy to get lodging resistance is the development of ‘semi-leafless’ pea cultivars (Fig. 10.3) using ‘afla’ leaf type, which proved superior to ‘leafless’ in photosynthetic capacity; equivalent to that of the wild type is considered possibly the best achievement made in pea breeding (Duparque 1996). The lodging changes



**Fig. 10.3** Semi-leafless tall and dwarf dry pea varieties. (a) Variety, Aman. (b) Variety, IPFD 12-2

the canopy microclimate congenial for fungal disease development, condenses photosynthetic ability of the plants, declines harvest efficiency and amplifies harvest cost; consequently, it is considered as a serious constraint towards field pea production (Heath and Hebblethwaite 1985; Warkentin et al. 2001a; Xue and Warkentin 2001; Taran et al. 2003; Zhang et al. 2006). Given situations can cause up to 74% grain yield loss in some dry pea cultivars and also affect quality of seed (Armstrong et al. 1999; Warkentin et al. 2001b; Amelin and Parakhin 2003; Hashemi et al. 2003; Singh and Srivastava 2018). The semi-leafless plant type significantly increased lodging resistance or standing ability of pea cultivars which reduced grain yield losses and canopy disease severity (Wang et al. 2002; Banniza et al. 2005; Singh and Srivastava 2018). Thus, the semi-leafless type is preferred by most pea producers and has become the dominant leaf type in commercial cultivars. Such cultivars also increased the interest of farmer towards cultivating pea as a quality food and feed at worldwide. Most cultivars released during the recent decades have the semi-leafless leaf type (Mikić et al. 2006, 2011). Complete to partial shift has been made in many countries from 'leafy' cultivars to 'semi-leafless' cultivars. The first commercial deployment of the semi-leafless (*afila*) trait was done during the 1970s in Europe with the development of Solara cultivar. During recent period, 'semi-leafless' pea cultivars accounted 95%, 80% and 30% of the total dry pea production in Canada, European Union and Russia, respectively. It has to be noticed that *afila* improve the lodging resistance, but increased stem strength is also a very important trait (Banniza et al. 2005; Tayeh et al. 2015). In addition, it is also suitable for cultivation under diverse climatic conditions, particularly low and high temperature (McPhee and Muehlbauer 2007; MCPhee et al. 2007; Mikić et al. 2011). Such cultivars contributed significantly in substantial increment of the total pea cultivation area in many countries, i.e. Canada, India, Australia and China (Mikić et al. 2007; Warkentin et al. 2015). A number of varieties were released with semi-leafless trait which helps



in increased production potential of dry pea in India (Dixit and Parihar 2014; Dixit et al. 2014; Gupta and Parihar 2015; Parihar and Dixit 2017; Parihar et al. 2019).

### 10.5.2 *Breeding for Dwarf Type*

Wild pea and most of the older cultivated varieties have tall plant type, which had high biomass and severe lodging problems leading to disease severity (Donald and Hamblin 1983). One developmental mutant (*le-1*) shortened internode length by reducing 3 $\beta$ -hydroxylation of GA<sub>20</sub> to GA<sub>1</sub> (Ingram et al. 1984; Ross et al. 1989; Martin et al. 1997). The most of modern varieties have shortened internodes or dwarf plant type due to the incorporation of dwarf gene (*le-1*). A similar phenomenon has been exploited during *Green Revolution* in wheat and rice, which is associated with gibberellin (GA) pathway (Martin et al. 1997). Vasileva et al. (1980) reported that dwarf cultivars have greater lodging resistance than tall cultivars since they have short internode length. The dwarfing Mendel's *le-1* mutation, affecting gibberellin biosynthesis, seems to be the only dwarf gene/allele that has been used by pea breeders; another allele, *le-3*, is described as less severe than *le-1* (Ross and Reid 1991). Its effect on yield and lodging resistance is also assessed since *le-1* adds a slightly depressing effect on yield (Burstin et al. 2007) while having a highly beneficial effect on lodging. The dwarfing gene has been successfully incorporated in pea breeding especially in India which enhanced productivity through improved response to fertilizers, irrigation and dense plant population. The first dwarf and semi-leafless variety HFP 4 (Aparna) has been developed in 1988 from the cross of T 163 with an exotic line EC 109196. Later, HFP 4 in combination with EC 109185 and Flavanda led to the development of dwarf variety, HFP 8909 and Swati, respectively (Dixit and Gautam 2015). It also resulted in the development of dwarf leaflet less variety KPMR 144-1 (Sapna) from hybridization with Rachna. At the end of the twentieth century, a dwarf and landmark variety of dry pea HUDP-15 developed which is the product of the cross (PG 3 X S 143) X FC 1 and has resistance against powdery mildew and good tolerance to rust and ruled the seed chain for long time span (Dixit et al. 2014). Sincere efforts have been made, and a number of high-yielding dwarf type varieties, viz. IPFD 99-13, IPFD 1-10, IPFD 10-12, IPFD 12-2, IPFD 11-5 and IPFD 6-3, have been developed (Anonymous 2019).

### 10.5.3 *Breeding for Biotic Stresses*

The productivity of dry pea is limited by large number of biotic stresses. These included fungal, viral, bacterial pathogen causing diseases and various insect-pests and nematodes. Of them, fungal diseases with more than 28 fungi species are the most common and devastating (Reiling 1984). Some of these are powdery mildew, rust, root rots, wilt, stem/pod rot, ascochyta blight, etc. (Bohra et al. 2014). These

diseases occur in almost all pea-growing regions of the world and can cause significant crop losses when conditions are favourable for their development. Keeping this in view, the progress made in breeding for diseases resistance in field pea has been presented in this section of chapter.

### 10.5.3.1 Powdery Mildew

Powdery mildew is a serious constraint to dry pea production in pea-growing areas worldwide and largely incited by *Erysiphe pisi* (Gritton and Ebert 1975; Smith et al. 1996; Kraft and Pflieger 2001; Sun et al. 2016, 2019). Earlier, only *Erysiphe pisi* was the only known causal agent of dry pea powdery mildew, but during recent past two other fungi such as *Erysiphe baeumleri* and *Erysiphe trifolii* have also been designated as casual organism for powdery mildew disease with similar symptoms on pea plant (Ondřej et al. 2005; Attanayake et al. 2010; Fondevilla and Rubiales 2012; Sun et al. 2019). This is an airborne disease and turns into more serious threat in temperate and tropical climatic conditions with warm dry days and cool nights (Smith et al. 1996; Davidson et al. 2004; Fondevilla and Rubiales 2012; et al. 2016). It causes 25–80% losses in total grain yield and also reduces total biomass, number of pods per plant, number of seeds per pod, plant height, number of nodes and seed quality under congenial conditions for disease expansion (Munjal et al. 1963; Singh et al. 1978; Warkentin et al. 1996; Katoch et al. 2010; Fondevilla and Rubiales 2012; Ghafoor and McPhee 2012). As symptoms this disease basically developed a white powdery coating on surface of leaves, stems and pods (Fig. 10.4) (Singh et al. 1978; Bilgrami and Dube 1982; Agrios 1988; Kazmi et al. 2002). The delayed planting and late-maturing varieties are more vulnerable to powdery mildew (Gritton and Ebert 1975; Tariq et al. 1983; Davidson et al. 2004; Fondevilla and Rubiales 2012).

Owing to their economic importance, a large number of methods to control powdery mildew have been proposed, including cultural practices, the use of resistant



**Fig. 10.4** Powdery mildew infected plants of dry pea

varieties and fungicide application. However, the control efficacy of chemical and agronomic practices is restricted by many factors. Therefore, use of resistant varieties has become the first choice due its efficiency, low cost, eco-friendly and qualitative resistance nature (Fondevilla and Rubiales 2012; Ghafoor and McPhee 2012). First time powdery mildew resistance was recognized by Harland (1948) in the pea landrace Huancabamba which genetically controlled by a single recessive gene. Since then, screening and genetic analysis of resistance to pea powdery mildew have been performed almost for more than 60 years (Fondevilla and Rubiales 2012; Sun et al. 2016). Many resistant pea accessions have been identified and characterized their gene(s) for resistance to *E. pisi*. Different levels of resistance to *E. pisi* have been reported, but only three genes for resistance have been reported so far, of them two recessive, namely, *er1* and *er2*, and one dominant *Er3* (Heringa et al. 1969; Fondevilla et al. 2007c; Parihar et al. 2013). Among them *er1* gene exists in maximum resistant pea accessions, while *er2* gene is harboured only in few resistant accessions (Tiwari et al. 1997). The *Er3* is a recently identified dominant gene from a wild relative of pea (*P. fulvum*) that has recently been successfully introduced into cultivated pea (*P. sativum*) (Fondevilla et al. 2007a, b, c; Fondevilla and Rubiales 2012). Most pea breeding programmes depend on *er1*, and it is based on pre-penetration resistance (Fondevilla et al. 2006). Both monogenic and digenic recessive models for powdery mildew resistance have been reported by many researcher (Harland 1948; Heringa et al. 1969; Saxena et al. 1975; Kumar and Singh 1981; Liu et al. 2003; Sharma 2003). Several researchers reported linkage between the *er1* locus and various morphological and molecular markers and used them to place the *er1* gene on pea chromosome VI (Sarala 1993; Dirlewanger et al. 1994; Timmerman-Vaughan et al. 1994). Similarly, different types of marker, i.e. RAPD, SCAR and SSR, linked with powdery mildew resistance gene *er1* have been reported as given in Table 10.2 (Tiwari et al. 1998; Rakshit et al. 2001; Janila and Sharma 2004; Ek et al. 2005; Pereira and Leitão 2010; Tonguc and Weeden 2010; Nisar and Ghafoor 2011). The recessive *er1* locus due to loss-of-functional alleles of plant-specific *MLO* (*Mildew Resistance Locus O*) governed powdery mildew resistance in pea (Humphry et al. 2011; Pavan et al. 2013).

Most recently, a new allele of *er1* which is named as *er1-6* has been reported by using cDNA sequence of *PsMLO1* gene. Subsequently, the resistance allele *er1-6* in landrace G0001778 has been confirmed by resistance inheritance analysis using mapping populations derived from G0001778 × Bawan 6. Finally, a SSR marker specific to *er1-6* has been developed which could be used in pea breeding for marker-assisted selection (Sun et al. 2016). Similarly, Sun et al. (2019) reported two novel *er1* alleles, *er1-8* and *er1-9*, in the germplasm accessions G0004839 and G0004400, respectively. These alleles were identified using inheritance analysis and genetic mapping with F2- and F2:3-derived populations, respectively. In addition, codominant functional markers specific to *er1-8* and *er1-9* have been developed and validated in populations and pea germplasms. These results would improve our understanding of *E. pisi* resistance in pea germplasms worldwide and provide powerful tools for marker-assisted selection in pea breeding.

**Table 10.2** Details of markers/QTLs linked with different important traits in pea

Trait	Marker name/marker type	Gene/QTLs	References
Fusarium root rot ( <i>Fusarium solani</i> f.sp. <i>pisii</i> )	AA416/SSR, AB60/SSR	<i>Fsp-Ps 2.1</i> ; <i>Fsp-Ps3.2</i> ; <i>Fsp-Ps3.1</i> , <i>Fsp-4.1</i> <i>Fsp-Ps3.3</i> ; <i>Fsp-Ps7.1</i>	Coyne et al. (2015, 2019), Feng et al. (2011)
Rust ( <i>Uromyces fabae</i> )	AA446/SSR, AA505/SSR, AD146/SSR, AA416/SSR	<i>Qruf</i> , <i>Qruf1</i> , <i>Qruf2</i>	Singh et al. (2015), Rai et al. (2016)
	SC10–82360/RAPD, SCRI-711000/RAPD	<i>Ruf</i>	Vijayalakshmi et al. (2005), Rubiales et al. (2011)
	F7XEM4a/SRAP		Saha et al. (2010), Rubiales et al. (2011)
Rust ( <i>U. pisi</i> )	OPY111316/RAPD, OPV171078/RAPD	<i>Up1</i>	Barilli et al. (2010), Rubiales et al. (2011)
	AD280/SSR, 3567800/ DArT, 3,563,695/ DArT, 3,569,323/ DArT	<i>UpDSII</i> , <i>UpDSIV</i> , <i>UpDSIV.2</i>	Barilli et al. (2018)
Fusarium wilt ( <i>Fusarium oxysporum</i> . f.sp. <i>Pisi</i> ), race 1	H19/RAPD, Y14/RAPD, Y15/RAPD, p254/RFLP, p248/RFLP, p <sup>2</sup> 27/RFLP, p10μ/RFLP		Dirlewanger et al. (1994)
	Y15_999/SCAR, Y15_1050/RAPD/ACG: CAT_222/AFLP, ACC: CTG_159/AFLP		Okubara et al. (2005), McClendon et al. (2002)
	AD134_213/SSR, AA5_225/SSR, AA5_235/SSR, AB111/SSR, AD73/SSR, AA484/SSR, AD85_178/SSR		Loridon et al. (2005)
	THO/CAPS, AnMtL6, Mt5_56, PR X1TRAP13, TC112650/SSR, TC112533/SSR		Jain et al. (2013, 2015)
	<i>Fw_Trap_480/SCAR</i> , <i>Fw_Trap_340/SCAR</i> , <i>Fw_Trap_220/SCAR</i>		Kwon et al. (2013)
	PSAS/SSR		Burstin et al. (2001)
Fusarium wilt, race 2	PSMPSAD171/ SSR		McPhee et al. (2004)
	AC22_185/SSR, AD171_197/SSR, AB70_203/SSR, AD180_60/SSR	<i>Fnw 4.1</i> , <i>Fnw 3.1</i>	McPhee et al. (2012)
Fusarium wilt, race 5	U693_400Fwf/ SCAR		Okubara et al. (2002)

(continued)

**Table 10.2** (continued)

Trait	Marker name/marker type	Gene/QTLs	References
Powdery mildew	p236/RFLP, PD10650(RAPD to SCAR)	er-1, er-2, er-3	Dirlwanger et al. (1994)
	Sc-OPO-181200/SCAR, Sc-OPE-161600/SCAR		Frew et al. (2002)
	OPU-17/RAPD, ScOPD-10 <sub>650</sub> /SCAR, ScOPL61600/SCAR, OPO-18 <sub>1200</sub> /RAPD, OPE-16 <sub>1600</sub> /RAPD, OPL-6 <sub>1900</sub> /RAPD		Janila and Sharma (2004), Tiwari et al. (1998), Loridon et al. (2005)
	AB71/SSR, AD59/SSR, AD60/SSR/SCAR, ScOPO18-1200/SCAR, ScOPX04-880/SCAR, ScOPE16-1600/SCAR		Timmerman-Vaughan et al. (1994), Tiwari et al. (1998), Sun et al. (2019)
	PSMPSAD51/SSR, PSMPSA5/SSR, PSMPSAA374e/SSR		Ek et al. (2005), Pereira and Leitão (2010), Sun et al. (2019)
	PSMPSAA369/SSR, c5DNAmel/gene marker		Sun et al. (2015)
	AD60/SSR, ScOPX04880/SCAR, ScOPD-10 <sub>650</sub> /SCAR		Srivastava et al. (2012), Sun et al. (2019)
	SNP1121/SNP		Sun et al. (2016)
Common root rot	OPW04_637/RAPD, OPC04_640/RAPD, OPF14_1103/RAPD, OPAH06_539/RAPD, SCW4637/SCAR, SCAB1874/SCAR		Fondevilla et al. (2008a)
	N14.950/RAPDs, U326.190/RAPD, E7M4.251/AFLPs, E2M4.292/AFLP, E3M3.167/AFLP	<i>Aph 1</i> , <i>Aph 2</i> , <i>Aph 3</i>	Pilet Nayel et al. (2002, 2005)
	P393/RFLP, PgmF <sub>-390</sub> Isozyme		Weeden et al. (2000)
	PSARGDECA_F/SSR	<i>Ae-Ps4.5</i> , <i>Ae-Ps7.6</i> , <i>Ae-Ps2.2</i> , <i>Ae-Ps5.1</i> , <i>Ae-Ps3.1</i> , <i>Ae-Ps1.2</i> , <i>Ae-Ps4.1</i>	Hamon et al. (2011, 2013), McGee et al. (2012), Lavaud et al. (2015, 2016), Desgroux et al. (2016, 2018), Kwon et al. (2012)
	Ps115429/SNP		Desgroux et al. (2018)
AA505/SSR, AB101/SSR	Ae26, Ae27	Boutet et al. (2016)	

(continued)

**Table 10.2** (continued)

Trait	Marker name/marker type	Gene/QTLs	References
Ascochyta blight	p227/RFLP, p105/RFLP, p236/RFLP	QTL	Dirlewanger et al. (1994)
	c206/RFLP, M02-835/RAPD, sM2P5-234/SCAR M27/SCAR, J12-1400/RAPD, C12-680/RAPD, W17-150/RAPD, P346/RFLP, sY16-112/SCAR1 M2P2-193/AFLP sB17-509/SCAR, S15-1330/RAPD	<i>Asc1.1, Asc2.1, Asc3.1, Asc3.2, Asc4.2, Asc4.3, Asc5.1, Asc7.1, Asc7.2, Asc7.3</i>	Timmerman Vaughan et al. (2002, 2004)
	V03-1200/RAPD, PSm PSAA175/SRR, PSMPSAA 163.2/SSR, PSMPSAA399/SSR, G04-950/RAPD	<i>mpIII-1, mpIII-3, mpVa-1, mpVII-1, mpVI-1</i>	Prioul et al. (2004)
	Sc33287_25420/SNP, Sc34405_60551/SNP, Sc33468_44352/SNP, Sc12023_67096/SNP	<i>abIII-1, abI-IV-2, abI-IV-2.1, abI-IV-2.2</i>	Jha et al. (2017)
	<i>Ilccta2</i> /AFLP, <i>Ivcccl1</i> /AFLP, <i>VIACCT1</i> /AFLP		Taran et al. (2003)
	Drought	A6/SSR, AA175/SSR, AC74/SSR, AD57/SSR, AB 141/SSR, AB64/SSR, <i>Psblox2</i> /SSR, <i>PsAAP2-SNP4</i> /SSR, <i>DipeptIV-SP1</i> /SSR	<i>rwclF-1, rwclF-3, rwcsF-1, audpc_rwcs-2, rwcsF-2, rwclF-2, audpc_rwcs-1, audpc_rwcl</i>
Frost	AD59/SSR, AD141/SSR, AA200/SSR, AD159/SSR	<i>WFD3.1, WFD 5.1, WFD 6.1</i>	Lejeune-Henaut et al. (2008)
	AA67/SSR, <i>AGL20a</i> /SSR, AD141/SSR, <i>SucSyn</i> /SSR, AA475/SSR, I01.600/SSR, AB64/SSR, <i>AGL20a</i> /SSR	<i>WFDcle.a, WFDmon.a, WFDcle.b, WFDmon.b, WFDcle.c, WFDmon.c, FD164.a, FD164.b, FD164.c</i>	Dumont et al. (2009)
	EST1109/SSR		Liu et al. (2017)

A second monogenic recessive resistance locus *er2* was identified earlier by several researchers (Heringa et al. 1969; Ali et al. 1994; Tiwari et al. 1997). It has not been used commercially since the resistance breakdown because the pathogen virulence influenced by day/night temperatures and age of plants (Tiwari et al. 1997; Fondevilla et al. 2006; Rana et al. 2013). The resistance governed through *er2* gene is mainly based on post-penetration cell death complemented by a reduction of percentage penetration success in mature leaves (Fondevilla et al. 2006). Different molecular markers like AFLP, RAPD and SCAR linked to *er2* gene are available

which can be used in breeding programme for marker-assisted selection after validation (Tiwari et al. 1999; Katoch et al. 2009).

The availability of saturated consensus map, associated molecular markers and diagnostic marker for different important traits are very precious resources for dry pea breeding programme. Recently, Sudheesh et al. (2014) developed saturated genetic linkage maps using SNP and SSR markers in two RIL populations. A consensus map constructed by combining data of these maps with previously published integrated map. The consensus structure has 2028 loci scattered across seven linkage groups (LGs), with a cumulative length of 2387 cM at an average density of one marker per 1.2 cM. Trait dissection of powdery mildew resistance identified a single genomic region (PsMLO1) of large size in the same genomic region on Ps VI, which is inferred to correspond to the *er1* gene. The identified candidate gene validated in resistant and susceptible genotypes as putative diagnostic marker for powdery mildew resistance which would be used in dry pea molecular breeding programmes.

The third gene dominantly inherited powdery mildew resistance (*Er3*) identified in *Pisum fulvum* and has been introduced successfully into the adapted *P. sativum* (Sharma and Yadav 2003; Fondevilla et al. 2007a, c). Its resistance mechanism mainly based on the high frequency of cell death that occurs both as a rapid response to infection and a delayed response that follows the colony establishment (Fondevilla et al. 2007a, c). Dominant molecular marker like RAPD that tightly linked to *Er3* has been identified and converted into SCARs (Fondevilla et al. 2008a) for their utilization in pea breeding. Still, breeders are dependents on a single gene *er1* for powdery mildew resistance which is not safe; therefore, pyramiding of more than one gene in a single background is instantly required. In addition, other species including *E. trifolii* also infects pea and breaker1 resistance, which deserves urgent attention to sustain dry pea production (Fondevilla et al. 2013).

### 10.5.3.2 Rust

Pea rust has been considered as a serious disease since the mid-1980s, and it is scattered around the world in all pea-growing countries (Barilli et al. 2010). This disease incited either by *Uromyces viciae-fabae* (Pers.) j.Schrot (Arthur 1934) or *U. pisi* (Pers.) Wint (EPPO 2009; Barilli et al. 2009a, b, c, 2010, 2018; Rubiales et al. 2011, 2019; Singh et al. 2015; Das et al. 2019). In the tropical and subtropical regions *U. viciae-fabae* is prevalent, where weather is warm-humid which remains suitable for the manifestation of both uredial and aecidial stage (Pal et al. 1980; Singh et al. 2004; Kushwaha et al. 2006). These conditions usually coincide with the flowering or podding stage of crop and favour rust outbreak (Kushwaha et al. 2007; EPPO 2009; Singh et al. 2015). Contrarily, in temperate regions, it infected pea at seedlings stage and later developed under field conditions (Emeran et al. 2005; Barilli et al. 2007, 2010). *U. viciae-fabae* is an obligate parasitic fungus that only infected legume species crops such as pea, faba bean, lentil and vetches (Cummins 1978), whereas *U. pisi* is heteroecious fungi ubiquitous in cool climatic condition, and it completes life cycle on *Euphorbia esula* and *Euphorbia*

*cyparissias* (Pfunder and Roy 2000; Rubiales et al. 2019). This disease under amiable environmental circumstances vigorously spread over aerial part, i.e. leaves, stipules, pods and stem and distressed physiological and biochemical processes of plants which subsequently lead to reduction in photosynthesis (Fig. 10.5). Consequently, most of leaves fall down, and pods remain undeveloped, which resulted into more than 30% yield losses (EPPO 2009; Barilli et al. 2010). The best strategy to stabilize the productivity of pea crop is to go for host plant resistance and grow rust-resistant varieties. Complete resistance for rust yet to be reported and partial resistance or incomplete resistance is the only best available option. However, sincere efforts have been made for screening pea germplasm towards rust, but none of the genotype was found completely free from infection, while genotypic differences for rust intensity were observed (Narsinghan et al. 1980; Singh and Srivastava 1985; Gupta 1990; Anil Kumar et al. 1994). Sources of incomplete resistance to *U. pisi* from 2759 pea accession have been identified under both field and controlled conditions (Barilli et al. 2009c). Resistance to pea rust is mainly due to a restriction of haustorium development, and none of the pea accessions is observed free from rust infection (Singh and Srivastava 1985; Chand et al. 2006; Barilli et al. 2009a, b, c).

The number of genotypes with incomplete or partial resistance against *U. viciae-fabae* has been reported (Vijayalakshmi et al. 2005; Chand et al. 2006; Kushwaha et al. 2006; Das et al. 2019). Rust resistance in pea is governed by single dominant gene (*Ruf*) (Katiyar and Ram 1987; Tyagi and Srivastava 1999; Vijayalakshmi et al. 2005). Further, this trait seems to be controlled by polygenic nature of gene action in addition to the reported oligogene *Ruf* (Singh and Ram 2001). Singh et al. (2012) found that single gene shows partial dominance along with minor and 2–3 additive genes. Pea breeders have used the reported partial resistance sources in their breeding programme and developed number of high-yielding varieties with partial rust resistance suitable for different agro-climatic conditions.

The occurrence of rust is significantly influenced by environmental conditions during disease contamination and further development. This is the major constraint



**Fig. 10.5** Rust infected plant parts of dry pea



in proper screening and identification of rust resistance stable genotypes. Therefore, use of molecular marker and QTL would allow indirect selection of genotypes independent of weather conditions. Some rust-associated marker and QTL with rust have been reported as presented in Table 10.2, which seems to be controlled by one major gene and one minor QTL (Vijayalakshmi et al. 2005; Barilli et al. 2010; Rai et al. 2011). A single major gene *Ruf* responsible for this partial resistance has been identified which is flanked by two RAPD markers, SC10-82<sub>360</sub> and SCRI-71<sub>1000</sub>, with 10.8 and 24.5 cM distance, respectively, but both markers were not close enough to the gene of interest to allow marker-assisted selection for rust resistance (Vijayalakshmi et al. 2005; Rai et al. 2011). A linkage map was developed by Barilli et al. (2010) using a F<sub>2</sub> population of two *Pisum fulvum* lines. A QTL (*Up1*) associated with resistance to pea rust caused by *U. pisi* was identified on LG III. The two RAPD flanking markers OPY 11<sub>1316</sub> and OPV17<sub>1078</sub> are located at the position 26.9 and 46.3 cM, respectively. Both the markers are not close to QTL; therefore their subsequent conversion in SCAR markers could permit a reliable marker-assisted selection for rust resistance. Rai et al. (2011) reported the quantitative nature of resistance of pea rust caused by *U. fabae*. ARIL population was used (population size – 136) which derived from the cross between HUV P 1 (susceptible) and FC 1 (resistant) pea genotypes. A linkage map was developed using simple sequence repeat (SSR), sequence-tagged site (STS) and random amplified polymorphic (RAPD) markers covering 634 cM of genetic distance on the seven linkage groups of pea with an average interval length of 11.3 cM. They reported one major (Qruf) and one minor (Qruf1) QTL associated with rust resistance located on LG-7 using composite interval mapping (CIM). Also reported two flanking SSR markers AA505 and AA446 (10.8 cM) for major QTL. The minor QTL was environment-specific and only detected in polyhouse. It was flanked by two SSR markers, AD146 and AA416 (7.3 cM). Therefore, the SSR markers flanked QTL Qruf would be useful in future for marker-assisted selection for pea rust (*U. fabae*) resistance.

The validation of associated marker and QTLs is quintessential step before accommodation of them in marker-assisted programme to reduce risk and cost of programme. Therefore, the four reported SSR markers (AA446 and AA505 flanking the major QTL, Qruf; AD146 and AA416 flanking the minor QTL, Qruf1) by Rai et al. (2011) were validated in 30 diverse pea genotypes. The results revealed that the QTL, Qruf flanking markers were able to identify all the resistant genotypes when used together, except for Pant P 31, while SSR markers AD146 and AA416 flanking the minor QTL, Qruf1 were able to identify all the pea resistant genotypes during validation, except for HUDP-11 by AD146 and Pant P 31 by AA416. Similarly, SSR markers AA446 and AA505 were able to cull all the susceptible pea genotypes, except IPFD 99–13, HFP 9415 and S-143. SSR markers AD146 and AA416 were together able to identify all the pea susceptible genotypes used for validation, except KPMR 526, KPMR 632 and IPFD 99–13. The validation clearly indicated that the above-mentioned SSR markers can be used in MAS of pea rust resistance (Singh et al. 2015).

Recently, Barilli et al. (2018) used RIL population of *P. fulvum* for *U. pisi* and genotyped by Diversity Arrays Technology. Finally, an integrated linkage map was

developed using total 12,058 markers (9569 high-quality DArT-Seq and 8514 SNPs) which were distributed into seven linkage groups. The CIM revealed three QTLs (UpDSII, UpDSIV and UpDSIV.2) distributed over two linkage groups that were associated with the rust disease. First two QTLs were constantly detected both in adult and seedling plants under controlled conditions. The third QTL (UpDSIV.2) was environmentally specific and also situated on the LGIV identified only in seedlings plant under controlled conditions.

### 10.5.3.3 Ascochyta Blight

Ascochyta blight (AB) (commonly acknowledged as ‘black spot disease’) is incited by a complex of fungal species that includes *Ascochyta pisi*, *Peyronellaea pinodes* (syn. *Mycosphaerella pinodes*), *Phoma medicaginis* var. *pinodella*, *P. koolunga* and *P. glomerata* (Kraft and Pflieger 2001; Davidson et al. 2009; Aveskamp et al. 2010; Li et al. 2011; Khan et al. 2013; Liu et al. 2013; Tran et al. 2014; Sivachandra Kumar and Banniza 2017). Of them *P. pinodes* is the most prevalent and devastating fungus caused 28–88% yield damage under wet climatic conditions (Bretag et al. 1995a; Tivoli et al. 1996; Xue et al. 1997; Garry et al. 1998; Rubiales et al. 2019). It is one of the most severe diseases of field peas, and it is distributed worldwide, including almost all of the major pea-growing areas (Bretag et al. 2006; Parihar et al. 2013). In general ascochyta blight complex reduces grain yield 10–60% depending on environmental conditions in different growing regions (Wallen 1965, 1974; Tivoli et al. 1996; Xue et al. 1996; Bretag et al. 2006; Liu et al. 2016). This disease complex develops range of symptoms on seedling and all aboveground pea plant parts, including necrotic leaf spots, stem lesions, shrinkage and dark-brown discoloration of seeds, blackening of the base of the stem, foot rot and pod spot. It also causes slightly hollow, circular, tan coloured lesions with dark brown margins that occur on the leaves, pods and stems (Chilvers et al. 2009; Davidson et al. 2009; Li et al. 2011; Tran et al. 2014). All the pathogens are seed-borne in nature that can also survive on infected plant debris which play a crucial role in disease transmission in uninfected areas of developing crop (Tivoli and Banniza 2007; Parihar et al. 2013; Liu et al. 2016;). Seed-to-seedling transmission under controlled conditions is up to 100% for *P. pinodes* (Xue 2000) and 40% for *A. pisi* (Maude 1966). Most importantly *Ascochyta* spp. can survive on pea seed coats for several years (Bretag et al. 1995b), and particularly for *A. pisi*, it was estimated that the fungus will be dissect from seed after 5–7 years of seed storage in cool and dry conditions (Wallen 1955).

The incidence of the disease under field conditions is highly influenced by agronomic traits including lodging and plant height (Taran et al. 2003; Banniza et al. 2005; Le May et al. 2009; Jha et al. 2013, 2016). Therefore, development of resistant cultivars is the best management strategy for ascochyta blight in peas since it is most practical, effective and economical approach (Zimmer and Sabourin 1986). However, sincere efforts have been made, but none of the material from cultivated pea could show complete resistance against ascochyta blight fungi. Therefore,

cultivars that are highly resistant to ascochyta blight have not yet been developed. Although, some potential genotypes out of more than 3500 cultivated pea accessions with low- to moderate-level resistance were identified (Kraft et al. 1998; Zhang et al. 2006). On the contrary, high level of resistance was reported in wild pea (*P. fulvum*) accession (Clulow et al. 1991; Wroth 1998; Fondevilla et al. 2005; Jha et al. 2012). Further, Fondevilla et al. (2005) also identified the high level of resistance in accession P651 (*P. fulvum*) compared to other wild peas (*P. sativum* ssp. *elatius* and *P. sativum* ssp. *syriacum*) accessions. Later on, Jha et al. (2012) recognized promising accessions AB resistance from *P. fulvum* and *P. sativum* ssp. *elatius* through appraisal of 44 wild pea accessions. Of them, the most promising accession was P651 belong to *P. fulvum* and utilized for resistance breeding (Sindhu et al. 2014; Jha et al. 2016). The nature of inheritance so far reported for AB resistance is polygenic (Xue and Warkentin 2001; Prioul et al. 2004; Fondevilla et al. 2007b; Prioul Gervais et al. 2007; Carrillo et al. 2014; Timmerman Vaughan et al. 2016; Jha et al. 2017), and this has hampered the AB resistant cultivar development programme (Rubiales and Fondevilla 2012). Furthermore, different QTL mapping studies have identified numerous genomic regions (see Table 10.2) involved in the control of resistance and confirming the polygenic nature of resistance (Timmerman Vaughan et al. 2002, 2004; Taran et al. 2003; Prioul et al. 2004; Fondevilla et al. 2008b). QTLs were also identified from a cross involving wild pea, *P. sativum* subsp. *syriacum* (Fondevilla et al. 2008a, b, 2011; Carrillo et al. 2014). The candidate genes co-locating with QTL for resistance to *M. pinodes* have also reported (Prioul Gervais et al. 2007). Further, Jha et al. (2015) reported a significant association of SNPs detected within candidate genes PsDof1 (PsDof1p308) and RGA-G3A (RGA-G3Ap103) for AB resistance. Similarly, Jha et al. (2016) reported nine QTLs associated with AB resistance in an interspecific pea population (PR-19) developed from a cross between Alfetta (*P. sativum*) and wild pea accession P651 (*P. fulvum*), of them two QTLs abIII-1 and abI-IV-2 were consistent across locations and/or years.

QTL mapping in several pea crosses designated genomic regions associated with AB resistance; nevertheless, these QTLs cover large regions and may not be effective for use in MAS programme. Similarly, the large number of markers associated with resistance genes has been identified, but none of them tightly linked to the targeted gene of interest (Michelmore 1995). Recombination could occur between a marker and QTL if markers are not tightly linked to genes (Collard et al. 2005). Therefore, high resolution or fine mapping of QTLs should be used to recognize more tightly linked that can be precisely used for MAS (Mohan et al. 1997). For fine mapping, an advanced mapping population, like near-isogenic lines (NILs), through consecutive backcrossing is need to be developed. An alternative and more efficient method proposed by Tuinstra et al. (1997) is development of heterogeneous inbred family (HIF) populations, which is more efficient method than the NILs.

Most recently, Jha et al. (2017) fine mapped the abIII-1 and abI-IV-2 QTLs using a high-density SNP-based genetic linkage map and examine identified markers in HIF populations. Selective genotyping was performed in 51 PR-19 recombinant inbred lines using genotyping-by-sequencing (GBS), and the resultant high-density

genetic linkage map was utilized to recognize eight new SNP markers within the *abI-IV-2* QTL, whereas no additional SNPs were identified within the *abIII-1* QTL. Two HIF populations HIF-224 (143 lines) and HIF-173 (126 lines) were developed from F6 RILs PR-19-224 and PR-19-173, respectively. The HIF populations ascertained under field conditions in which a wide range of variation for reaction to AB resistance observed. HIFs were genotyped using SNP markers within targeted QTLs. The genotypic and phenotypic data of the HIFs were used to identify two new QTLs, *abI-IV-2.1* and *abI-IV-2.2* for AB resistance within the *abI-IV-2* QTL. These QTLs individually accounted for 5.5–14% of the total phenotypic variation. Resistance to lodging was also associated with these two QTLs. In addition, five and three additional SNP markers identified in QTLs *abI-IV-2.1* and *abI-IV-2.2*, respectively, by fine mapping will be useful in marker-assisted selection for development of pea cultivars with improved AB resistance. This approach has been extensively adopted in several species such as *Arabidopsis*, soybean and maize for fine mapping of QTLs (Meng et al. 2008; Bai et al. 2010; Todesco et al. 2010; Coles et al. 2011; Dwiyananti et al. 2011; Watanabe et al. 2011; Bouteillé et al. 2012).

#### 10.5.3.4 *Fusarium* Root Rot

*Fusarium* root rot, caused by *Fusarium solani* f.sp. *pisi*, is a cosmopolitan disease of pea occurred in almost all pea-growing areas around the world and considered as major limiting factor in production (Kraft et al. 1988, 1996; Backhouse et al. 2001; Kraft and Pflieger 2001; Grunwald et al. 2003; Hamid et al. 2012; Porter et al. 2015). *Fusarium* root rot of peas, caused by *F. solani* f.sp. *pisi*, was first reported as a serious pathogen in the USA (Bisby 1918; Jones 1923). The disease may damage peas produced in both dry and wet fields and has been reported that it reduced yield up to 60% under suitable circumstances (Kraft and Pflieger 2001; Kraft 2001; Chang et al. 2004; Porter 2010). This disease is distinct from *Fusarium* wilt, caused by *F. oxysporum* f.sp. *pisi*, but sometimes occurs in combination with other diseases of peas also (Zaumeyer and Thomas 1957). The compact and warm soil (18–24 °C soil temperature) conditions are most suitable for *Fusarium* root rot development in pea-growing regions around the world (Kraft and Roberts 1969; Kraft and Giles 1979; Kraft and Wilkins 1989; Kraft and Boge 2001). The symptoms above the ground include yellowing of aerial parts start from the base and progress towards upper side. The black to brown lesions developed on stems where the cotyledons are attached to the stem and eventually, and it causes root and stem rot followed by necrosis and death of leaves (Kraft 1994). In case of *Fusarium* root rot, wilting of plants is not commonly happened, but it shortened the growth of plants and induces force maturity (Hagedorn 1991; Oyarzun 1993; Hamid et al. 2012; Porter et al. 2015).

Breeding disease resistance varieties is considered to be the basic prerequisite for improving and stabilizing yield of grain legumes (Ranalli 2003). But, so far complete resistance to this disease has not been reported in pea, but a number of sources of partial tolerance have been found (Kraft et al. 1988; Gretenkort and Helsper 1993; Hwang et al. 1995; Grunwald et al. 2003; Porter 2010; Porter et al. 2015).

Interestingly, most of the accessions with pigmented flowers have tendency of greater partial resistance to *F. solani* f.sp. *pisi* than white-flowered cultivars (Kraft 1975; Grunwald et al. 2003). Detailed data about sources of resistance to *Fusarium* diseases in wild *Pisum* species and accessions are not available. However, a set of ten accessions of wild *P. sativum* subspecies along with varieties was examined for resistance to *F. Solani* under controlled conditions (Kraft and Roberts 1970; Lebeda and Švábová 1997; Grunwald et al. 2003; Coyne et al. 2008; Porter et al. 2014). Genetic resistance offers one of the best strategies to control this root-rotting fungus. Complete resistance to pathogen was not recorded, but very high level of resistance was observed (Lebeda and Švábová 1997). It has polygenic nature of inheritance; therefore, development of resistant varieties becomes more complicated (Lockwood 1962; Muehlbauer and Kraft 1973; Kraft 1992). However, the genetics of the quantitative partial resistance is little bit studied with just few QTL reports published so far for *Fsp* as given in Table 10.2 (Hance et al. 2004; Feng et al. 2011). First a RIL population derived from cross between JI 1794 and Slow (*P. sativum* subsp. *sativum*) has been used. The segregation patterns results revealed that the tolerance to *F. solani* was multigenic in JI 1794 and also identified one QTL for *Fusarium* root rot tolerance that near to *Le* gene.

Feng et al. (2011) developed RIL population (71) of dry pea, derived from crosses between a resistant cultivar 'Carman' and a susceptible cultivar 'Reward'. To discover markers linked with the resistance, a total of 213 SSR markers were used, and of them only 14 markers were polymorphic between the two parents. QTL analysis reported a QTL that explained 39.0% of the phenotypic variance in the RIL population and flanked by markers AA416 and AB60 on LG VII. The microsatellite markers that are closely linked to this QTL may be useful for marker-assisted selection to develop cultivars with superior *Fusarium* root rot resistance. Additionally, five QTL were also reported on pea LGs II, III, IV, VI and VII (Hance et al. 2004; Weeden and Porter 2007).

Recently, Coyne et al. (2015) used a RIL population and employed composite interval mapping (CIM) for QTL detection. A total of five QTL were identified, and of them one QTL is detected consistently over the years. The multiyear QTL *Fsp-Ps2.1* contributed a significant portion of the phenotypic variance (22.1–72.2%), while a second QTL, *FspPs6.1*, contributed 17.3% of the phenotypic variance. The other single growing season QTLs are of additional interest as they co-segregate with previously reported pea-*Fusarium* root rot resistance QTL. QTL *Fsp-Ps2.1*, *Fsp-Ps3.1*, *Fsp-4.1* and *Fsp-Ps7.1* are flanked by codominant SSRs and may be useful in marker-assisted breeding of pea for high levels of partial resistance to *Fsp*. Most recently the previously identified QTL *Fsp-Ps 2.1* has been confirmed in two RIL populations by Coyne et al. (2019). They identified three QTLs such as *Fsp-Ps 2.1*, *Fsp-Ps3.2* and *Fsp-Ps3.3* using CIM. The first QTL *Fsp-Ps 2.1* explains 44.4–53.4% of the variance with a narrow confidence interval of 1.2 cM. The second and third QTL *Fsp-Ps3.2* and *Fsp-Ps3.3* are closely linked and explain only 3.6–4.6% of the variance. All of the alleles are belong to the resistant parent PI 180693. The confirmation of *Fsp-Ps 2.1* now in two RIL populations and SNPs associated with this region makes it a good target for marker-assisted selection in

pea breeding programmes to develop high levels of partial resistance for *Fusarium* root rot caused by *Fusarium solani* f.sp. *pisi*.

### 10.5.3.5 *Fusarium* Wilt

*Fusarium* wilt is inflicted by soil-borne fungus *Fusarium oxysporum* f.sp. *pisi* (van Hall) Snyd. & Hans., which is a serious production threat and dispersed around the world (Haglund 1984; Kraft 1994; McClendon et al. 2002; Sharma et al. 2010; Rubiales et al. 2015). It enters into the host vascular system through root tips or wound, subsequently causes chlorosis of the leaves and stems, wilting, and collapse of the root systems (Bishop and Cooper 1983; Beckman 1987; Correll 1991; Benhamou and Garand 2001; Haglund and Kraft 2001; Haglund 2001; Zvirin et al. 2010). *Fusarium* wilt is an economically significant disease causes losses in dry pea up to 100% under favourable conditions (Aslam et al. 2019). The early symptoms are yellowing of lower leaves and reduced plant growth which eventually leads towards wilting of complete plants. A soil temperature of 23–27 °C is most suitable for proper wilt development. This fungal species has a total of 11 different races which are described on the basis of virulence (Armstrong and Armstrong 1974; Gupta and Gupta 2019). Of these, races 1 and 2 are widely distributed, while races 5 and 6 are, to date, scattered only in some specific regions (Infantino et al. 2006; Bani et al. 2018). *Fusarium* wilt race 1 is one of the major races among the four pathogenicity groups on pea (Kraft and Pflieger 2001). This pathogen is soil-borne and can survive in the soil in the absence of pea crop for longer time (Skovgaard et al. 2002; Roncero et al. 2003; Bani et al. 2018; Gupta and Gupta 2019). The soil-borne fungal diseases are mainly controlled by the integration of different disease management procedures. Among these methods, the use of resistant cultivars is widely recognized as the safest, most economical and most effective crop protection method (Ciancio and Mukerji 2008; Rubiales et al. 2015; Gupta and Gupta 2019). Mcphee et al. (1999) reported 62 and 39 resistance accessions for race 2 and race 1, respectively, from core collection. One of the wild progenitors, PI 344012, possessed resistance to races 1 and 2. The genetic resistance to Fop races 1, 5, and 6 is conferred by single dominant genes, whereas resistance to race 2 is quantitative (Mcphee et al. 1999, 2012; Bani et al. 2012, 2018; Risipail and Rubiales 2014). The resistance controlled by a single dominant gene has been incorporated successfully into many varieties (Mcphee 2003). The transfer of quantitative resistance in susceptible cultivar is complicated where molecular marker can play a crucial role because the selection process is time-consuming and labour-intensive for such traits. Therefore, recent developments in genomics research have provided scope for searching, using, and selecting naturally occurring resistance against *Fusarium* wilt in cool season food legumes with the help of molecular tools (Kamboj et al. 1990; McClendon et al. 2002; Infantino et al. 2006; Kumar et al. 2011; Smýkal et al. 2012).

Therefore, numerous dominant molecular markers like RAPD, SCAR and AFLP for race 1 (*Fw*) locus have been identified as presented in Table 10.2 (McClendon et al. 2002; Okubara et al. 2005). The inheritance of resistance to race 5 is conferred

by single dominant gene, *Fwf* (Hagedorn 1989). For race 5 Coyne et al. (2000) used a RIL population and identified a locus which was associated with resistance for race 5 (*Fwf*). Similarly, a total of 14 markers including 5 morphological, 1 isozyme and 9 RAPD co-segregated with *Fusarium* race 5 resistance gene (*Fwf*) within a 123 cM interval. Of these, one tightly linked RAPD marker, i.e. U693a, located at distance of 5.6 cM and about 8.5 cM closer than previously identified marker (Okubara et al. 2002). The gene conferring resistance to *F. oxysporum* race 1 in pea, *Fw*, which is located on linkage group (LG) III and widely used in breeding programmes. Similarly, Loridon et al. (2005) placed *Fw* between two SSR markers AA5\_225 and AD134\_213 at 2.7 and 2.5 cM distances, respectively. Because both the markers situated at relatively larger distance from *Fw*, hence, both the SSR markers are not suitable for reliable marker-assisted selection (MAS) of *Fw*. Later on, three sequence-characterized amplified region (SCAR) markers were developed using the target region amplified polymorphic (TRAP) marker technology and mapped close to *Fw* in a population developed from PI 179449 and 'Green Arrow' using a bulk segregant analysis approach (Kwon et al. 2013). These three markers, *Fw\_Trap\_480*, *Fw\_Trap\_340* and *Fw\_Trap\_220*, are located only 1.2 cM away from *Fw* locus. However, use of these markers in MAS is dubious because of the dominant nature of these markers. Codominant markers such as CAPS makers are more suitable for MAS in plants since they can distinguish heterozygotes from homozygotes (Jiang 2013). Therefore, Jain et al. (2015) developed a breeder-friendly functional codominant cleaved amplified polymorphic sequence (CAPS) marker, THO, which can be used in pea breeding programmes for selection of resistance to *F. oxysporum* race 1. By using this marker, dry pea breeder can select lines with more than 94% accuracy from mapping populations and advanced pea breeding lines. This marker, in combination with other gene-based markers (AnMtL6, Mt5\_56 and PRX1TRAP13) developed from conserved sequences of closely related legume species, lays the foundation for candidate gene identification through comparative mapping.

#### 10.5.3.6 Common Root Rot

Common root rot of field pea incited by the soil-borne fungus *Aphanomyces euteiches* Drechs. is one of the serious constraints to pea production (Jones and Drechsler 1925; Mcphee 2003; Pilet Nayel et al. 2005; Desgroux et al. 2016; Wu et al. 2018). This pathogen has been mainly reported as a yield-limiting factor in major dry pea cultivation countries such as the USA, Europe and most recently in Canada (Papavizas and Ayers 1974; Wicker and Rouxel 2001; Wicker et al. 2003; Chatterton et al. 2015; Desgroux et al. 2016). This pathogen can cause severe root damage, wilting and substantial yield losses under wet soil conditions (Wu et al. 2018). Two main pathotypes of *A. euteiches* were reported, and both pathotypes cause honey brown necrosis symptoms on pea roots and epicotyls, resulting in dwarfism, foliage yellowing and then death of plants in highly infested fields (Wicker and Rouxel 2001). The conventional disease management strategies, such as crop rotations and

seed treatments, are unable in full prevention of this disease under favourable conditions, due to the durability of the pathogen oospores (Papavizas and Ayers 1974), which can infect field pea plants at any growth stage. Therefore, development of resistant cultivars has been considered as a major objective in dry pea breeding programme. Pea lines partially resistant to *A. euteiches* were identified from germ-plasm screening and breeding programmes (Gritton 1990; Kraft 1992; Davis et al. 1995; Malvick and Percich 1998a, b; Kraft 2000; Kraft and Coffman 2000a, b; Pilet Nayel et al. 2007; Conner et al. 2013). The reported resistant accessions were incorporated into breeding programmes during the last three decades to develop breeding lines (Roux-Duparque et al. 2004; Moussart et al. 2007), recombinant inbred lines (RILs) (Pilet Nayel et al. 2002, 2005; Hamon et al. 2011, 2013; McGee et al. 2012) and near-isogenic lines (NILs) (Lavaud et al. 2015). But breeding for tolerance to common root rot has been difficult because of the polygenic nature of the tolerance and also associated with some undesirable traits like long internodes, anthocyanin content and late-flowering (Marx et al. 1972; Pilet Nayel et al. 2002). Therefore application of different molecular marker has become important to speed up and reduces the cost of breeding programme. Different types of molecular marker are identified (Table 10.2), for example, Weeden et al. (2000) found a gene MN313 located on the linkage group IV near P393 which has a significant influence on the expression of tolerance to common root rot. The nature of inheritance of partial resistance to *A. euteiches* in pea has not been extensively studied. Therefore, RIL population (127) derived from the cross Puget (susceptible) × 90-2079 (partially resistant) was used and genotyped using automated AFLPs, RAPDs, SSRs, ISSRs, STSs, isozymes and morphological markers. Subsequently, developed genetic map and identified seven genomic regions, including a major quantitative trait locus (QTL), *Aph1*, along with two other year-specific QTLs, namely, *Aph2* and *Aph3* associated with partial resistance to *Aphanomyces* root rot and explained 47%, 32% and 11% of the variation, respectively. The remaining two QTLs were environment-specific and mapped near the *R* (wrinkled/round seeds) and *af* (normal/afila leaves) genes. However, the integration of these QTL for MAS in European breeding programmes has been questionable, since partial resistance of 90-2079 was not effective in French field conditions (Pilet Nayel et al. 2002). To evaluate the specificity and consistency of already identified QTLs in previous study (*Aph1*, *Aph2* and *Aph3*), the same mapping population was evaluated under greenhouse and field conditions with two isolates (the USA and French). By using previously reported genetic map, a total of ten QTL were identified for resistance in greenhouse conditions to the two isolates. Among these *Aph1*, *Aph2* and *Aph3* were previously detected for partial field resistance in the USA. *Aph1* and *Aph3* were detected with both isolates and *Aph2* with only the French isolate. The consistency of the detected resistance QTL, i.e. *Aph1*, *Aph2* and *Aph3*, suggested the usefulness of these in marker-assisted selection (Pilet Nayel et al. 2005). Hamon et al. (2011) used two RIL mapping populations (178 individual in each), derived from crosses between 552 or PI180693 (partially resistant) and Baccara (susceptible), to identify QTL for *Aphanomyces* root rot resistance. They identified a total of 135 additive-effect QTL corresponding to 23 genomic regions and 13 significant epistatic interactions



associated with partial resistance to *A. euteiches* in pea. Of the 23 additive-effect genomic regions identified, 5 were constantly detected and showed high stability towards *A. euteiches* strains and other external factors. These results confirmed the complexity of inheritance of partial resistance to *A. euteiches* in pea and suggested to use steady QTL in marker-assisted selection programme to increase current levels of resistance to *A. euteiches* in pea breeding, since development of durable plant genetic resistance to pathogens through QTL pyramiding and diversification requires in-depth knowledge of polygenic resistance within the available germplasm. The polygenic partial resistance to *Aphanomyces* root rot, caused by *Aphanomyces euteiches*, is already confirmed in individual mapping populations (Pilet Nayel et al. 2002, 2005; Hamon et al. 2011). However, there are no data available regarding the diversity of the resistance QTL across a broader collection of pea germplasm. Therefore, Hamon et al. (2013) performed a meta-analysis using previously reported 244 individual QTL in three mapping populations (Puget × 90–2079, Baccara × PI180693 and Baccara × 552) and in a fourth mapping population in this study (DSP × 90–2131), which detected 27 meta-QTL for resistance to *A. euteiches*. In addition, 11 stable meta-QTL have been identified which highlight 7 highly steady genomic regions. Furthermore, seven resistance meta-QTLs were identified; of them six were highly consistent, co-segregated with morphological/phenological alleles. Alleles accountable for the resistance were often associated with unwanted alleles for dry pea breeding (Marx et al. 1972; Pilet Nayel et al. 2002).

QTL validation is an important and often ignored step prior to subsequent research in QTL cloning or marker-assisted breeding for disease resistance in plants. Therefore, Lavaud et al. (2015) validate seven recently identified QTL in different genetic backgrounds and also assess the effects of various resistance alleles. In this study near-isogenic line (NIL) population was evaluated for resistance to two reference strains of the main *A. euteiches* pathotypes under controlled conditions. The NILs carrying resistance alleles at the major-effect QTL *Ae-Ps4.5* and *Ae-Ps7.6*, either individually or in combination with resistance alleles at other QTL, showed significantly condensed disease severity compared to NILs without resistance alleles. Resistance alleles at some minor-effect QTL, especially *Ae-Ps2.2* and *Ae-Ps5.1*, were also validated for their individual or combined effects on resistance. The effect of QTL × genetic background interactions were observed high for QTL *Ae-Ps7.6* in the winter cultivar. The pea NILs are a novel and valuable resource for further understanding the mechanisms underlying QTL and their integration in breeding programmes.

The proper understanding of the effects of resistance QTL on pathogen development is an important concern for the construction of QTL combination strategies to increase durability disease resistance in plants. Therefore, recently, Lavaud et al. (2016) investigated the effect of the main *A. euteiches* resistance QTL in NILs on different steps of the pathogen life cycle. Significant effects of several resistance alleles at the two major QTLs *Ae-Ps7.6* and *Ae-Ps4.5* were observed on symptom appearance and root colonization by *A. euteiches*. Some resistance alleles at three other minor QTLs (*Ae-Ps2.2*, *Ae-Ps3.1* and *Ae-Ps5.1*) significantly decreased root colonization. The combination of resistance alleles at two or three QTLs including

the major QTL *Ae-Ps7.6* (*Ae-Ps5.1/Ae-Ps7.6* or *Ae-Ps2.2/Ae-Ps3.1/Ae-Ps7.6*) had an increased effect on delaying symptom appearance and/or slowing down root colonization by *A. euteiches* and on plant resistance levels, compared to the effects of individual or no resistance alleles. This study recommended that single resistance QTL can affect different steps of the disease growth cycle and that their actions could be pyramided to increase partial resistance in future pea varieties. Further studies are needed to investigate QTL effects on different steps of the pathogen life cycle, as well as the efficiency and robustness of pyramiding strategies with QTL which come out to act on the similar stage of the pathogen cycle.

Genome-wide association (GWA) mapping has recently emerged as an important move towards refining the genetic basis of polygenic resistance to plant diseases, which are being used in integrated strategies for durable crop protection. Linkage mapping studies reported quantitative trait locus (QTL) controlling resistance to *A. euteiches* in pea (Pilet Nayel et al. 2002, 2005; Hamon et al. 2011, 2013). Nevertheless, the confidence intervals (CIs) of these QTLs remained large and were often linked to undesirable alleles, which limited their application in breeding. Therefore, to refine and validate the previously reported QTLs and to identify new loci, Desgroux et al. (2016) used GWA with 13,204 SNPs from the recently developed GenoPea Infinium® BeadChip. The GWA analysis identified total 52 QTL of small confidence intervals associated with resistance to *A. euteiches*, using the recently developed multi-locus mixed model. The analysis validated six of the seven previously reported main *Aphanomyces* resistance QTLs and detected novel resistance loci. The previously reported linkages between resistance alleles and undesired late-flowering alleles for dry pea breeding were mostly confirmed, but the linkage between loci controlling resistance and coloured flowers was broken due to the high resolution of the analysis. A high proportion of the putative candidate genes implicit resistance loci encoded stress-related proteins, and others suggested that the QTLs are concerned in diverse functions. Similarly, Desgroux et al. (2018) used a comparative genome-wide association (GWA) of plant architecture and resistance to *A. euteiches* in a collection of 266 pea lines contrasted for both traits. The collection was genotyped using 14,157 SNP markers from recent pea genomic resources. A total of 11 genomic intervals were significantly associated with resistance to *A. euteiches* confirming several reliable formerly known major QTLs. One SNP marker, mapped to the major QTL *Ae-Ps7.6* was associated with both resistance and root system architecture (RSA) traits. This marker is associated with the resistance-enhancing allele along with an increased total root projected area. The identify pea lines, QTL, closely linked markers and candidate genes for RSA loci can be used to reduce *Aphanomyces* root rot severity in future pea varieties.

### 10.5.4 *Breeding for Abiotic Stresses*

The major abiotic stresses which are now becomes serious issue in sustainable production of dry pea under climate change scenario are extremities of temperature (low and high), moisture extremities (drought and flood) and salinity conditions (Rubiales et al. 2019). These stresses have full potential to negatively affect the seed yield and its quality at significant levels. The selection of resistance genotypes for abiotic stresses is cumbersome owing to the oscillation of environmental conditions over the locations and years. Besides, the growth stage of the crop at the time the stress comes can result in dramatic changes in response and injury level (Monti et al. 1993). Therefore, evaluations of crop in controlled environments have been commenced to estimate precisely plant response to a specific stress. Importantly, the testing of pea materials in extreme field situations where a specific stress is assured while other abiotic stress can be avoid or minimized can be productive and improve breeding efficiency (Sadras et al. 2012). In this section we will discuss status of the major abiotic stresses such as heat/high temperature, drought and frost.

#### 10.5.4.1 Heat Stress

Grain legumes play a vital role in different cropping systems towards ensuring food security for alarmingly increasing human population (Foyer et al. 2016; Considine et al. 2017). However, according to the IPCC report in 2018, global average temperature over the last 5 years (2014–2018) has been increased by 1.04 °C compared to the preindustrial base line and will reach 1.5 °C as soon as by 2030 (IPCC 2018). Accordingly, legume growth and development will be subjected to recurrent and harsh heat stress (Zinn et al. 2010; Vadez et al. 2012).

The elevated temperature beyond the threshold level especially at critical growth stages causes a significant loss in productivity and quality of produces (Wahid et al. 2007; Bitá and Gerats 2013; Farooq et al. 2017; Liu et al. 2019a). The optimal temperature for grain legume crops range 10–36 °C, above which severe losses in grain yield can take place (Siddique 1999). High leaf temperatures condense plant growth and limit crop yields. It is estimated up to 17% decrease in grain yield for each degree Celsius increase in average growing season temperature (Lobell and Asner 2003). On the basis of climatic requirements, dry pea comes in cool season category of grain legumes (Oram and Agcaoili 1994). Cool season grain legumes are more sensitive to high temperature than warm season grain legumes (Hall 2001).

Elevated ambient temperature above 25 °C during dry pea life cycle reduces seed yield by reducing plant growth, number of flowering nodes, number of pods per plant and abortion of flowers and young pods and by speeding up the crop life cycle towards maturity (Boswell 1926; Lambert and Linck 1958; Karr et al. 1959; Stanfield et al. 1966; Nonnecke et al. 1971; Jeuffroy et al. 1990; Guillioni et al. 1997, 2003; Sadras et al. 2012; Bueckert et al. 2015). The high temperature negatively affects photosynthesis and growth of pea with substantial genotypic difference

(McDonald and Paulsen 1997). In pea (*Pisum sativum* L.), photosynthetic activity is detained at 40 °C (Georgieva and Lichtenthaler 1999). Similarly, reduction in net photosynthesis rate beyond 35 °C temperature in pea leaves has been noticed, and at 45 °C net photosynthesis reduced up to 80% (Haldimann and Feller 2005).

The heat stress exaggerated under field conditions by other environmental and management factors (Bonada and Sadras 2015). The increased temperatures caused seed yield reduction in dry peas by reducing flowering to maturity period (Bueckert et al. 2015) indicating that earlier flowering with prolonged flowering duration would result in greater heat tolerance (Huang et al. 2017). The longer flowering period supports the idea that greater plasticity in crop phenology would contribute to greater yield under stress conditions proposed by Turner et al. (2001). The severe heat stress (33 °C day–30 °C night for 2 days) caused rapid abortion and abscission of reproductive organs in pea under controlled conditions (Guilioni et al. 1997). Using a 12 h photoperiod, high night temperatures (24 °C day–30 °C night) caused 25% yield loss in dry pea, as opposed to 8% loss for high day temperatures (32 °C day–15 °C night) (Karr et al. 1959). In other experiments, elevated day temperatures ranging from 24 to 33 °C did not affect the number of seeds per pod nor the seed to ovule ratio in dry pea, whereas severe heat stress significantly reduced these parameters when day temperatures increased from 33 to 36 °C (Jiang et al. 2015). It was also suggested that seed development was most affected to the exposure of high temperatures for 5–10 days after opening of the flower at the first node (Jeuffroy et al. 1990). High temperatures during flowering caused reduction in pea grain yield by reducing fruitful node and number of pods per plant (Pumphrey et al. 1979; Duthion et al. 1987; Laconde et al. 1987). Exposure to high temperatures reduces in vitro pollen germination percentage and pollen tube length in field pea (Petkova et al. 2009; Lahlal et al. 2014; Jiang et al. 2015, 2017a). Therefore, exposure to a severe temperature of 36 °C in a growth chamber under cool fluorescent lights was recommended for future screening of pea genotypes for assessment of their heat tolerance using in vitro pollen germination (Jiang et al. 2015, 2017a).

Most importantly, every 1 °C increase in mean temperature during flowering stage could reduce yield to the tune of 0.6 tonnes/ha (Ridge and Pye 1985). Similarly, Pumphrey and Raming (1990) suggested yield loss in pea varying from 16 to 67 kg/ha vis-à-vis a temperature increase between 27 and 35 °C. Additionally, biological nitrogen fixation is severely affected above 40 °C in pea (Michiels et al. 1994). Pea production starts to suffer a reduction when the maximum daytime air temperature exceeds 25 °C (Guilioni et al. 2003). When air temperature is over 30 °C for just a few hours a day, the damage to plants is regarded as moderately severe and severe when maximum air temperature exceeds 35 °C for similar periods (Munier Jolain et al. 2010). Sousa Majer et al. (2004) found that high temperature reduced the protective capacity of the transgenic peas by reducing the production of  $\alpha$ -amylase inhibitor 1 ( $\alpha$ -AI-1). The plants exposure to high temperatures produces 27% less seeds than the controls. In the transgenic peas, the level of  $\alpha$ -AI-1 as a percentage of total protein was reduced on average by 36.3% in the high-temperature treatment. If crop exposed to high temperature during flowering and seed filling stages under field condition, it reduces membrane stability index (28.8%), plant height (60.2%),

total biomass yield (61.7%), seed yield (68.9%) and harvest index (19.3%). Based on the minimum reduction in observed traits, genotypes, KPF 103, DMR 15 and IPFD 4-6, were found to be having comparatively higher amount of resistance towards high temperature stress. IPFD 99-7, IPFD 3-17, IPFD 2-6, IPFD 1-10, HUDP 16 and DPR 13 were adjudged to moderately resistant for high temperature stress as they were having more than 75.0% yield stability index (Vijaylaxmi 2013). Jiang et al. (2018) used two cultivars ('CDC Golden', 'CDC Sage') and exposed them to 24/18 °C (day/night) continually or to 35/18 °C for 4 or 7 days. The given heat stress altered stamen chemical composition, reduced pollen and ovule viability. Pollen appears susceptible to stress, and ovule fertilization and embryos are less susceptible to heat, but further research is warranted to link the exact degree of resilience to stress intensity. Recently, Jiang et al. (2019) reported that the heat stress reduced the number of pollen grains per anther, induced smaller pollen grains and increased ROS production in pollen grains, but it did not affect ROS accumulation in ovules and ovule number per ovary. Heat exposure when young floral buds were visible at the first reproductive node was more detrimental to flower retention, seed set, pod development and seed yield compared to heat exposure started later when flowers at the second reproductive node were fully open. Overall, the high temperature stress negatively affects pollen development and seed set. Heat stress reduced pollen viability, in vitro pollen germination and pollen tube length in field pea (Jiang et al. 2015, 2018, 2019; Jiang 2016).

Since flowering stage is the most sensitive for heat stress, therefore, to increase seed setting efficiency, pea genotypes are being selected for viable pollen production (Jiang et al. 2017a), viable ovules, successful pollination (Jiang et al. 2015, 2017a). The preliminary screening methods are only based on limited number of genotypes (2–24 genotypes), and easily observable traits are being adopted for the examined material (Jiang et al. 2017a). In addition, drought and heat normally comes together causes severe reduction of grain yield (Bueckert et al. 2015). Other traits that are being used to develop heat and drought resistance are pod wall ratio and proxy measurements for crop growth rate from vegetation indices such as NDVI (Sadras et al. 2013), PRI and WBI and leaf wax (Bueckert and Clarke 2013; Tafesse 2018). Most recently, it has been well established that the knowledge of structural-chemical composition of the leaf cuticle is of immense interest in stress physiology (Sánchez et al. 2001), because when pea leaves are exposed to high temperature, the cuticular compounds may respond to heat stress by changing composition or amount, as emphasized in Suseela and Tharayil (2018) and Heredia-Guerrero et al. (2018). Liu et al. (2019b) used attenuated total reflection (ATR)-Fourier-transform infrared (FTIR) spectroscopy, a non-invasive technique, to investigate and quantify changes in adaxial cuticles of fresh leaves of pea varieties exposed to heat stress. Results reported considerable diversity in spectral-chemical makeup of leaf cuticles within commercially available dry pea varieties, and they responded differently to high growth temperature, revealing their diverse potential to resist heat stress. The ATR-FTIR spectral technique can, therefore, be further used as a medium-throughput approach for rapid screening of superior cultivars for heat tolerance.

In addition, other measurements which are suitable for automation of phenotyping are canopy temperature (infrared thermometry and thermal images), lodging (red green blue images) and height, either for abiotic stress impact or for a factor linked to biotic stress (disease and disease ratings). The studies conducted in controlled conditions are in unnatural environments and expensive but still remains valuable as screening methods for trait validation. In contrast, more genotypes can be evaluated in the field condition with low cost and precise phenotyping for canopy measurements, but environmental affects need to be understood for proper interpreted.

Based on visual observation, the selection of physiological traits associated with plant response to high temperature, selection for grain yield and more recently marker-assisted selection (MAS) are the important selection methods mainly used to develop heat-tolerant materials through breeding (Howarth 2005). Of them selection and improvement through MAS is most précised and robust technique with better efficiency compared to other approaches. Therefore, a panel of 92 diverse pea cultivars was evaluated across 9 environments and genotyped using 1536 single-nucleotide polymorphisms (SNPs) arranged in a GoldenGate array. The population structure analysis developed three subpopulations, and association analyses identified a total of 60 SNPs significantly associated ( $-\log_{10} p \geq 4.3$ ) with various reproductive development-related traits. Of them, 33 SNPs were associated with the onset of flowering, 8 SNPs with pod development and 19 SNPs with the number of reproductive nodes. Also found 12 SNPs linked with days to flowering and 2 SNPs associated with duration of flowering which were overlapped with the SNP markers associated with the number of reproductive nodes. Genomic regions associated with variation for reproductive development-related traits identified in this study provide grounds for future genetic improvement in pea (Jiang et al. 2017b). Heat tolerance is a quantitative trait, therefore, identification of associated QTLs and their judicious utilization is an important strategy for accelerating breeding programme for the development of heat tolerant genotypes. So far, any QTLs directly related to heat tolerance have not been reported in dry pea. However, considerable progress has been made regarding QTL mapping for heat tolerance in major crops including wheat (*Triticum aestivum* L.) (Mason et al. 2010; Pinto et al. 2010) and rice (*Oryza sativa* L.) (Jagadish et al. 2010; Ye et al. 2012). In case of legume crops, QTLs associated with heat tolerance have been detected in chickpea and cowpea (Lucas et al. 2013; Paul et al. 2018). In case of pea with the use of next-generation sequencing technology and high-density genetic maps (Leonforte et al. 2013; Duarte et al. 2014; Sindhu et al. 2014), identification of QTLs linked to heat tolerance traits has become possible. Recently, Huang et al. (2017) used a RIL populations (107) developed from the cross of dry pea cultivars CDC Centennial and CDC Sage and developed a genetic linkage map consisting of 1024 loci with a total coverage of 1702 cM using SNP markers. Ten QTLs were found constantly over more than one environment, five for flowering traits and five for yield component traits. A stable QTL at Linkage Group 6b for days to flowering was detected over four environments. The QTLs for flowering duration, TSW and reproductive node number were different

between normal and late seeding, which implies that different mechanisms were involved under the contrasting environments.

#### 10.5.4.2 Drought Stress

Drought or water stress is an imperative environmental limitation that reduces quality and quantity of the yield (Boyer 1982; Ali et al. 1994). The reduction in grain yield due to moisture stress is reported 25% under field conditions (Sánchez et al. 1998). Water stress causes reduction in plant growth rate, stem elongation, leaf expansion and stomatal movements (Hsiao 1973). Furthermore, it causes changes in a number of physiological and biochemical processes governing plant growth and productivity (Daie 1988). Previously, it was reported by many researcher that peas are more sensitive to moisture stress during flowering and pod filling stage than the vegetative stage (Salter 1962, 1963; Maurer et al. 1968; Pumphrey and Schwanke 1974). Later on it was found that the timing of water stress is less important than the actual intensity of the deficit (Zain et al. 1983; Jamieson et al. 1984; Martin and Jamieson 1996). The timing of water stress does not influence the decline in pea grain yield but affects the total dry matter (DM) production. If moisture stress occurred before flowering, the total DM is reduced more than if it occurred after flowering. However, this total yield reduction is completely compensated by an increase in individual seed weight and consequently an increased harvest index (Martin and Jamieson 1996). The shoot-to-root ratio of drought-resistant cultivars remains significantly smaller than the sensitive plants in both control and drought treatments (Grzesiak et al. 1997). In case of plant type, the dwarf types have more drought resistant than tall type (Iwaya-Inoue et al. 2003).

Earlier, the semi-leafless type were considered more tolerant to water stress than conventional leafy- type varieties and it was supposed that the reduced leaf area of the semi-leafless varieties is the main factor (Semere and Froud Williams 2001). But, Gonzalez et al. (2001) examined the background of phenomena and noticed that total leaf area and transpiration rate per plant are not significantly different in both plant types. In addition, osmolarity at tissue level is similar among different leaf structure, whereas at the epidermal vacuole level, tendrils of the semi-leafless have a higher osmolarity than conventional plant type of pea. On the semi-leafless plants, the tendrils are about 40% of the total leaf; thus, its more efficient osmotic adjustment might be involved in improving water use efficiency under water stress (Gonzalez et al. 2001). Nevertheless, under water stress only, stipules of semi-leafless pea plants exhibited significantly better ability to boost osmolarity by accumulation of potassium, magnesium and chloride as compared to other leaf structures (Gonzalez et al. 2002). The Epicuticular wax load of cultivars increased significantly under drought conditions, and it is supported by increased residual transpiration rate (Sánchez et al. 2001). In drought condition, the roots of field pea go deeper in the soil than those under irrigated conditions (Benjamin and Nielsen 2006). However, osmotic stress induced by PEG 6000 resulted in shortening of primary root and increase of lateral root number (Kolbert et al. 2008).

Drought stress negatively affects the number and distribution of seeds developed on the basal phytomers of drought-stressed pea plants than on control plants (Guilioni et al. 2003). If moisture stress appears 1 week after development of first pods, then it leads to 79% reduction in number of seeds than the controls (De Sousa-Majer et al. 2004). According to Iturbe-Ormaetxe et al. (1998), severe water stress almost completely inhibited photosynthesis and damaged the photosynthetic system. Net photosynthesis was also decreased by water stress during the stress period. The relationship between total seed numbers and plant growth rate during critical period for seed set suggests that pea can adjust the number of reproductive sinks in a balance with assimilate availability in the plant (Guilioni et al. 2003). Although yield was reduced when drought stress exists during flowering and pod filling, the size and distribution of seeds are not affected constantly (Sorensen et al. 2003). The plant height and leaf area are not influenced significantly, but the drought stress decreased the fresh and dry weight of the pea and especially the relative leaf water content. The decrease in relative leaf water content is the main factor in reduced growth in drought-treated plants (Alexieva et al. 2001).

In moisture stress conditions, pea demonstrated major reduction in photosynthesis (78%), transpiration (83%) and glycolate oxidase activity (44%) and minor reduction in the chlorophyll a, carotenoids and soluble protein content (Moran et al. 1994) which might lead to reduction in various morphological traits and overall grain yield. The chlorophyll content to some extent increased, while the amounts of anthocyanins were not affected in water-stressed pea plants. The soluble phenols in leaves increased noticeably under drought stress (Alexieva et al. 2001). Water stress led to full disruption of the chiral macroaggregates of the light harvesting chlorophyll a/b pigment-protein complexes (LHCII<sub>s</sub>) measured by circularly polarized chlorophyll luminescence (CPL) in detached pea leaves (Gussakovskiy et al. 2002). Sucrose content of seeds is also increased by water stress (Sorensen et al. 2003). Contrarily, recently it has been reported that chlorophyll and protein contents in leaves decreased significantly with increased water stress, while the proline and malondialdehyde (MDA) contents elevated as a result of water shortage in pea leaves. Drought stress noticeably improved the activities of superoxide dismutase, catalase and peroxidase but slightly changed the activity of ascorbate peroxidase (Karatas et al. 2012).

The capability of plant to cope up with moisture stress condition determines its yield potential in a specific environment. There are mainly three strategies, i.e. escape, avoidance and tolerance of crops to sustain in moisture restricted conditions (Turner et al. 2001). Of these strategies, the initial two stand firm against stresses, while in third crop it has to survive with sizeable loss in productivity. Given approaches can be used in breeding programme to develop genotypes that would perform well under limited water conditions. The avoidance by escape approach is mainly based on earliness in terms of flowering and maturity, and therefore, it is the first preference of breeders. Because early flowering is often associated with early maturity, early flowering-early maturing crops cannot respond to higher moisture environments, and the yield performance of early-flowering genotypes can be low (Khan et al. 1996). Dry pea can perform well under water stress conditions if the



crop flowers early and pod filling occurs when plant water status is adequate (drought escape mechanism); accordingly, development of genotypes with vigorous early growth, flowering and pod set is necessary (Khan et al. 1996). Therefore, greater plasticity in phenology with early flowering would be always helpful (Turner et al. 2001). The pea breeding programme in many countries is presently selecting more stress-resistant cultivars with high yield potential by earliness and prolonged flowering duration. The drought avoidance tactic is delayed water loss using stomatal conductance, leaf area and any non-transpirational water loss from leaves. Leaf area is a significant factor due to extensive adaptation of the *afila* trait. The semi-leafless trait has many advantages in water-deficit situations owing to reduced leaf area (Rodríguez Maribona et al. 1990, 1992; Sánchez et al. 2001). Semi-leafless genotypes are enabled to maintain stomatal conductance for long time in water stress, maintain canopy temperature low and yield more compared to the normal leafed genotype under water stress condition (Alvino and Leone 1993). Genetic diversity exists for root architecture and water uptake ability (Armstrong et al. 1994; Thorup Kristensen 1998), but none of the programme has selected superior genotypes based on rooting. The increased ABA production was a preferred feature in the mid-1980s and successful in maize and wheat, which is related with stomatal control in stress and results in high yield, but only in some environments (Read et al. 1991). Thus, breeder must be cautious when selecting for improved ABA. Association between yield performance under water stress and osmoregulation ability of pea genotypes has been proved (Neumann and Aremu 1991; Rodríguez Maribona et al. 1992). The relationship between growth and osmotic adjustment and turgor maintenance was observed at seedlings stage under water stress induced by 46 mM polyethylene glycol (PEG) 6000 (Sanchez et al. 2004). The assessment of turgor maintenance at the early stages of development could be used to recognize drought-tolerant genotypes (Sanchez et al. 2004).

In dry pea, grain numbers are most decisive, and crop has maximum sensitivity to stress in the period between the start of flowering and the beginning of seed filling (Guilioni et al. 2003; Jeuffroy et al. 2010; Lecoeur and Guilioni 2010; Sadras et al. 2012). Overall, water stress in pea crop reduced plant height, internode length and leaflets size. The canopy colour changes to pale green since drought reduces nitrogen fixation or uptake. Tips of leaflets can die, flower buds and flowers may abort, and the life cycle is shortened resulting in fewer pods with fewer seeds (Rubiales et al. 2019). Therefore, development of new varieties with wider adaptation ability including drought tolerance is the prime endeavour of pea breeding programme (Abd-El Moneim et al. 1990). Drought tolerance is a multifaceted phenomenon in which different adaptations mechanism are involved (Sánchez et al. 2001); thus, it is quintessential to reveal mechanisms responsible towards drought tolerance and enhancement of crops performance in water stress situations. The use of molecular markers for the indirect selection of breeding lines reduces the time required for selection process compared to direct screening under greenhouse and field conditions (Dita et al. 2006). So far none of the study has been addressed the genetics of adaptation to drought in pea or reported QTLs for this trait. Therefore, recently Iglesias-Garcia et al. (2015) reported the quantitative genetics of drought adaptation

in pea and identify the genomic regions controlling the trait. They assessed drought symptoms and relative water content in soil (RWCS) and leaves (RWCL) in a RIL population. They identified 10 quantitative trait loci (QTLs) associated with the traits accounted individually between 9% and 33% of the phenotypic variation depending on the variable ascertained and altogether between 20% and 57%. A set of reproducible markers linked to these QTLs (*A6*, *AA175*, *AC74*, *AD57*, *AB141*, *AB64*, *Psbox2*, *PsAAP2-SNP4* and *DipeptIV-SNPI*) has been dissected. The SSR marker associated with the drought adaptation QTLs could be useful for MAS in drought adaptation breeding programmes.

### 10.5.4.3 Frost Stress

Frost stress is one of the major abiotic stresses causing a significant problem at vegetative and reproductive stage in pea (Shafiq et al. 2012; Liu et al. 2017). In temperate environment, frost during winter or early spring can severely damage or kill seedlings (Swensen and Murray 1983; Badaruddin and Meyer 2001; Meyer and Badaruddin 2001; Stoddard et al. 2006). Frost is the situation in which temperature goes abruptly below 0 °C during the vegetative and reproductive stage according to planting time, and this shock is usually experienced with low overnight temperatures that last between 2 and 8 h before the cold acclimation of crop (Rubiales et al. 2019). Frost damage cause permanent injury such as destruction of cell membrane system or loss of photosynthetically active tissue when plants are not acclimated (Chen et al. 2004; Menon et al. 2015; Liu et al. 2017). In cold acclimation, crop plants developed an adaptation mechanism to withstand cold which is induced by low, non-freezing temperatures (Levitt 1980; Xin and Browse 2000). Severe radiant frost is a hazard during reproductive stages, causing ice formation within plant cells or tissues (Ridge and Pye 1985). Frost at reproductive stage can damage or kill buds, flowers and pods and can reduce seed weight which leads to overall reduction in grain yield (Ridge and Pye 1985). Under wet conditions, physical frost damage on plants can promote infection by *Pseudomonas syringae* pv. *pisi*, the causal agent of bacterial blight (Knott and Belcher 1998). The frost tolerant of field pea at the vegetative stage decrease gradually with increasing age (Badaruddin and Meyer 2001; Meyer and Badaruddin 2001), and the sensitivity towards frost increases after flower initiation (Lejeune-Henaut et al. 1999).

Genetic variation has been reported for frost tolerance in dry pea for seedling (Bourion et al. 2003), vegetative stage (Lejeune-Henaut et al. 2008) and reproductive stage (Shafiq et al. 2012). Shafiq et al. (2012) identified five accessions ATC 104, ATC 377, ATC 968, ATC 3992 and ATC 4204 originated at different countries, which showed the highest frost tolerance at flowering stage with the production of least numbers of abnormal seeds. Dry pea is exhibited moderate freezing tolerance with LT50 (temperature that kills 50% of seedlings) of -4.5 °C in comparison to forage legumes (Meyer and Badaruddin 2001), while some winter hardy varieties of pea are found to be able to adapt to a temperature range between -8 and -12 °C (Homer and Sahin 2016; Auld et al. 1983). The evaluation was also done for winter

hardiness in a set of 58 accessions of pea germplasm under both field and laboratory conditions in Turkey and identified genotypes with differential survival at  $-8\text{ }^{\circ}\text{C}$  among which the higher level of winter hardiness was selected for future cultivar development (Homer and Sahin 2016). Recently, a large-scale evaluation of 3672 pea germplasm for cold tolerance was executed in the field condition in China and found that genotypes from winter production regions showed a higher level of cold tolerance than those from spring production regions and identified a collection of genetic resources for cold tolerance of pea in China (Zhang et al. 2016). Selecting frost-resistant genotypes is possible in controlled conditions up to  $-5\text{ }^{\circ}\text{C}$  (Shafiq et al. 2012) and in the field under naturally occurring radiation frost (Davies and Pham 2017).

The frost-tolerant accessions identified in these studies may be useful as parents for developing resistant cultivar for frost and mapping population for identification and tagging of candidate gene for frost tolerance, since understanding of the genetic nature of frost tolerance is a prerequisite for the development of frost-tolerant pea cultivars. In addition, breeding winter cultivars requires the combination of freezing tolerance as well as high seed productivity and quality. The flowering locus *Hr* is suspected to influence winter frost tolerance in pea by delayed floral initiation until the main winter freezing periods over (Avia et al. 2013; Dhillon et al. 2010). In pea, Lejeune-Henaut et al. (2008) reported six QTL region referred to as *WFD 1.1* to *WFD 6.1*, among which three (*WFD 3.1*, *WFD 5.1* and *WFD 6.1*) are steady among the different experimental conditions, confirming oligogenic determinism of frost tolerance in pea. A major QTL of pea frost tolerance on LGIII is located in the vicinity of the *Hr* locus. *Hr* is a gene controlling plant response to photoperiod (Weller et al. 2012). This gene is an essential component of frost avoidance, since it delays the vegetative to reproductive stage transition until longer days, when the risk of frost occurrence is lower. The co-locations between WFD QTL and QTL for physiological traits have been also discovered on LGV and VI (Dumont et al. 2009). Klein et al. (2014) also confirmed the quantitative inheritance of frost tolerance and detected a total of 161 QTLs which explained 9–71% of the phenotypic variation across the six environments for all traits studied. Two clusters of QTL mapped on the linkage groups III and one cluster on LGVI revealed the genetic links between phenology, morphology, yield-related traits and frost tolerance in winter pea. QTL clusters on LGIII highlighted major developmental gene loci (*Hrand Le*), and the QTL cluster on LGVI explained up to 71% of the winter frost damage variation. This suggests that a specific architecture and flowering ideotype defines frost tolerance in winter pea. However, two reliable frost tolerant QTL on LGV were detected, and these are independent of phenology and morphology traits, showing that different protective mechanisms are involved in frost tolerance. These results suggest that frost tolerance can be bred independently to seed productivity and quality. Most recently, Liu et al. (2017) performed marker-trait association analysis for frost tolerance with 267 informative SSR markers and identified 16 accessions as the most winter-hardy based on their ability to survive. Population structure analysis revealed two subpopulations plus some admixtures in the 672 accessions. Association analysis detected seven markers that repeatedly had associations with frost tolerance in at

least two different environments with two different statistical models. In addition, one of the markers is the functional marker EST1109 on LG VI which is supposed to co-segregate with a gene involved in the metabolism of glycoproteins in response to chilling stress and may provide a novel mechanism of frost tolerance in pea. These winter-hardy germplasms and frost tolerance-associated markers will play a vital role in marker-assisted breeding for winter-hardy pea cultivar. The consistently reported QTLs/marker can be used as interesting targets for marker-assisted selection.

## 10.6 Future Perspectives

Notwithstanding considerable efforts have been made to improve its productivity; the average productivity of this crop at global level is now to the tune of 1.9 tonnes/ha which is quite low as compared to other leading countries likely the Netherlands, Denmark, Belgium, Ireland, Germany, France and Canada. In dry pea, breeders around the world have been focused largely on three traits, viz. tendril (*afila*), dwarf plant type and powdery mildew resistance. These three traits are being extensively used in the breeding programme, which has resulted in the development of a number of high-yielding varieties resistant to powdery mildew with diverse plant type. In spite of that, during the last five decades, the yield gain is just 15.3 kg/ha/year in dry pea at global level, which is much lower than other crops, indicating that least attention was paid towards pea research. On the contrary, the yield gain in Canada is 2.0% which is greater than the yield gain in most of the crops at global level witnessed large investment in pea breeding programme over the years (Rubiales et al. 2019). Therefore, there are few more areas of interest that need greater attention in future and are discussed below.

1. *Multiple disease resistance*: Dry pea is vulnerable towards different biotic stresses such as powdery mildew, rust, ascochyta blight, *Fusarium* root rot, *Fusarium* wilt and *Aphanomyces* root rot which have increased over the years. Therefore, it is urgently required to incorporate multiple diseases resistance (region-specific) in a single variety in future dry pea breeding programme to increase the productivity.
2. *Better standing ability*: The stem of pea plant is very weak and coupled with huge mass of foliage on the upper side and accumulation of massive pods which leads to lodging as the lean stem is unable to hold it in upright position. Hence, any heritable system that can make the base of pea plant anatomically strong and thick enough, which can keep plant standing erect till full maturity, will undoubtedly boost its yield potential.
3. *New uses*: The dry pea crop in some parts at global level has a limited direct consumption pattern, and the uses of grain are not diversified. Therefore, new uses of dry pea have to be found if the popularity of this crop has to increase.

4. *Multi-purpose*: Another aspect of pea breeding could be to initiate breeding programmes for multipurpose (food-feed-fodder). There is no doubt that in addition to its use as protein source for human being, the demand for cattle/poultry feeds and fodder will increase manifold in this country. A cheap pulse like dry pea could play a crucial role in such a situation.
5. *Earliness*: Nowadays, earliness is becoming another trait of economic importance in every crop; through that, the productivity per unit time and per unit area can be increased. The early varieties with dwarf semi-leafless type plant can be planted with higher crop density and good standing ability. Perhaps this would be the most ideal situation to maximize yields not only per unit time but also per unit area.
6. *Abiotic stress tolerance*: The major abiotic stresses which are now become serious issue in sustainable production of dry pea under climate change scenario are high temperature, drought and frost (Rubiales et al. 2019). Therefore, there is urgent need to develop abiotic stress tolerance varieties with high yield potential to get more production of field pea.
7. In future the extensive utilization of similar kind of parent should be avoided in hybridization programme and needs to include diverse parents in dry pea breeding programme to develop new plant types with high levels of resistance to biotic and abiotic stresses, and earliness, thus, dry pea would be adapted better to the changing climatic scenario. The value added dry pea varieties, i.e. low flatulence, high iron and zinc are the quintessential need of future to popularize this crop. Thus, the future needs of dry pea breeding will be to develop lodging resistance, early maturing, biofortification, heat, drought and frost tolerance and disease-free varieties with yellow and green cotyledon (for human consumption) as well as pigmented and mottled seed coat (for feed and fodder purpose). Furthermore, most importantly, major/minor genes or QTLs have been identified responsible for different traits including important biotic and abiotic stresses. It would be advisable to concentrate further on large-scale high-throughput screening of germplasm for identification of genes/QTLs and their tightly linked markers for various targeted traits with high precision using different advance mapping populations. Further, the introgression of these resistant sources in good genetic agronomic background should be done with the help of marker-assisted selection to accelerate field pea breeding programme efficiently and more precisely. It is believed that conventional breeding approaches will remain the mainstay in combating these stresses. However, new tools of genomic selection, genome editing, gene mapping, gene cloning and genetic transformation offer opportunities to create new gene combinations to overcome losses due to biotic and abiotic stresses.

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