Chapter 3
Development of Biotic Stress Resistant Pea in the Post-genomics Era

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Abstract Pea (*Pisum sativum* L.) is an annual pulse crop which is eaten fresh and dried form around the world. It is widely accepted as a culinary best due to its protein rich nature. Pea crop is devastated due to various abiotic and biotic factors, which sometimes leads to complete crop loss worldwide. Biotic stresses in pea are mainly characterized by pathogens like virus, fungus, bacteria, insect pests, and nematodes. Infestation of insect pests not only damages the pea crop but also acts as vectors to spread viruses. These pathogens cause severe productivity loss if proper control measures and integrated disease management (IDM) strategies are not implemented. Various measures like cultural, chemical and biocontrol methods help reduce the crop damage. Severe damage by these pathogens leads to more than 80% crop loss in pea. Utilization of the available genetic resource for resistant sources from the *Pisum* genera will make the introgression of novel traits to develop disease resistant lines. It mainly includes primary, secondary, and tertiary gene pool sources which

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could be included for developing lines resistant to various biotic stresses. Classical genetics and conventional breeding techniques have doubled the pea productivity during the last six decades. Various molecular markers like SSR, STMS and SNP have been developed in pea for various biotic stresses which have led to marker assisted breeding. QTLs for various fungal, bacterial and insect resistance have been detected though mapping studies in pea. Whole genome sequencing of pea has been accomplished and this has opened a plethora of opportunities to carry out genomics-assisted breeding for developing resistant varieties against various pests and diseases in pea. Functional genomics techniques by reverse genetics approach like TILLING by sequencing (TbyS) has increased the relevance of development of non-GMO by utilizing conventional mutation breeding techniques along with next generation sequencing (NGS) technologies for obtaining variability in pea germplasm. Development of transgenics has been done in pea by *Agrobacterium* mediated transformation techniques for insect and fungal resistance. Gene editing techniques with CRISPR-Cas9 has been used in pea for precisely editing genes of importance for developing resistant lines for various biotic stresses. Bioinformatics tools with the development of various databases have increased the knowledge of genomics, proteomics and metabolomics in pea for biotic stresses. With the advent of modern tools like gene editing the conservation of wild type and landraces has raised concern with regulatory framework drafted in different countries. A combined effort from the conventional breeding with utilizing the modern biotech tools along with nanotechnology and speed breeding will help molecular breeders to design climate resilient pea varieties with resistance to biotic stress.

Keywords Biotic stress · *Pisum sativum* L. · Fungus · Virus · Insects · Nematodes · Molecular breeding · Resistance genes · QTL

3.1 Introduction

Pisum sativum L. ssp. *sativum*, commonly known as 'pea' or 'garden pea', is an annual, cold-season legume crop which belongs to Family the Fabaceae (Elzebroek and Wind 2008; Mahajan et al. 2018). There are mainly two types of peas grown—fresh green peas and dry peas. Green peas are mainly produced in China, India, and USA, whereas in case of dry peas, majority of the production happens in Canada, Russia, China, USA and India (Anonymous 2018). Pea is one of the major legumes consumed in both fresh and dry forms. It is also among the top five pulses in the world, i.e., dry beans (32%), chick pea (17%), peas (14.6%), cowpea (9%) and lentils (6%) (Rawal and Navarro 2019).

Peas have very high nutritional value with typically carbohydrates, proteins, minerals (Iron and Potassium) vitamins (Thiamine, Pantothenic acid, Folate) and fibers (USDA 2019). Culinary use of peas, mainly from fresh garden peas in various dishes throughout the world shows the wide dietary acceptance as nutritious food.

In fact, the dry pea production has doubled during the past six decades due to rising demand for consumption (Rawal and Navarro 2019).

Global climate change scenario shows greenhouse house gas (GHG) emissions and land use by dairy beef are about 36 and 6 times greater than peas (Poore and Nemecek 2018). This shows the relevance of pulse crops like peas in the future climates with challenges like global warming, rising sea levels, new virulent strains of viruses, fungus and bacteria in crops.

The impact of biotic and abiotic stresses on pea yield has been drastic; the data show that there has been a decline in both yield and area under pea cultivation in France. During 1990–2015, the cultivated area of pea has declined from a peak of more than 700,000 ha to 1,000,000 ha. In fact the total pea productivity has declined from 55 q/ha in 1999 to 38 q/ha in 2015 (Bénézit et al. 2017). This has not been a local feature, but such drastic effects due to climate change have been observed in other countries as well. The overall global production of dry pea has tremendously increased from 10.7 to 13.5 M tons during 2000 to 2018, whereas in case of green pea 12.2 to 21.2 M tons in the same period (FAOSTAT 2018) (Fig. 3.1). These have been mainly because of the adoption of improved varieties with productivity management practices.

Another major challenge is the increasing population, which is putting pressure on both farm and the field with more and more land getting used for housing, and fertile

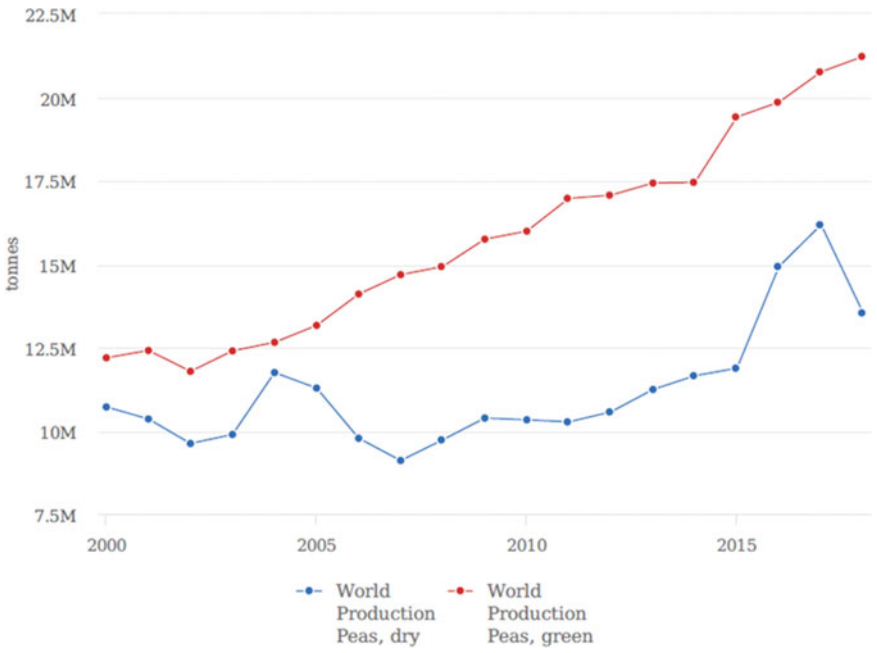

Fig. 3.1 World pea production from 2000 to 2018; dry pea (blue dotted lines) and green pea (red dotted lines)

fields turning infertile due to continuous use of pesticides and chemical fertilizers. By the end of 2050 the world population will reach about 9.7 billion and by the end of 2100 to 10.9 billion (World Population Prospects 2019). Challenges like climate change and population explosion will exert mounting pressure on governments across the world to work towards sustainable crop production. Abiotic and biotic stresses in crops have put a lot of pressure on the productivity of crops thereby leading to nonfulfillment of sustainable development goals (mainly in case of Goal 13—Climate action) laid down by the United Nations (UNDP 2017).

Changes in the environmental conditions lead to severe pressure on crop productivity. Disease and insect pest incidence increases tremendously due to climate change. Biotic stresses lead to complete loss of crops when combined with abiotic stresses like drought, heat, salinity, waterlogging, etc. Traditional breeding has been utilized for biotic stresses by crossing wild species with biotic stress resistance with elite parents with quality traits like better yield, nutritive traits, etc. But due to continuous breeding and development of new pests and diseases the resistance build up is broken. Utilizing modern biotechnological tools like marker assisted selection (MAS), transgenic development, high through-put 'omics' approaches and gene editing can help to overcome the drawbacks of conventional breeding in pea. Integrated pest and disease management (IPDM) strategy with biotechnological interventions for improving various pea cultivars will help growers to maximize pea productivity (Smýkal et al. 2012).

Modern biotechnological tools are helping the breeders to design new varieties which will be more resilient towards climate change challenges and various biotic stresses. This chapter will give an overall enumeration of various fungi, bacteria, virus, insect pests and nematodes infecting and infesting pea. Various aspects included like genetic resources for resistance genes, mapping studies, diversity analysis in germplasm, and strategies for development of improved varieties of pea in the future. Novel biotech tools to develop resistant pea lines, utilizing artificial intelligence for precision farming in pea have been discussed.

3.2 Biotic Stresses

Biotic stress is a condition created by various microorganisms and pests including bacteria, viruses, fungi and insects, infecting and infesting crops leading to complete crop loss in some cases. Biotic and abiotic stress together aggravate the crop damage and will have a grave impact towards food security in the future. Crop productivity of pea is impacted by infections by various diseases and pests, which mainly lead to abnormal growth, non-productive crop and permanent damage. In this section we discuss in detail regarding various insect pests and diseases affecting pea crop with details of taxonomy, races, isolates, biotypes of pathogens, insect pests and nematodes. Further the stages and extent of crop damage along with various methods of controlling the incidence and proper management by IPM strategies have been detailed.

3.2.1 Viruses

3.2.1.1 Main Groups of Viruses Affecting Pea

Garden pea is prone to a large number of vector transmitted viruses, leading to individual disease or in multiple combinations. There are three distinct groups of viruses infecting different pea species—first group with pea enation mosaic virus, second group with pea streak and red clover vein mosaic virus and the third group with bean yellow mosaic, clover yellow vein and pea seed borne mosaic virus (Zitter 1984).

***Pea Enation Mosaic Virus* (PEMV)**

Pea enation mosaic virus (PEMV) (Fig. 3.2a–c) generally infects pea in temperate part of the world, with unique features of symptoms and is spread by aphid (*Acrythosiphon pisum*). It also infects other legumes including broad bean, sweet pea, and *Medicago*. It is a single stranded RNA virus from family Luteoviridae. Main symptoms include mainly veinal enations in the abaxial side of leaves with chlorosis, necrotic lesions, and stipulated leaves with distortion in plants. PEMV infection leads to accumulation of abscisic acid and salicylic acid and have enhanced accumulation of nitrogen dioxide near the veins, which causes leaf enations (Kyseláková et al. 2013). Controlling PEMV infection in pea is mainly through control of aphid spread by spraying insecticides. Alternative hosts from legume family can be removed to check the spread of the disease (Zitter 1984).

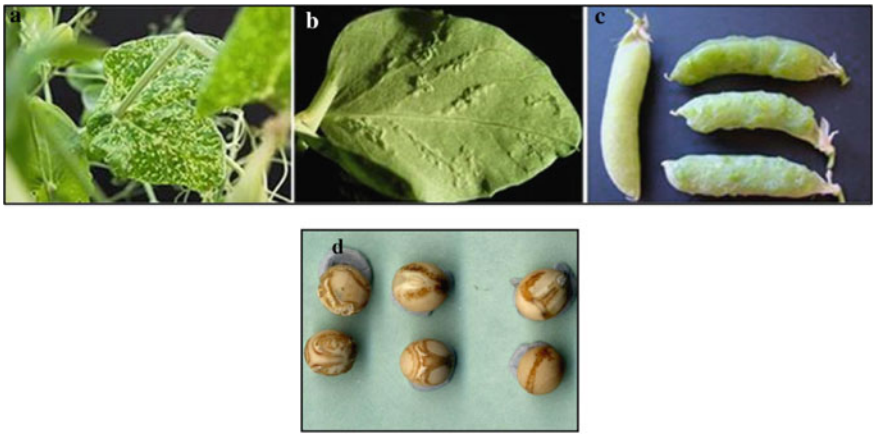

Fig. 3.2 *Pea enation mosaic virus* (PEMV) infection symptoms in pea (*Pisum sativum*). **a** Leaf flecks on leaves. **b** Enations in the abaxial side of leaf. **c** PEMV infected pea pods compared with healthy pod (left) (Porter 2014). **d** PSbMV infection in pea seeds showing symptoms of necrotic rings and line patterns (tennis-ball' like symptoms) on seed coat (https://www.agric.wa.gov.au/field-peas/pea-seed-borne-mosaic-virus-field-peas).

***Pea Seed-borne Mosaic Virus* (PSbMV)**

Field pea crops have been severely affected by *Pea seed-borne mosaic virus* (PSbMV) (Fig. 3.2d), which is generally a seed-borne and aphid-borne virus from family Potyviridae. It mainly spreads from infected field pea seed to healthy seedlings and further to the next generation and causes yield loss and defective seeds (tennis-ball like symptoms) (DPIRD GOWA 2018). Seed-born PSbMV infection in field pea causes yellow, stunted plants. When young plants are infected with PSbMV aphids, the symptoms of the leaf include slight moth and low rolling, and the affected plants show moderate inhibiting and delayed maturity and mild malformation. Infection observed in seeds includes seed discoloration, deformed pods and malformed terminal rosette, splitting of seed coats (Wunsch et al. 2014; DPIRD GOWA 2018).

***Bean Yellow Mosaic Virus* (BYMV)**

Bean yellow mosaic virus (BYMV) infects many leguminous plants like peas, red clover, and lupin. BYMV is a single-stranded, positive-sense, RNA (Group IV) virus from family Potyviridae. It is mainly found in most pea producing regions of the world and the main vector is the aphid**.** General symptoms are distinct dark and yellowish green areas on leaves and infected plants mostly have bright yellow spots which intensifies in color with plant age.

***Clover Yellow Vein Virus* (ClYVV)**

Clover yellow vein virus is from family Potyviridae and infects pea and other legumes generally transmitted by aphids (pea aphid). Symptoms of ClYVV disease in pea are intense vein chlorosis, white patches to extreme yellowing on leaves, accompanied by apical necrosis and early death. Plants appear to be stunted and deformed pods are observed during severe infections of ClYVV.

***Alfalfa Mosaic Virus* (AMV)**

Alfalfa mosaic virus (AMV) has damaging affect in pea and also other pulse crops like faba beans, lentils and chickpeas. AMV belongs to family Bromoviridae and it is transmitted from plant to plant through aphids. Damp conditions aggravates the spread of aphids whereby leading to increase in AMV infections in pea. Chlorosis and necrosis in new shoots are general symptoms of AMV infection. Older leaves show necrotic spots or streaks and generally plants are stunted as compared to healthy plants. Pods are malformed and seed set is poor thereby leading to lower production. Chemical control is not so effective to control AMV and pesticides for aphid control will indirectly help to lower the incidence of AMV. Other methods to control AMV are cultural methods like using healthy seeds, and proper weed management to contain the spread (Trebicki 2020a)**.**

***Cucumber Mosaic Virus* (CMV)**

Cucumber mosaic virus (CMV) causes severe disease in lupin, chickpea, lentil besides pea. CMV belongs to family Bromoviridae and is transmitted by more than 80 different aphid species (Trebicki 2020b).

3.2.1.2 Strategies for Viral Disease Control

Integrated disease management (IDM) strategies with integrating cultural, chemical and other control measures will facilitate to minimize the extent of crop damage in pea. Seed fractionation technique could be used as a phytosanitary method for lowering the levels of PSbMV infection in seeds and thereby lowering the spread to other pea crop (Congdon et al. 2017). Selection of tolerant and resistant pea lines helps to lower the incidence of virus. PSbMV resistant lines with recessive *eIF4e* gene have been developed from utilizing resistant source available in numerous pea accessions. These lines hold potential to improve productivity in pea (Smýkal et al. 2010). Resistance gene, *wlv* for *Bean yellow mosaic virus* (BYMV) and potyviruses were identified from pea genotypes, which are mapped to linkage group VI in pea. The *wlv* gene corresponds to the *sbm1* allele of *eIF4E* gene, which also confers resistance towards PSbMV in pea (Gao et al. 2004; Bruun-Rasmussen et al. 2007). Protein-wide analysis in pea PSbMV resistant (B99) and susceptible (Raman) lines showed that 116 proteins were differentially expressed in resistant lines. These data could be utilized for advancement of the development of specific gene markers for pea breeding programs (Cerna et al. 2017).

3.2.2 Fungi

3.2.2.1 Types of Fungi

Powdery Mildew *(Erysiphe pisi)*

Powdery mildew disease (Fig. 3.3a) caused by *Erysiphe pisi* is the major disease of pea. Morphological characterization of *E. pisi* done by scanning electron microscope revealed hyaline colored conidia, with shape varying from oblong (young) to cylindrical (matured) (Fondevilla et al., 2012; Parthasarathy 2017). Typical symptoms of powdery mildew in pea are white powdery spots on the upper surface of the leaves, stipules, stems and pods. Disease spreads quickly during warm dry days and cool night weather conditions (Beck and Mathew 2019).

Ascochyta Blight (*Aschochyta pinodes, A. pinodella, A. pisi, Phoma kooolunga*)

Ascochyta blight (Fig. 3.3b) is spread by a complex of *Ascochyta pinodes*, *Ascochyta pinodella*, *Ascochyta pisi*, and/or *Phomakoolunga*, which has devastating effects in field peas growing regions (Skoglund et al. 2011). IPM strategies for Ascochyta blight control includes mainly use of fungicides such as tebuconazole, boscalid, iprodione, carbendazim, and fludioxonil, which displayed more than 80% disease control efficacy under the recorded conditions (Liu et al. 2016). Other control strategies are use of biocontrol such as strains of *Bacillus* sp. and *Pantoeaagglomerans*, which were isolated from pea-related niches and had significantly reduced the severity of disease under greenhouse and field conditions (Jha et al. 2016; Liu et al. 2016).

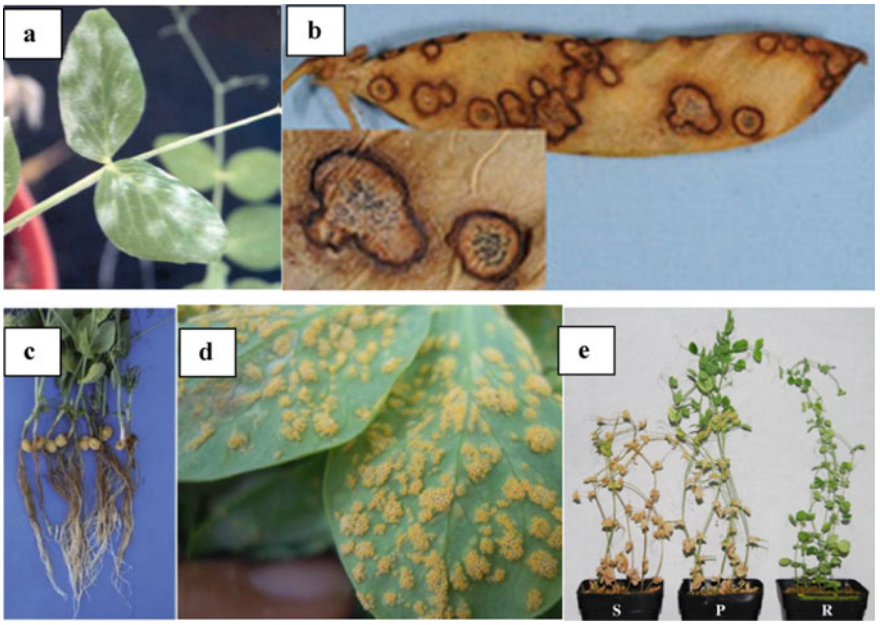

Fig. 3.3 **a** Powdery mildew in pea. **b** Ascochyta blight in pea pods. **c** Aphanomyces infection on pea pods with honey-brown dicoloration. **d** Pea Rust (Skoglund et al. 2011; Fondevilla and Rubiales 2012; Barilli et al. 2014; Castillejo et al. 2015; Wu et al. 2018). **e** Pea fusarium wilt Castillejo et al. (2015)

Root Rot *(Aphanomyces euteiches)*

Aphanomyces euteiches Drechs. causes damping off and root rot diseases (Fig. 3.3c) in pea and leads to more than 80% yield loss. It is a soil-borne pathogen which survives in soil for many years and till now there has been no efficient chemical control available. To control this disease, it is recommended not to grow pea in fields where legumes or pea has been grown earlier. The genus *Aphanomyces* comes under the order Saprolegniales and family Saprolegniaceaes. l. or Leptolegniaceae. Generally root rot is quite widespread in legumes like alfalfa, snap bean, clover and vetch including field pea (Gaulin et al. 2007; Wu et al. 2018). Glossary of information has been documented in European Union: Grain legume Integrated project areas http://www.indexfungorum.orr/Names.asp and http://www.medicago.org.

Pea Rust *(Uromyces viciae-fabae* Pers. de Bary*)*

Pea rust (Fig. 3.3d) is caused by *Uromyces pisi* and *U. viciae-fabae*, mostly at the reproductive stage of the crop during mid-spring season. In pea rust, severe infections lead to leaf and pod drop immaturely, as a result yield loss upto 30% is observed (Barilli et al. 2014). Resistance towards this pathogen is mostly partial and is influenced a lot by environmental conditions. *U. fabae* is an autoecious and heterothallic fungus, which forms all types of spores (pycniospores/ spermatiospores,

aeciospores, urediospores and telliospores) in pea (Das et al. 2019; Upadhyay et al. 2019). Common symptoms of pea rust are appearance of yellow aecia (powdered spots) under the leaf surface, with browning during severe infections (Chand et al. 2006; Das et al. 2019).

Fusarium wilt

Fusarium oxysporum f. sp. *pisi* causes Fusarium wilt (Fig. 3.3e) in pea with 30–40% yield loss. Generally, the symptoms are yellowing of leaves and stunted plant growth. There are various strains and races of *F. oxysporum*based on the virulence. It is a soil borne fungi which can even survive for more than 10 years in soil due to the presence of thick walled chlamydospores. Different cultural practices, use of resistant varieties and biological and chemical methods could be used to control Fusarium spread in pea fields (Castillejo et al. 2015; Gupta and Gupta 2019).

3.2.2.2 Management of Fungal Diseases

Management of powdery mildew is mainly by utilizing resistant varieties. Other method includes utilization of fungicides like treatments with carbendazim (0.1%), followed by Neem Seed Kernel Extract (NSKE) (5%) and nimbicidin (0.3%) (Abhishek and Simon 2017). Other management strategies are use of biocontrols such as mycolytic bacteria, mycophagous arthropods, and yeasts (Fondevilla and Rubiales 2012). Severe conditions of powdery mildew could be controlled by foliar sprays of *Trichoderma* or Karathane, while the former is more beneficial as compare to the toxic impact on health and environment of the latter (Maharjan et al. 2015). IPM strategies have to be strictly followed for control of this complex disease, chemical control up to 80% could be achieved by the use of fungicides like tebuconazole, boscalid, iprodione, carbendazim and fludioxinil are recommended (Liu et al. 2016).

Pea rust could be controlled by altering sowing dates of pea seeds during different seasons. Three different factors which affect the disease development are host, pathogen and environment. Crop rotation with other crops like mustard, wheat and linseed helps the containment of leaf rust in pea (Upadhyay et al. 2019). Due to the lack of durability to manage and wide host range in pea rust disease, chemical control is not recommended (Barilli et al. 2009; Das et al. 2019). Partial resistance to pea rust is available which shows reduced disease severity and low necrosis in host cells (Xue and Warkentin 2001; Chand et al. 2006; Barilli et al. 2009).

Resistant germplasm of pea for Fusarium wilt disease expressed differential proteins, which were taken from roots of susceptible, partially resistant and resistant varieties. This knowledge will help molecular breeders to utilize such resistant sources for developing better breeding materials (Castillejo et al. 2015).

3.2.3 Bacteria

Bacterial diseases are very common threat for major crop loss in legumes. Pea bacterial blight, bacterial pustule and bacterial wilt are more commonly found bacterial diseases in pea.

3.2.3.1 Pea Bacterial Blight (*Pseudomonas syringae* pv. *pisi*)

Pseudomonas syringae pv. *pisi* is the major causative agent for field pea bacterial blight. It is a gram-negative, aerobic, non-spore-forming rod shaped bacteria (Fig. 3.3a) (CABI 2019). Bacterial blight is most noticeable when necrotic patches arise inside the crop. Around the edge of the dead parts exhibit the characteristic water-soaked and the fan-shaped lesions of bacterial blight. In dry weather with occasional frost, symptoms on stems occur as elliptical water-soaked regions, which will be olive-green and eventually brown. Sometimes, these lesions circle the stem and can spread a few centimetres and infect the stem with both the stipulations and the leaflets. Severe infection of the stem may lead to plant death. Infection on the pod also shows the lesions on the pods which are normally sunken and become dark brown and shiny (Hollaway et al. 2007).

3.2.3.2 Pea Brown Spot (*Pseudomonas syringae* pv. *syringae*)

Pea brown spot is caused by *Pseudomonas syringae* pv. *syringae* which typically shows brownish spots on pea leaves and leaf sheath area and looks similar to infections of *P. syringae* pv. *pisi.* Bacteria can live on seed or pea trash, while *P. syringae* pv. *syringae* can live on a variety of host plants. Spread of bacterial blight is enhanced by rainfall, heavy precipitation, strong winds and cold temperatures (Victoria 2020). Acibenzolar-S-methyl (ASM) spray on plants significantly controls the bacterial blight disease (Akköprü 2020).

3.2.3.3 Bacterial Wilt Disease (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*)

Bacterial wilt and tan spot of dry beans (family Fabaceae), are caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, which, a gram positive bacteria, has also been reported to be infecting field pea (Fig. 3.3b). It is a common pea disease in USA, Brazil, Canada, eastern Australia, and Iran (Tegli 2011; Harveson et al. 2015; Harveson et al. 2015). Copper oxychloride, copper hydroxide, and copper sulphate are some of chemicals for controlling bacterial wilt by either field spray or seed treatment. Antimicrobial chemical like Kocide (copper hydroxide) and MasterCop (copper sulphate) are other anti-bacterial controls. Further, antibiotic

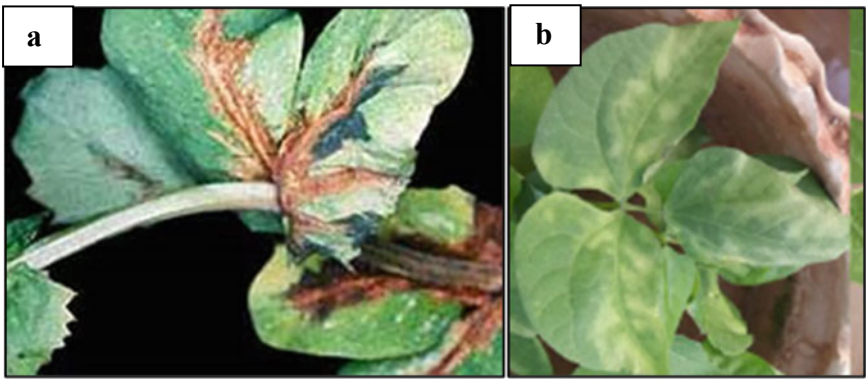

Fig. 3.4 **a** Bacterial blight infection in field pea. **b** Bacterial wilt in dry beans. https://agriculture.vic.gov.au/biosecurity/plant-diseases/grain-pulses-and-cereal-diseases/bacterial-blight-of-field-peas

seed treatment can reduce surface contamination of seeds. Slurry seed treatments using StreptomycinAgri-Strep 500 can give effective results on pea plants (Osdaghi et al. 2020) (Fig. 3.4).

3.2.4 Insect Pests

Insect-pests are biotic stresses which affects yield of pulses severely. It damages crop by chewing plant parts like leaves, stem, fruits and roots. They also act as carrier for virus, bacteria and fungi. They affect crop directly by damaging plant tissues or indirectly by damaging quality of the harvest and increased crop production cost (Bardner and Fletcher 1974). There are many pests which affect pea plants at different stages of growth and are as following.

3.2.4.1 Early Risk Pests

Pea and bean weevil (*Sitona lineatus*)

Pea weevil (Fig. 3.5a) belongs to family Curculionidae and genus *Sitona.* It causes damage when plants are small and growth is slow. Plants affected by weevil show 'U' shaped cut at the edges of leaves. Major damage is caused by larva nurturing on the root nodules (Processors and Growers Research Organisation—https://www.pgro.org/pests-diseases-peas/). Nitrogen availability for plant is highly reduced as *S. lineatus* larvae uses nitrogen fixing bacteria from root nodules for feed (Cárcamo et al. 2015, 2018).

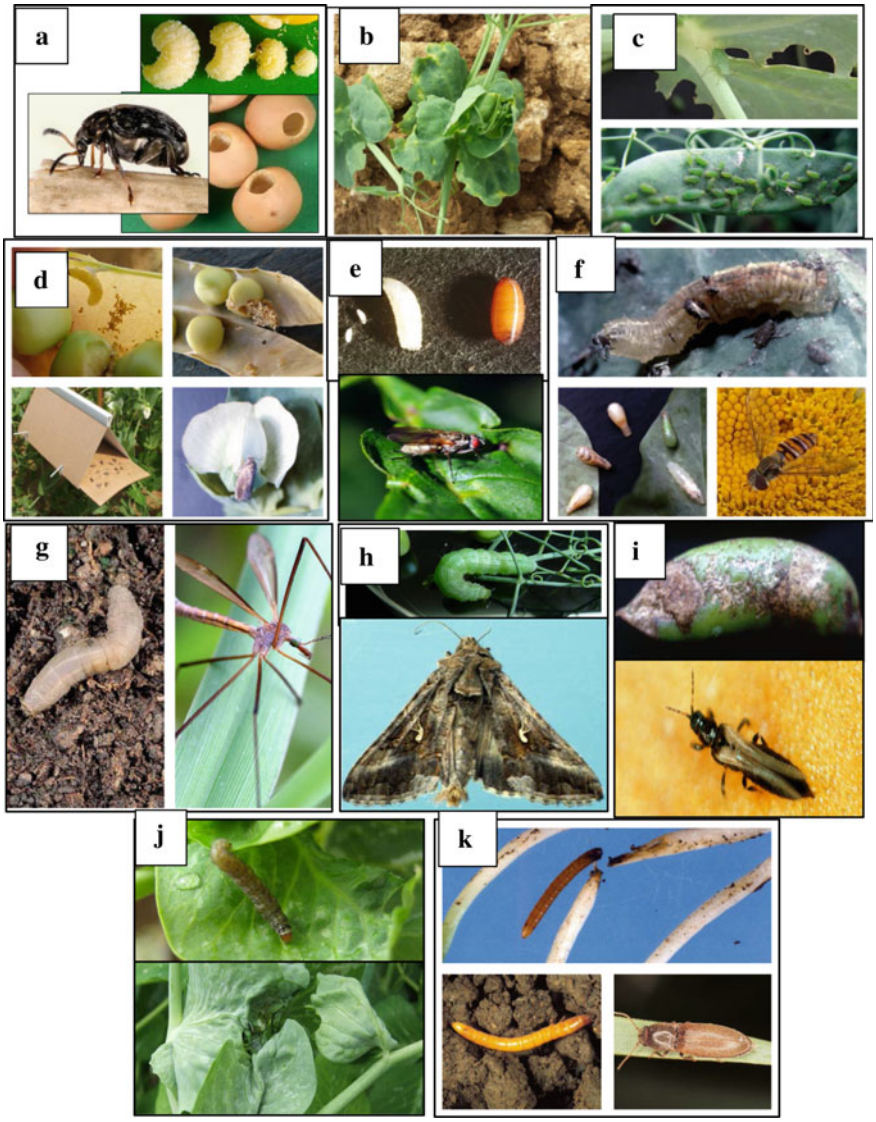

Fig. 3.5 Different pest infesting in pea. **a** Pea and bean weevil. **b** Field thrips. **c** Pea aphid. **d** Pea moth larvae. **e** Bean seed fly. **f** Hoverfly. **g** Leatherjacket. **h** Silver Y moth. **i** Thrips. **j** Tortrix moth and **k** Wireworm (Pea and Bean Crop Walkers' Guide 2018)

Field thrips (*Thrips angusticeps*)

Field thrips (Fig. 3.5b) belongs to family Thripidae and genus *Thrips*. It targets emerging plant leaf surface and make it thicker and wrinkled. Pale colored seedlings appeared (Processors and Growers Research Organisation—https://www.pgro.org/pests-diseases-peas/). *Thrips angusticeps*is considered as most dangerous pest on pea in parts of France and England (Pobozniak 2011).

3.2.4.2 Pre/Early Flower Pests

Pea aphid (*Acyrthosiphon pisum*)

Pea aphid (Fig. 3.5c) belongs to family Aphididae and genus *Acyrthosiphon*. Yield loss in pea is caused by large number of Aphids. It affects crop by acting as career for *Pea enation mosaic virus* (Processors and Growers Research Organisation—https://www.pgro.org/pests-diseases-peas/). Pea aphid *Acyrthosiphon pisum* causes crop damage of hundreds and millions of dollars every year. It is also used in laboratory. Many aphid populations became resistant from conventional pesticides (Sadeghi et al. 2009).

3.2.4.3 Late Flower/Early Pod Pests

Pea moth larvae (*Cydia nigricana*)

Pea moth (Fig. 3.5d) larvae belong to family Tortricidae and genus *Cydia*. Pea moth larva affects developing seeds in pods. It does not affect yield but reduce the quality of harvest. It reduces the value of harvest by reducing quality (Huusela-Veistola and Jauhiainen 2006).

3.2.4.4 Other Pests

Bean seed fly (*Delia platura*)

Bean seed fly (Fig. 3.5e) belongs to family Anthomyiidae and genus *Delia*. Flies put eggs on soil. Larvae from eggs attack seeds and seedlings during germination. It damages seeds, stems and upper root system. Damaged seeds cannot form seedlings (Valenciano et al. 2004).

Leatherjacket (*Tipula* spp.)

Leatherjacket (Fig. 3.5g) belongs to family Tipulidae. Larvae of crane flies are known as leatherjacket. It lives in soil and feeds on grassroots. During spring larvae feeds

on germinating seedlings and damage it. It damages stem at soil level. Presence of larvae in large number causes crop loss (Pea and Bean Crop Walkers' Guide 2018).

Silver Y moth (*Autographa gamma*)

Migratory moth *Autographa gamma* (silver Y moth), is a pest which affects wide range of crops including pea (Ingeborg Menzler-Hokkanen 2020). Silver Y moth (Fig. 3.5h) belongs to family Noctuidae and genus *Autographa*. It is a migratory pest which damages foliage and pods. Adult fly is having silver grey forewings with silver Y in the middle of each. Larvae vary from bright to dark green with white strips at sides and back (Pea and Bean Crop Walkers' Guide 2018).

Thrips (*Kakothrips pisivorus*)

Thrips (Fig. 3.5i) belong to family Thripidae and genus *Kakothrips*. Heavy presence of thrips in large pea production area is observed more in humid condition which damages the pods. Damage does not affect yield and pod quality but damaged pod shows silver and rutted surface (Pea and Bean Crop Walkers' Guide 2018).

Tortrix moth (*Cnephasia asseclana*)

Tortrix moth (Fig. 3.5j) belongs to family Tortricidae and genus *Cnephasia*. Tortrix feed on foliage but does not affect crop yield. It webs leaves together giving hooded appearance to plant. It leaves the crop before harvesting (Pea and Bean Crop Walkers' Guide 2018).

Wireworm (*Agriotes* spp.)

Soil dwelling pests causing high yield loss in various crops are known as wireworms (Barsics et al. 2013). Wireworm (Fig. 3.5k) belongs to family Elateridae. Click beetle larvae are known as wireworms. It is of yellow or brown color with smooth and rigid bodies. It affects on small area. It attacks on shoot and germinating seeds below ground level which fails to recover (Pea and Bean Crop Walkers' Guide 2018).

3.2.4.5 Beneficial Pests

Hoverfly (*Syrphidae*)

Hoverfly (Fig. 3.5f) belongs to family Syrphidae. Hoverflies benefits pea by predating aphids. It is present in infected crops. Larva and pupa contaminate vining peas during harvesting (Pea and Bean Crop Walkers' Guide 2018). Hoverfly is an efficient aphid-specific predator in natural eco-system which plays important role in population dynamics of their prey (Almohamad et al. 2007).

3.2.4.6 Integrated Pest Management Strategies for Managing Insects and Pests

Integrated pest management is very critical for optimum productivity, which includes cultural, biological and chemical control measures. Pea weevil is very active during spring and fall season. Cultural control mainly includes by keeping enough spacing between host and infested fields. Proper weeding and removal of infested plants are the best methods for temporary relief for the spread. Timely irrigation of pea field also helps to keep the pea weevil larval survival in check (Cárcamo et al. 2018). Pheromone traps are a useful technique which includes pheromone-baited pitfalls and ramp traps for capturing adults of pea weevil (Reddy et al. 2018). Main strategy to lower the aphid incidence in pea field is to clear out infested hosts. Utilization of aphid resistant pea lines could be beneficial for effective control to reduce production loss (https://ipmdata.ipmcenters.org/documents/pmsps/2016PulsePMSP_FINAL.pdf). Crop rotation is also an effective strategy for controlling *Thrips angusticeps* infestation in pea field. Resistant or tolerant varieties of pea could be grown in highly infested fields (https://www.plantwise.org/KnowledgeBank/datasheet/53727).

Chemical method includes synthetic pyrethroids spray on adult pea weevil before small pods are visible. Insecticides are effective on adults only. Border spray will control pea weevil from entering into crop. If heavy infection occurs, whole paddock shall be sprayed (https://www.agric.wa.gov.au/pest-insects/management-pea-weevil). Aphicides partially reduce the spreading of secondary infection by controlling *Acyrthosiphonpisum* (Pea aphid) (https://ipmdata.ipmcenters.org/documents/pmsps/2016PulsePMSP_FINAL.pdf). Chemical control of *Thrips angusticeps* in pea can be done by using insecticides pyrethroids and neonicotinoids. Treatments are mostly done during blooming stage and the same is repeated at 7 days interval (Pobożniak and Leśniak 2015).

Biocontrol methods with natural products and biotic agents also help to control pea insect pest infestation. Parasitoids, predators, entomopathogenic fungi, and entomopathogenic nematodes act as a biological control agent for *Sitona lineatus*. From laboratory experiments it is observed that some general predators also act on *Sitonalineatus*. It includes the large, adventive ground beetle, *Pterosticusmelanarius* (Illiger; Coleoptera: Carabidae) which eats *Sitonalineatus* adults and small species *Bembidionquadrimaculatum* (L.; Coleoptera: Carabidae) eats its eggs (Vankosky et al. 2011; Satyagopal et al. 2015). Some parasitoids species like *Hyperapostica* (Gyllenhal) Coleoptera: Curculionidae were released in North America for management of *Sitonalineatus*. *Anaphesdiana* (Girault; Hymenoptera: Mymaridae) is considered promising species to control *Sitona lineatus* which eats eggs of *Sitona* weevils which is established in the eastern United States (Cárcamo et al. 2018). Use of *Beauveria bassiana* (Mycotrol Ois listed by the Organic Materials Review Institute (OMRI) to control *Acyrthosiphon pisum* (O'Neal 2017). Implementation of IPM strategies will make sure the production loss due to insect pest is reduced to minimal. Timely decisions to counter the spread of the insects is the only way to efficiently implement the IPM strategies in pea crop production.

3.2.5 Nematodes

Nematodes cause severe problem for pea crop productivity with tremendous revenue loss. Nematodes can interact with other organisms like fungi, bacteria and viruses to form a disease complex (Singh et al. 2013). Nematodes mainly attack the root system leading to lowering of nutrient uptake and drastic decrease in plant growth and yield. More than 100 species of plant parasitic nematodes are published to be related with pea and other legumes, of which root-knot nematode (*Meloidogyne* spp.), lesion nematode (*Pratylenchus*spp.), reniform nematode (*Rotylenchulus* spp.), cyst nematode (*Heterodera* spp.), spiral nematode (*Helicotylenchus* spp.), stunt nematode (*Tylenchorhynchus* spp.) and lance nematode (*Hoplolaimus* spp.) are common. Needle nematode (*Longidorus* spp.) and dagger nematode (*Xiphinema* spp.) also act as vectors in transmitting viruses to the crops (Askary 2017). Most pea varieties are susceptible to root-knot nematode, which is the major cause of crop loss, so resistant pea lines need to be used for production in areas where the nematode infection is severe (Youssef and El-Nagdi 199). Nematode infection can be confusing to be diagnosed as it shows similar symptoms with fungal diseases and nutritional disorders (Askary 2015). Large, circular patches of stunted pea plants in field are often linked with nematode-infested areas in pea fields. Another common symptom is presence of only primary roots and no secondary roots in nematode infected pea plants. Severe nematode infection shows chlorotic leaves which discolors from the base to the top of the plant (Northwest et al. 2012).

3.2.6 Nematode Management Practices

Cultural methods used to minimalize root-knot nematode damage is soil tilling for two to three times and crop rotation. Optimal soil pH, fertility and soil moisture requirements for plant growth would reduce nematode pressures. Avoiding alternative hosts like faba bean crop near pea field will be good to keep a check on nematode spread (Inglis 1998). Common nematicides used for nematode control are methyl bromide, methyl isothiocyanate and chloropicrin treatment of the soil. Timely soil test from the pea field helps to know the rate of infection and for timely decision (PNW-VEG 2012). Intercropping of green manure crops such as sundan grass, sesame, rapeseed, white mustard and ryegrass in pea field helps to minimize the nematode population in general. Root galling of *M. incognita* in pulses could be reduced by a combined use of cow dung manure and egg shell powder and *Rhizobium* sp. during soil preparation before sowing (Rizvi et al. 2015).

3.3 Genetic Resources of Resistance Genes

Pisum sativum is a diploid species ($2n = 14$) and has a large genome size of 4.45 Gb, as compared to other legumes with smaller genome like *Medicago truncatula*, *Lotus japonicas* or *Glycine max* (Kreplak et al. 2019). About 82.5% of the genome is repetitive sequences as estimated after the complete pea genome sequencing. There are three distinct species of *Pisum* genus, first one is *Pisum sativum* subsp. *sativum* and wild subsp. *elaitus*, which is native in Europe and northwest Asia, second one is *Pisum fulvum* from middle-East Asia and the third one is *P. sativum* subsp. *abyssinicum* or *P. abyssinicum* from Ethiopia and Yemen (Smýkal et al. 2017; Trněný et al. 2018; Coyne et al. 2020).

3.3.1 Primary Gene Pool

Primary gene pool in pea predominantly includes wild pea *P. sativum* subsp. *elaitus* (var. *elaitus*, var. *brevipedunculatum* and var. *pumilo*) along with the cultivated garden pea (*P. sativum* subsp. *sativum*–var. *sativum* and var. *arvense*) (Zong et al. 2019). The intercrosses between the subspecies are difficult due to nuclear-cytoplasmic incompatibility which has hampered the gene flow in some cases (Bogdanova et al. 2014; Nováková et al. 2019; Coyne et al. 2020). But mostly the cross compatibility and rich genetic diversity have led to efficient use of the primary gene pool for genes imparting tolerance to both biotic and abiotic stresses (Zong et al. 2019).

Promising genotypes for pea weevil resistance with less damage were obtained by screening 602 pea accessions from Ethiopia. These germplasm lines from the primary gene pool of *P. sativum* subsp. *sativum* could be utilized in pea breeding program for obtaining enhanced resistance (Teshome et al. 2014). Expressed sequence tag (EST)—simple sequence repeat (SSR) marker analysis showed high differentiation among 46 *P. sativum* accessions and pathological screening of these accessions led to the identification of few less susceptible accessions towards pea weevil infestations (Teshome 2015).

Powdery mildew (resistance in pea has been characterized and markers have been developed with close link to *er1*, *er2* and *Er3* genes. These have been good candidate genes for conferring resistance and are widely utilized in pea breeding programs. As new pathotypes and strains of *Erysiphe* species have emerged, the efficacy of the resistance genes is challenged (Ghafoor and McPhee 2012). Two pea lines resistant to powdery mildew were identified, ILS657 (foliage and pod resistance) and UN6651 (pod resistance only). The study showed that the *er1* gene showed Mendelian recessive segregation pattern. The new sources were confirmed to be allelic to *er1* in case of foliage and pod resistance and another unique allele for pod resistance only (León et al. 2020).

3.3.2 Secondary Gene Pool

Secondary gene pool in the genus *Pisum* includes *P. fulvum* and *P. abyssinicum. P. abyssinicum* exhibits a distinct diversity and karyotype without being found in the wild (Trněný et al. 2018; Weeden 2018). Pea weevil resistance was searched in the *Pisum* secondary gene pool (*P. fulvum* Sm.) due to low detection of seed resistance in *P. sativum* and subspecies. Study was carried out to account for the extent of pod and seed damage in *P. fulvum* accessions and about 26 moderately resistant and resistant lines were obtained after the screening (Clement et al. 2002; Coyne et al. 2020). These lines could be utilized for introgression of these traits into cultivated species for obtaining resistant cultivars. Fusarium root rot leads to significance yield loss in North America where dry pea production has increased significantly over the last few decades. Partial resistance to *Fsp* was identified by developing recombinant inbred line (RIL) population and three associated quantitative trait loci (QTLs) were detected by using three disease criteria i.e. root disease severity, ratios of diseased versus healthy shoot heights and dry plant weights. *Fsp-Ps 2.1* QTL has been observed to be promising for partial resistance to *Fsp* and the single nucleotide polymorphisms (SNPs) associated with the same could be utilized for pea breeding program (Coyne et al. 2019). Rust resistance in pea could not be achieved due to lack of variability in pea germplasm. *P. fulvum*, wild relative of *P. sativum*, is an important source for allelic diversity mainly for rust resistance. Diversity arrays technology sequencing (DArT-Seq) was used for genotyping the RIL population, developed by crossing two *P. fulvum* accessions, IFPI3260 and IFPI 3251. Seven linkage groups with 12,058 markers were assembled, equivalent to both the parents haploid chromosome number. Three QTLs, *UpDSII*, *UpDSIV* and *UpDSIV.2* revealed by composite interval mapping were distributed over two linkage groups linked with the percentage of rust disease severity. These QTLs were found to be closely linked to pea rust disease resistance (Barilli et al. 2018).

3.3.3 Tertiary Gene Pool

The tertiary gene pool of *Pisum sativum* L. includes *Vavilovia Formosa* (Stev.) Fed. which is the common ancestor of tribe Fabeae and shares close phylogenetic relationship. Pea and Vavilovia are close relatives with similar chromosome number of 14 (2*n*). Utilization of Vavilovia germplasm for pea breeding activities will help to introgress essential traits for fighting against various biotic stresses (Zong et al. 2019; Coyne et al. 2020). Along with Vavilovia, grasspea (*Lathyrus sativus* L.) is also a part of the tertiary gene pool of pea. Previously various interspecific crosses were achieved between these two close relatives that has been reviewed earlier. Resistant source of fungal pathogens (*Erysiphe pisi*, *Uromyces pisi*, and *Mycosphaerellapinodes*) of pea has been observed in Lathyrus and could be utilized for obtaining fungus resistant

lines in pea. Some grasspea germplasm also have resistance to pea rust which is caused by *Uromyces pisi-fabae* (Kankanala et al. 2019; Zong et al. 2019).

3.4 Classical Genetics and Traditional Breeding

Peahas been utilized as one of the main crops for studying the inheritance of traits or genes. Further research in pea led to the development of classical genetics and this became the landmark study for development of traditional breeding. From Mendel's work on pea to the whole genome sequencing of pea has seen a remarkable change in the understanding of the inheritance of various traits (Smýkal et al. 2016, 2020; Kreplak et al. 2019). Crop improvement in pea mainly includes tending to biotic and abiotic stresses which significantly affect the crop productivity. It requires utilization of numerous crosses of diverse germplasm as parental lines and selection of important traits which are climate resilient and effective towards biotic stresses as well. Biotic stress in crops has been varied in different geographical areas and various abiotic stresses aggravate the rates of infection of pathogens. Biotic stress in pea is mainly due to fungi, virus, insects and in some cases parasitic plants (Tayeh et al. 2015b; Zong et al. 2019).

Conventional breeding have achieved significant increase in the production of pea consistently with approximately 2% increase per year (Warkentin et al. 2015). Limitation of the classical breeding are brought out by the increased challenges of climate change and new strains of various pathogen infections in pea, mainly due to unavailability of variability.

Availability and inclusion of resistance sources for pea breeding program is the key technique for pea improvement for biotic stresses (Hussain 2015). With increasing requirement for better quality and yield the classical breeding has transformed in to molecular breeding with the advent of new biotechnological tools for introgressing of new traits. Abiotic and biotic stresses have put more constraints on the crop improvement strategies in pea, but utilization of germplasm diversity in pea and genome edited lines will be utilized to overcome some of the challenges in future (Tayeh et al. 2015b; Ali et al. 2018; Zong et al. 2019). SRAPs (sequence-related amplified polymorphisms) were utilized for making linkage maps in pea by crossing cultivars, DDR11 and Zav25. The F_2 mapping population was screened with 25 SRAP markers and about 208 polymorphic markers were generated. Linkage map constructed had seven linkage groups with length ranging from 47.6 to 144.3 cm (mean 75.54 cm). These linkage groups could be utilized for mapping studies in pea (Guindon et al. 2016). Morphological and SSR-based marker approaches were studied to characterize pea germplasm at the Aegean Agricultural Institute of Turkey. About 40 cultivars collected from different geographical regions of Turkey were used for the study according to UPOV criteria and with 10 SSR markers. A total of 61 alleles were detected at 10 loci from the SSR markers and a UPGMA dendrogram was constructed with the morphological characterization. These studies could be utilized for better management of pea germplasm for breeding activities (Sarikamiş

et al. 2010). Resequencing of gene fragments in pea was done with 384-SNP set in different genotypes and genome sequence data were compiled using Illumina GoldenGate assay. About 92% allelic diversity and 37 new gene markers was obtained from the pea germplasm collection which included species and subspecies of *Pisum sativum* ssp. *Sativum* (Deulvot et al. 2010).

Isozyme electrophoresis analysis was carried out to find protein markers for reproducible characterization of individual genotypes of *Pisum sativa* L. Seed and leaf tissues from 45 cultivars were utilized to obtain isozyme profile, mainly with six enzyme systems i.e. acid phosphatase, amylase, esterase, leucine amino peptidase, shikimate dehydrogenase and phosphoglucomutase (Pošvec and Griga 2000).

Pea seed-borne mosaic virus resistant gene *sbm-4* in pea was found to be closely linked to the isozyme marker plastidic glutamine synthetase GS 185. Isozyme and restriction fragment length polymorphism (RFLP) mapping study also revealed that another allele, the *Prx-3* allozyme was loosely linked to the PSBMV resistance gene (Dhillon et al. 1995). These markers could be utilized for pea breeding program and a gene conferring PSBMV resistance could be introgressed in elite pea lines. Isozymes like amylase, esterase and glutamate oxaloacetate transaminase were examined in pea seeds from F_2 population with different crosses. Isozyme characterization and morphological markers were developed and linkage studies were also carried out in pea germplasm (Mahmoud et al. 1984).

Over the last decades conventional plant breeding has resulted in development of various open-pollinated (OP) and hybrid varieties but due to some limitations the biotechnological interventions was required. Limitations of conventional breeding were mainly the non-compatibility for interspecific crosses limiting the novel trait introgression and multiple undesirable traits get passed to the next generation which have harmful impact on other characters (ISAAA 2006).

Conventional plant breeding mostly focused on yield and resistance to pest and disease, by introgressing genes from various wild sources. Recent molecular genetic and biotechnological innovation for developing better crop varieties has tremendously enhanced in both quality and quantity. Conventional breeding techniques led to Green Revolution from 1960 to 80 in cereal crops mainly wheat and rice. Utilization of biotechnological tools such as marker-assisted selection and genetic engineering are the remarkable advancements in crop improvement strategies for sustainable agricultural development (Bhargava and Srivastava 2019).

Developing improved crop varieties quickly is the only way of alleviating food scarcity problems and increasing food security. Genetic variability has come down drastically in crops like pea, where novel molecular techniques are needed to make it more climate resilient as well as biotic stress resistant. Conventional breeding techniques have been improved by the inclusion of genetic selection, molecular breeding, somaclonal variations, genetic engineering, next-generation sequencing (NGS) approaches for whole genome and functional genomic tools. Recent advances in genome editing tools like clustered regularly interspaced short palindromic repeats with associated proteins Cas9 (CRISPR/Cas9) have helped to develop traits which were difficult to develop with conventional breeding. Speed breeding techniques and gene editing tools will help the molecular breeders to develop varieties which are

resistant to both abiotic and biotic stresses along with addressing concerns in case of yield and nutrition (Ahmar et al. 2020).

3.5 Genetic Diversity Analysis

Pisum sativum L. is a protein-rich legume crop with diverse germplasm distributed through the geographical region in the world. According to use there are mainly three different variants of *Pisum* species i.e. garden peas, fodder peas and field peas.

Various marker techniques has been utilized to document the diversity in pea germplasm. Marker techniques like SSR, retrotransposon based insertion polymorphism (RBIP) along with SNP markers have also been utilized for diversity analysis (Burstin et al. 2015). SSR marker results showed that in pea germplasm evolution with fastest rate but RBIP showed the slowest rate showing dissimilarity in mode of evolution. About 331 SNP markers were utilized to analyze to predict phenotypes like flowering, number of seeds and seed weight (TSW).

The origin and domestication of Abyssinian pea (*Pisum sativum* ssp. *abyssinicum*) and its phylogenetic relationship with other subspecies and species were studied with the help of 54 genes. A close relationship of Abyssinian pea with *P. sativum* subspecies i.e. *P. s.* ssp. *sativum* and *P. s.* ssp. *elaitus* was observed after the allele affinities were studied (Weeden 2018).

Identification of stable and high yielding field pea lines from about 12 pea genotypes in seven environments were carried out in Eastern Amhara, Ethiopia. Experiments showed that different genotypes exhibited different levels of productivity in different environments. Some genotypes like EH-03-002 (Yewaginesh) were identified with higher yield than normal checks and were released in drought areas (Kindie et al. 2019). Interspecific crosses between similar species had been done for introgressing traits for biotic stresses in pea. Increased yield by 30% was observed by crossing dwarf and *afila* plant type in field pea.

About six structural types were observed after diversity studies in mitochondrial genomes from 38 accessions of pea (*Pisum* sp.) from different geographical areas, ranging from wild relatives to elite lines. Six events of hybridization in the past were revealed by topology study of the phylogenetic trees. Discordant inheritance of organelles, both plastids and mitochondria was also observed and this has resulted in the plastid-nuclear incompatibility, which is very common in pea (Bogdanova et al. 2020).

Genome-wide analysis shows that *P. sativum* subsp. *elatius* was clustered into five geographical clusters and *P. fulvum* has been identified as well-supported species. Spatial and environmental patterns of these two species of Mediterranean pea, alongwith the genetic diversity study does not correlate much with the origin of these two species (Smýkal et al. 2017). To identify SNPs associated with Ascochyta blight resistance in pea, allele diversity analysis was conducted with about 54 *P. sativum* accessions from Australia, Canada and Europe. Genotyping and phenotyping for the disease reaction was conducted and 15 SNPs were detected within the candidate

genes for Ascochyta blight resistance. Two SNPs, PsDof1p308 and RGA-G3Ap103 showed significant associations with Ascochyta blight resistance in pea, which could be utilized for pea breeding program for Ascochyta blight resistance (Jha et al. 2019). Nematode (*Meloidogyne incognita*) resistance was studied in about 23 field pea selections in greenhouse conditions and root-knot indices (RKI) were analyzed after inoculation of nematodes. Selections HFP-990713, Pant P-25 and HFP-0129 were found to be resistant and no root-knot was observed as compared to other susceptible lines (Sharma et al. 2006).

Fusarium solani f. sp. *pisi* (*Fsp*) is the causative agent of Fusarium root rot resulting in drastic yield loss in pea. Pea accessions and commercial varieties were screened for *Fsp* resistance by analyzing the root disease severity (RDS) and pigmented lines under greenhouse conditions. Physiological data like plant height, shoot dry weight and root dry weight were compare with RDS and it was observed that plant height was negatively correlated with the RDS value (Bodah et al. 2016).

3.6 Association Mapping Studies

Pisum germplasm is varied and has been widely studied and documented through various bureaus of genetic resources. Association mapping studies is the mapping of QTLs by linking phenotypes to genotypes for the detection of genetic associations.

An annotated pea genome has now been has been published with chromosome-level genome assembly for the Cameor cultivar. Pea genome shows intense gene dynamics which is associated with divergence from other Fabeaesister tribes due to genome size expansion (Kreplak et al. 2019). Genome sequencing results shows that across pedigrees differential occurrence of translocation and transposition was observed during pea evolution.

Field pea agronomic traits, seed morphology, seed quality was analyzed to identify gene loci with genome-wide association study (GWAS) by utilizing a germplasm collection of 135 pea accession from 23 different breeding programs form Africa, Asia, Europe and North America (Gali et al. 2019a). Genotyping by sequencing (GBS) gave linkage disequilibrium (LD) decay across chromosome varying from 20 to 80 kb and about nine sub-populations were grouped after population structure analysis.

To determine the genetic loci associated with stress responsive traits of physiological and agronomic importance in pea, GWAS was carried out. Three different environments were considered for identifying 32 marker–trait associations. For developing heat resistance varieties of pea, these markers could be used for crop improvement strategy in pea (Gali et al. 2019a). GWAS study was carried out to understand pathogen-legume interaction and about 52 QTLs were identified associated with resistance to root rot caused by *Aphanomyces euteiches*, by screening about 175 *Pisum sativum* lines which were genotyped using 13,204 SNPs from the GenoPea Infinium BeadChip (Desgroux et al. 2016; Kankanala et al. 2019).

3.7 Molecular Mapping of Resistance Genes and QTLs

Extensive mapping studies in QTLs for various agronomic traits as well disease resistance were carried out in pea by various researchers but were poorly documented earlier (Smýkal et al. 2012). Genome-wide SNPs and genetic linkage maps were developed for identifying QTLs in pea for seed mineral nutrients, which could be utilized for marker assisted selection (MAS) for improving nutritional qualities of pea (Ma et al. 2017a). A total number of 1609 polymorphic SNPs were found in pea parental lines from an F_6-derived RIL population, which generated a linkage map size of 1310.1 cm. About 46 seed mineral concentration QTLs, 37 seed mineral content QTLs and 6 seed weight QTLs were detected in total showing phenotypic variance of 2.4–43.3% (Ma et al. 2017a, b). QTLs in pea for yield traits were identified by SRAP, SSR, and SNP markers and a total of 873 polymorphic markers for linkage mapping was identified. About 45 QTLs were detected by composite interval mapping (CIM) method and a map constructed with 9 linkage groups (LGs) covering 655.5 cm was obtained with 128 genetic markers. About 10% phenotypic variance was observed in most of the QTLs detected through different generations and environments (Guindon et al. 2019).

3.7.1 QTLs in Pea for Fungal Resistance

QTLs for resistance to various pathogens have been identified in various pulse crops including pea. ***Fusarium solani*** f. sp. *pisi* (Fsp) is an infectious fungus that leads to severe symptoms like root rot in pea. To identify resistance associated QTL for Fusarium root rot in pea genome CIM was carried out. About three disease reaction criteria were taken into consideration i.e. root disease severity, ratios of diseased to healthy shoot heights and dry plant weights, for identifying the QTLs. These QTLs could be utilized in pea breeding programs for obtaining partial resistance to Fusarium root rot (Coyne et al. 2019). QTL for partial resistance of root rot caused by ***Aphanomyces euteiches*** in pea accessions were screened by meta-analysis using 244 QTLs reported earlier from mapping populations and about 27 meta-QTLswere identified for resistance to *A. euteiches* (Hamon et al. 2013). Similarly QTLs for partial resistance and management strategies have been reviewed for developing better varieties of pea cultivars (Wu et al. 2018). ***Aphanomyces euteiches*** causes severe root rot in pea and there have been several QTLs associated with partial resistance in pea for root rot caused by the same. Linkage maps were developed from different crosses with different parents. RIL population was developed from a cross of pea genotypes, Puget and 90–2079 lines and about three stable QTLs—*Aph1, Aph2* and *Aph3* were identified. These QTLs were located on the linkage groups IVb, V and Ia in the pea genome (Pilet-Nayel et al. 2002; Wu et al. 2018).

Highly significant and reproducible QTLs by genotyping-by-sequencing (GBS) were developed for various traits in three RIL populations. High-density linkage

maps were constructed and 375 QTLs were identified for traits like flowering, crop maturity, resistance to lodging, *Mycosphaerella* blight (caused by *Mycosphaerella pinodes*) resistance, seed weight and yield (Gali et al. 2018). Mapping populations were created from the crosses of partially resistant lines (3147-A26 and 3148-A88) with susceptible lines (Rovar population) in Western Australia and New Zealand, and about 11 novel putative QTLs for Ascochyta blight resistance (caused by a complex of three fungal pathogens—*Mycosphaerella pinodes* (Berk. and Bloxham) Verstergren, *Phoma medicaginis* Malbr. & Roum var. *pinodella* (L. K. Jones) Boerema, and *Ascochyta pisi* Lib.) in pea were identified (Timmerman-Vaughan et al. 2004). QTL mapping was done by mapping different linkage groups and QTLs were detected in Linkage groups II, III, IV, V and VII. These mappings of the QTLs were also measured for plant reproductive maturity as Ascochyta blight is severe when plants start maturing. Six and five plant maturity QTLs in case of A26 x Rovar population and A88 x Rovar population respectively were associated with the linkage groups found earlier. Different linkage groups of the QTLs were linked with different phases i.e. repulsion and coupling phases, which was found to be the result of pleiotropic effects of plant-maturity genetic loci (Timmerman-Vaughan et al. 2004). A total nine QTLs were identified for Ascochyta blight resistance, which individually explained 7.5 to 28% of the total phenotypic variation. These QTLs could be useful for development of molecular markers associated with Ascochyta blight resistance (Jha et al. 2016). A total six QTLs associated with resistance to *M. pinodes* were found in linkage groups II, III, IV and V, which collectively explained between 31 and 75% of the phenotypic variation (Fondevilla et al. 2008).

3.7.2 QTLs in Pea for Bacterial Resistance

Bacterial blight disease caused by *Pseudomonas syringae* pv. *syringae* causes a yield loss of upto 70% in pea. TwoQTLs for bacterial blight resistance were identified in pea. RIL population created from a cross between P665 x 'Messire' was used for QTL identification associated with bacterial blight resistance genes *psy1* and *psy2* and 21 SSR markers in pea (Rubiales and Caminero 2012). In pea, a total of two and four QTLs for resistance to infection of *P*. pv. *syringae* were detected in case of crosses of Kaspa (Susceptible genotype) x PBA (Resistance genotype) Oura and Kaspa x Parafield populations, respectively. QTLs for resistance to both *P. syringae* pv. *syringae* and *P. syringae* pv. *pisi* race are co-located on Ps III cross of Kaspax PBA Oura, which is an important area for developing resistance to bacterial blight and also provides the basis for co-selection in genomics-assisted breeding activities (Sudheesh et al. 2015).

3.7.3 QTLs in Pea for Insect Resistance

QTLs in pea have been identified for resistance against pea weevil (*Bruchus pisorum*) from a high-density integrated DArT-Seq SNP-based genetic map developed from a RIL population (RIL F8:9). Both pea weevil larval development and seed infestation were screened in five different environments. Genetic linkage mapping allowed to identify three QTLs from the study associated with the pea weevil resistance. Expression of the QTLs varied with different environmental conditions. Seven markers co-located with QTLs are potential markers which could be utilized for MAS in pea breeding program (Aznar-Fernández et al. 2020). Similarly with DArT-Seq SNP-based QTL mapping, candidate genes were identified for aphid tolerance in wild relatives of pea (*Pisum fulvum*). A total of eight QTLs linked with eight linkage groups were identified which were associated with tolerance towards aphid infestation (Barilli et al. 2020). Genomic studies with the help of published pea genomic sequence will help molecular breeders to generate more resistant and tolerant genes associated with various pests in pea (Kreplak et al. 2019).

3.8 Marker-Assisted Breeding for Biotic Stress Resistance

Marker-assisted breeding for introgressing genes into elite commercial pea lines have been done by molecular breeders in various pea breeding programs happening in various institutes and commercial companies to address various biotic stresses.

3.8.1 Pea Markers Developed for Virus Resistance

Mutagenesis has helped to develop various gene markers for resistance towards various biotic stresses, mainly pathogens like viruses in pea. Mutation in eukaryotic initiation factor 4E (eIF4E) confers resistance in both for *Pea seed-borne mosaic virus* (PSbMV) with *sbm-1* gene and *Clover yellow vein virus* (ClYVV) with *cyv-2* gene in pea. About 202 pea lines were screened by sequencing *eIF4E* gene and the resistant lines were generated for ClYVV (Andrade et al. 2009). PSbMV resistance is conferred by a single recessive gene of *eIF4E* which is localized on LG VI i.e. *sbm-1* locus. Resistant donors were obtained from 43 different pea varieties by sequencing the *eIF4e* genomic sequence. Markers for *eIF4E* allele were developed and PSbMV infection data were used to confirm resistance in 60 pea accessions (Smýkal et al. 2010). The RFLP marker (GS185) for PSbMV resistance, which is closely linked to *sbm-1* locus, was developed from screening different pea accessions. These markers could be used for introgressing the PSbMV resistance gene into the elite pea varieties (Timmerman et al. 1993). KASP (Kompetitive allele-specific PCR) markers were developed for resistance to PSbMV with endpoint genotyping for speedy testing new

breeding lines without requirement of greenhouse facilities for screening or ELISA testing. Breeders will benefit with such KASP markers for obtaining varieties with resistant lines, which in turn could be utilized for pea breeding programs (Grimm and Porter 2020). Other diagnostic assays such as tissue blot immunoassay (TBIA) has been used for virus diagnosis in pulses, which are manly reliable, fast and cost-effective methods for screening of large numbers of plant samples (Kumari et al. 2001).

3.8.2 Pea Markers for Fungus Resistance

A novel genomic region was identified for controlling cellular mechanism involved in pea resistance to Ascochyta blight which is useful for marker associated screening (Carrillo et al. 2014). Validation for Ascochyta blight resistance in pea was carried out with SNP markers in 36 cultivars of pea from Saskatchewan, Canada. KASP assays and SNP marker association studies were carried out and SNP makers like RGA-G3Ap103, PsC8780p118, and PsC22609p103 were found to be associated significantly with the Ascochyta blight scores (Jha et al. 2019). These markers could be further utilized in pea breeding programs for improved lines with Ascochyta blight resistance in pea. MAS for developing cultivars for resistance to powdery mildew (caused by *Erysiphe pisi*) resistance has been done with *er1, er2* and a new dominant *Er3* gene. A new pathogen, *E. trifolii,* for pea powdery mildew has been reported and for ita new resistant source has been searched in pea germplasm (Ghafoor and McPhee 2012). A total of 24 pea lines were evaluated for high yield and resistance to powdery mildew. ANOVA results showed that grain yield of 24 lines ranged from 22.87 to 102.54 g and were also highly resistant to powdery mildew (Iqbal et al. 2017). Mutagens like methylnitroso urea (MNU) and ethylnitroso urea (ENU) were utilized for obtaining variability in pea (*Pisum sativum* L.) germplasm. Two novel mutations related to powdery mildew (*Erysiphe pisi* Syd.) resistance were obtained by ENU treatment, which could be utilized for marker development for screening a large number of pea germplasm (Pereira and Leitão 2010). These *er1* mutants have inheritance as monogenic recessive trait, which exhibits Mendelian mode of inheritance. Several powdery mildew resistant lines were developed by mutation in two novel *er1* alleles. The first such allele is *er1-8*; germplasm accession G0004839 has which a 3-bp (GTG) deletion of the wild-type *PsMLO1* cDNA, that affects the *PsMLO1* protein sequence. Another mutation in accession G0004400 was caused by a 1-bp (T) deletion of the wild-type *PsMLO1* cDNA sequence, resulting in a truncated *Ps*MLO1 protein. Theses result concluded that *E. pisi* resistance in pea germplasm could provide a powerful tool for MAS in pea breading (Sun et al. 2019). The KASPar assay is useful tool for development of powdery mildew resistance line (accession PI 142775) in pea by phenotyping and genotyping to carry the allele *er* 1-1 (Ma et al. 2017b).

3.8.3 Pea Marker Developed for Insect Resistance

Pea weevil (*Bruchuspisorum* L.) infestation is a global problem for the pea crop production, various resistance sources from wild pea (*Pisum fulvum* Sibth. & Sm.) were introgressed into cultivated field pea (*Pisum sativum* L.). F2:3 families showed mortality rates of larva on pods similar to resistant parents, but complete resistance to pea weevil was not observed in the progenies (Clement et al. 2009).

3.9 Map-Based Cloning of Resistance Genes

Pulses are relatively minor crops on a global scale when compared with cereals and others when global production and field area sown are taken into consideration. The nutritive value of pulses are immense but fewer studies are being conducted in various legume species as compared to other major crops. Traits from wild relatives have been utilized in conventional breeding for introgressing into elite parents. Bacterial artificial chromosome (BAC) libraries of pulsed crops are essential genetic resources that will quicken gene discovery and augment molecular breeding in pulse crops especially in pea. Various BAC libraries in pulses like mungbean (*Vigna radiata* L.), cowpea (*V. unguiculata* L.), pigeonpea (*Cajanus cajan*L.), field pea (*Pisum sativum* L.), Lima bean (*Phaseolus lunatus* L.) and common bean (*P. vulgaris* L.) has been reviewed by Yu (200). *Hind* III BAC libraries in pea (Pea plant inventory (PI) accession 269818) were developed to isolate genes involved in plant disease resistance and other economically important traits. About 65,280 clones were obtained from a single-copy oriT-based T-DNA vector (pIndigoBAC-5) library. Two replication methods was developed to analyze the usefulness of the library, one by probing high-density filters with low copy number sequences and the second by amplifying 7 of 9 published pea resistance gene analogs (RGAs) with BAC plate-pool DNA (Coyne et al. 2007).

3.10 Genomics-Aided Breeding for Biotic Stress Resistance

Crop improvement in pea has been done by utilizing multiple numbers of genomics aided breeding techniques. Breeding tools like genome selection (GS) are being utilized for achieving novel trait development, where trait measurements are difficult due to environmental influence and occurrence of multiple pathogens during infection, especially in case of biotic stresses. Due to climate change and adaptation of insect pests the crops are on high risk of biotic stresses. To overcome this challenge there is a high requirement of availability of genomic resources which help researchers to improve crops.

3.10.1 Genome Sequencing

Individual and consensus genetic maps were constructed by genotyping of 12 pea (*Pisum sativum* subsp. *pisum*) RIL populations (French cultivar Cameor) by GenoPea 13.2 K SNP array. High resolution consensus map consisting of 12,802 transcript-derived SNP markers was constructed thereby revealing the duplication sites in pea genome. These SNP array data helps breeders to analyze genetically and physiologically for crop improvement strategy in pea (Tayeh et al. 2015a). Transcriptome analysis was done in pea by generating full length de novo assembly of RNA sequencing data from 20 cDNA libraries produced from plant tissues collected at various developmental stages from plants grown under different nitrogen levels. CameorUnigene set of a total number of 46,099 contigs were identified and further online search engine was developed (http://bios.dijon.inra.fr/FATAL/cgi/pscam.cgi) for annotation of candidate genes, transcript expression study, identifying uncharacterized genes and gene ontology study (Alves-Carvalho et al. 2015). Physical mapping in pea was assembled by constructing BAC contig libraries from about 295,680 BAC clones. Whole genome profiling (WPG) sequence tags were utilized to assemble 220,013 BACs into contigs which helped to construct a robust physical map of pea (Gali et al. 2019b).

An international consortium was formed to sequence the whole genome of *Pisum sativum* L. with the inclusion of latest NGS technologies, which are both time saving and cost-effective. The inbred pea cultivar used for sequencing was 'Cameor' which was released by French Breeding company Seminor in 1973 (Kreplak et al. 2019). The first annotated chromosome-level reference genome assembly for pea was reported by Kreplak and team (Kreplak et al. 2019). About 82.5% (3.23 Gb) of the estimated pea genome size (about 4.45 Gb) was assembled into seven pseudomolecules and about 14,266 unassigned scaffolds (685 Mb), with the size gap being highly repeated sequences (Fig. 3.5).

As pea production and storage is prone to be affected by several pests, development of resistant variety is required to match the increasing demand. As the pea draft genome sequenced, identification and annotation of agronomical important genes boosted programs of resistance breeding. In pea, gene annotation was done by combined ab initio and homology based methods. Using these methods 44,756 complete and 29 truncated genes were predicted. The average gene length is 2,784 bp. The average coding sequence length is 1,016 bp. The average exon numbers are 6.33 exons (Tayeh et al. 2015a; Kreplak et al. 2019).

Evaluation of Ascochyta blight resistance in pea germplasm was evaluated with GBS, Bayesian Reproducing Kernel Hilber spaces regression (RKHS) and genome based linear unbiased prediction (GBLUP) modeling techniques. Ascochyta blight disease score (ASC) of 0.56 was obtained in case of GBLUP analysis, after screening of 215 pea accessions originating from The New Zealand Institute for Plant and Food Research Limited (PFR) pea breeding program and other commercial cultivars from other sources (Carpenter et al. 2018).

3.10.2 Application of Functional Genomics

Identification and isolation of genes underlying functional genomics studies were carried out in pea by developing a fast neutron (FN)-mutagenized population. Mutant population developed will give a pool of variability due to deletions in associated gene for various functions which are not found in wild relatives of pea. Forward genetics screening with NGS helps molecular breeders to identify genes with deletions rapidly and steadily (Domoney et al. 2013).

Agrobacterium-mediated transformation is a challenge in pea, as it is a species which is recalcitrant to the same. Mutant populations of peas were created by treating the seeds with EMS (ethyl methane sulphonate) mutagen and TILLING (targeting induced local lesions in genomes), a high throughput reverse genetic tool, was developed. UTILLdb, a phenotypic peaphenotypic database was created from the mutant populations with sequence information on the mutant genes. UTILLdb is an online searchable database developed by INRA, France, which gives a platform where mutant gene sequence information can be searched through BLAST tools and also associated phenotypes (Till et al. 2007; Dalmais et al. 2008). TILLING has been applied for obtaining numerous mutants in different model plants and crops like *Medicago truncactula* (Carelli et al. 2013), *Phaseolus vulgaris* (Porch et al. 2009), and *Cicer arientinum* L. (Amri-Tiliouine et al. 2018), including other crops (Kumar et al. 2013). Screening for mutants with NGS technology to mutagenized TILLING populations as a tool for functional genomic study is known as TILLING by sequencing (TbyS). TbyS could be utilized in pea breeding program for screening large mutant population to identify and characterize induced mutations in gene of interest. TbyS will accelerate the functional genomics platform together with rapid increase in genome editing capabilities and enhance the quality and number of genome sequencing (Kumar et al. 2017). These mutant populations developed will allow plant breeders to utilize the resources for pea breeding program to obtain enhanced variability for various traits not only for yield and nutrition but also for biotic and abiotic stresses.

3.11 Recent Concepts and Strategies

Conventional breeding in pea has contributed significantly for improving yield and nutritional traits, but addressing issues pertaining to biotic stresses have been lagging behind (Warkentin et al. 2015). Pulses are generally climate smart, as they adapt to and mitigate the effects of abiotic stress like salinity, drought and heat. Low genetic diversity in pulses has been the main drawback for crop improvement efforts. Biotech techniques like transgenics by genetic transformation, gene editing, and nanotechnology could be utilized for accelerating the effort for developing climate smart pulses (Kumar et al. 2019).

3.11.1 Gene Editing

The process of delivering site-directed nucleases (SDNs) and single guide RNA to explants in culture for editing specific region of gene is known as gene editing (van de Wiel et al. 2017; Maher et al. 2020). Further these gene-edited cells are grown in plant growth medium with plant hormones (Cytokinins and auxins) for cell differentiation by tissue culture. SDNs mainly lead to small deletions/insertions (indels) and modification or replacement of genes. Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALEN) and CRISPR/Cas9 nucleases are SDNs which could be used for gene editing in plants. CRISPR (Clustered regularly interspaced short palindromic repeat) and Cas9 (associated protein 9) gene editing system is an effective technique which has been applied for targeted trait development in plants. There are different techniques of gene editing, viz. targeted gene mutagenesis, cytosine-based editing (CBE) and adenine-based editing (ADE), which are very efficient for obtaining gene-edited plants with desired phenotype (Fig. 3.6) (van de Wiel et al. 2017; Mao et al. 2019; Maher et al. 2020). Gene editing has provided a new cutting-edge strategy for development of traits against biotic and abiotic stress mainly because it is simple, specific, consistent, and very have very high efficiency, which was not feasible with conventional breeding techniques (Fig. 3.7).

Innovative modes of gene editing techniques are being developed for effective editing of complex traits in various crops. Virus-mediated gene editing is one such techniques which overcomes drawbacks of conventional transgene-mediated CRISPR-Cas reagent delivery method, such as unexpected genome changes,

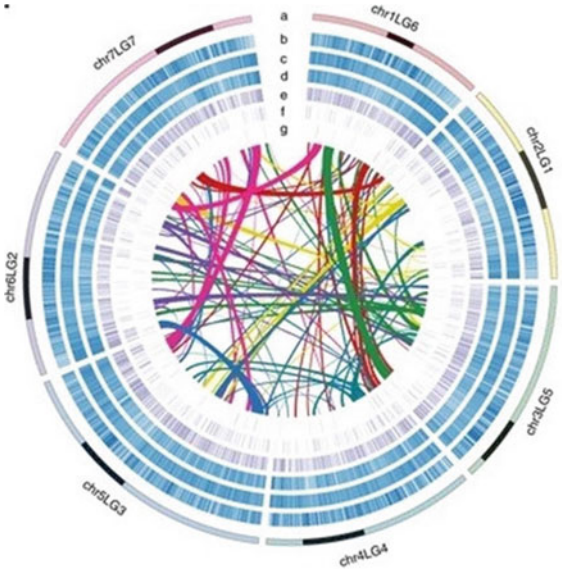

Fig. 3.6 Pea genome showing all the seven chromosomes; centromere position is colored in black. Circular representation of pseudomolecules in the lane (**a**), density of retrotransposons (**b**), transposons (**c**), genes (**d**), ncRNA (**e**), tRNA (**f**) and miRNA. Synteny-selected paralogues are linked in the inner circle with lines (Kreplak et al. 2019)

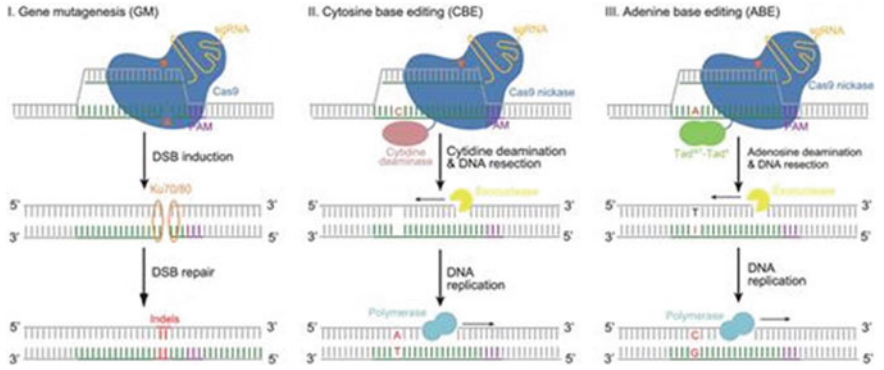

Fig. 3.7 CRISPR/Cas-mediated gene mutagenesis and base editing models. I—Gene mutagenesis, II—Cytosine base editing and III—Adenine base editing (Mao et al. 2019)

prolonged breeding cycles comprising foreign DNA segregation along with regulatory restrictions. Virus-mediated gene editing utilizes plant negative-strand RNA virus-based vector for DNA-free plant delivery of CRISPR-Cas9 cassette in tobacco. More than 90% of the plants contained targeted mutations, in which about 57% had tetra-allelic, inheritable mutations (Ma et al. 2020). *Tobacco rattle virus* (*TRV*) and *Pea early browning virus* (*PEBV*) were engineered to deliver multiple ssRNAs into *Nicotiana benthamiana* and *Arabidopsis thaliana* (Col-O) plants for inducing targeted mutations (Ali et al. 2018).

CRISPR/Cas9 gene editing and other latest techniques have enormously enhanced the characterization of complex genes mainly for biotic and abiotic stresses and other traits. Utilization of targeted mutagenesis with CRISPR/Cas9 helps to obtain novel lines with improved traits, gene regulation, breeding virus resistance and high-throughput editing mutant libraries (Chen et al. 2019).

Clover yellow vein virus (*ClYVV*) (Family: Potyviridae) infections in pea leads to crop damage in pea, mutated eIF4E proteins confers potyvirus resistance. CRISPR-Cas9–cytidine deaminase technology was used to edit *eIF4E* gene and *eIF(iso)4E* to develop various mutants (W69L, T80D, S81D, S84A, G114 and N176K substitutions) which gave *ClYVV* and potyvirus resistance in pea (Bastet et al. 2019) (Fig. 3.8).

Pea aphids (*Acyrthosiphon pisum*) are major pest in pea and other crops which also act as vectors for plant viruses which leads to a large extent of crop loss. Gene editing by CRISPR-Cas9 in pea aphid was carried out to obtain stably edited line. *Stylin-01* gene in aphid was edited, which mainly controls the transmission of *Cauliflower mosaic virus* (*CMV*), which will help in controlling crop loss due to the aphid transmitted viruses (Le Trionnaire et al. 2019).

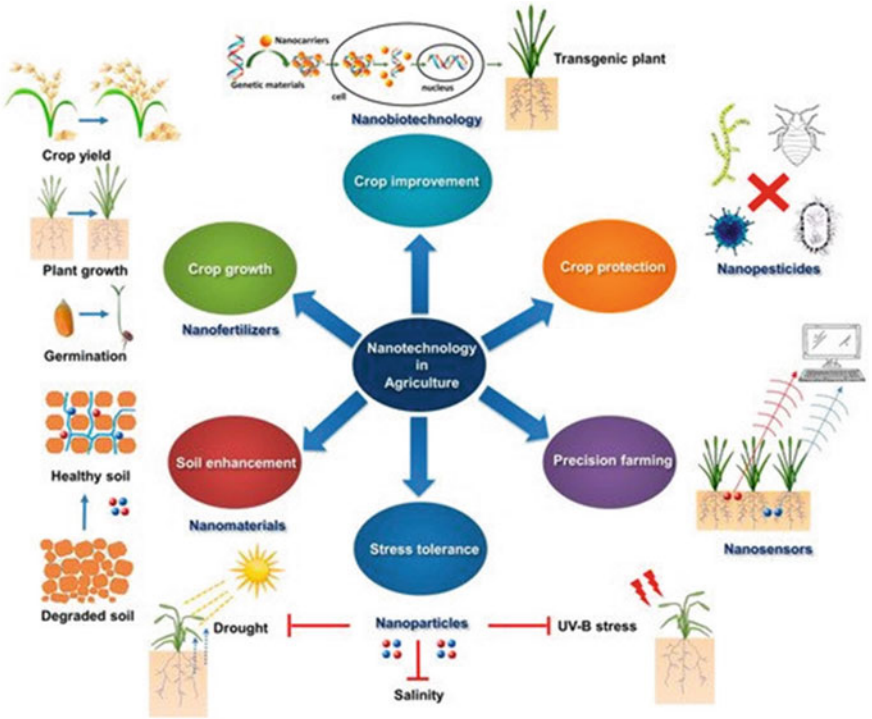

Fig. 3.8 Applications of nanotechnology in agriculture. Nanotechnology is in applied various sectors of agriculture mainly for crop improvement (nano carriers for genetic engineering), crop protection (nanopesticides), precision farming (nanosensors), stress tolerance (nanoparticles), soil enhancement (nanomaterials), and crop growth (nanofetilizers) (Yifen et al. 2019)

3.11.2 Nanotechnology

Another major inclusion of modern technology in sustainable crop development is nanotechnology. Nanotechnology enhances the efficiency by improving the agriculture productivity and by lowering the yield loss. Nanomaterials are those materials which efficiently site-directly delivers the pesticides and fertilizers and help to improve the crop output. Nanotools such as nanobiosensors help to collect intricate crop developmental data which could not be collected otherwise. Nanotechnology enhances crop care mitigation by sensing and identifying environmental impacts both from biotic and abiotic stress (Shang et al. 2019). A detailed diagrammatic representation of all the main areas were applications of nanotechnology in agriculture has been shown in Fig. 3.7 (Shang et al. 2019).

Nanotechnology in crop science is still in infancy, mainly in areas like plant-nanomaterials (NM) interactions, uptake of NM in cellular level, mobilization of NMs into target sites and accumulation in cellular vesicles. Enhanced research in crop improvement with NM is required to fill the void of information in this area.

Apart from NM, development of nano carriers with gene editing capability is also revolutionizing the genome variability of desired biotic and abiotic traits. This will help future molecular breeders to develop climate resilient pea genotype along with addressing challenges of biotic stresses (Sanzari et al. 2019).

3.12 Brief on Genetic Engineering for Biotic Stress Resistance

Genetic engineering have been utilized to improve pea crop by various techniques. Transgenic techniques like direct and indirect transformations, anti-sense RNA, RNAi and VIGS have been utilized for attaining biotic stress resistance in pea.

3.12.1 Transformation in Pea

Transgenic pea for resistance against wax moth (*Galleria mellonela*) was developed by screening small inhibitors with antibacterial and antifungal activities (gmSPl-2) which were isolated from labial glands of wax moth. Czech and French pea cultivars were used for *Agrobacterium tumefaciens* mediated transformation with construct EHA105::pWell11 bearing GUS and GFP reporter genes (Svabova et al. 2010).

Transgenic pea was developed by inserting *cry1Ac* gene from *Bacillus thuringenesis* for tobacco budworm resistance and *Bar* gene from *Streptomyces hygroscopicus* for herbicide resistance. The transgenic was taken up to T4 generation and results of insect bioassay showed complete mortality of tobacco budworm (Negawo et al. 2013). *Agrobacterium tumefaciens*-mediated transformation was carried with antifungal genes coding for polygalacturase-inhibiting protein (PGIP) from raspberry and stilbene synthase (*Vst1*) from grapes in pea. Stable transgenics were developed and antifungal activities were detected by various experiments (Richter et al. 2006). Similarly, transgenic lines with antifungal genes (1–3 β glucanase (G), endochitinase (C), polygalacturonase inhibiting proteins (P) and stilbene synthase (V) were created for Fusarium root rot (*Fusarium avenaceum*) resistance in pea. Transgenic lines developed could be utilized for gene stacking and field trials for overall crop development in pea (Kahlon et al. 2018). But drawbacks of transgenic development such as regulatory cost, time requirement for trials and labor required have to be taken for consideration for efficient implementation.

Transgenics expressing multiple genes for various biotic and abiotic stresses will enhance and broaden the resistance of crops. Transgenic pea was developed by transforming the cultivar with antifungal gene i.e. chitinase and glucanase (Amian et al. 2011).

3.12.2 Gene Silencing

Gene silencing techniques like virus-induced gene silencing (VIGS) have been utilized for different crop species for characterizing genes controlling abiotic stresses like drought, salt, nutrient deficiency related stresses (Ramegowda et al. 2014). VIGS is an efficient method to change the expression of genes in host plants. *Pea white clover mosaic virus* (WClMV) was utilised as base virus vector and VIGS was demonstrated with *phytoene desaturase* (PDS) in pea. PDS mRNA and subgenomic RNAs of WClMV were reduced and photo-bleached tissues were obtained (Ido et al. 2012). VIGS is also an effective reverse genetic tool for silencing genes in certain plants that are difficult to transform. Pea early-browning virus (PEBV) has been developed as a VIGS vector and has been used in pea for functional analysis of several genes. Genes involved in symbiosis with mycorrhizal fungi (AMF) were the targets for gene silencing and results obtained showed early and late stages of AMF symbiosis (Grønlund et al. 2010).

Powdery mildew (PM) in pea caused by *Erysiphe pisi* (*Ep*) is a fungus which secretes a plethora of effectors, primarily through specialized infection structures termed haustoria, to establish a dynamic relationship with its host. To identify *Ep* effector candidates, a cDNA library of enriched haustoria from *Ep*-infected pea leaves was sequenced. The functional roles of EpCSEP001, EpCSEP009 and EpCSP083 were probed by host-induced gene silencing (HIGS) via a double-stranded (ds) RNA-mediated RNA interference (RNAi) approach. Foliar application of individual EpCSEP/CSP dsRNAs resulted in a marked reduction in PM disease symptoms. Microscopic and molecular studies also shows similar results, suggesting that these Ep CSEP/CSPs play major roles in pea PM infections. This study also recognizes and functionally validate candidate effectors from the agriculturally relevant pea PM, and highlights the utility of transcriptomics and HIGS to elucidate the key proteins associated with Ep pathogenesis (Sharma et al. 2019).

RNAi techniques like miRNA, siRNA and piRNA pathways have been utilized for developing pea aphid resistant lines in pea. The role of the genes were studied by targeting 25 core RNAi genes, which in turn were expressed at various developmental stages and in various tissues during aphid infestation (Yang et al. 2020). Pea pathogen resistance to mainly virus, fungus, bacteria and insects have been attained by genetic engineering techniques but due to prevalence of new strains and biotypes of these pathogens the resistance built-up gets broken. Continued research in developing molecular tools like gene-editing will help to overcome such challenges.

3.13 Role of Bioinformatics as a Tool

3.13.1 Gene and Genome Databases

Climate change indirectly affects pea yield by biotic stresses like insect-pests and diseases. Genomic resource for pea is very crucial for developing resistant varieties. Several genomic resources generated for pea include pea gene atlas (Alves-Carvalho et al. 2015), whole-genome polymorphism data for multiple genotypes, and BAC libraries developed for the genotype Cameor (http://cnrgv.toulouse.inra.fr/fr). Databases developed to access genome data includes NCBI (National Centre for Biotechnology Information), The Legume Information System (LIS) (Dash et al. 2016), URGI (INRA) and Pulse Crop Database (PCD).

3.13.2 Comparative Genome Databases

Comparative analysis helps to develop novel varieties using knowledge of known resistant genes for several biotic stresses like disease resistance and insect resistance in other species. Comparative studies have been reviewed in detail earlier and have revealed conservation between various pulses (mainly alfalfa, chickpea, pigeonpea, lentil and soybean) and pea (Tayeh et al. 2015a). Comparative analysis of known powdery mildew resistance between pea and other species supports loss of function mutation in *MLO* (*Mildew Resistance Locus O*) gene (Humphry et al. 2011). Alignment tools such as BLAST (Basic Local Alignment Search Tool) from NCBI database, help to identify gene orthologs in other species (Altschul et al. 1990). There are several other comparative genomic analysis tools likeInParanoid (Remm et al. 2001) and OrthoMCL (Li et al. 2003).

3.13.3 Gene Expression Databases

To study the expression of genes related to Biotic stresses transcriptome data provide good resource. The high quality pea transcriptome data was generated and raw reads were submitted to NCBI Sequence Read Archives (SRA) (http://www.ncbi.nlm.nih.gov/sra/). Assembly created for some data was submitted to NCBI Transcriptome Shotgun Assembly (TSA) database. This data can be used to do BLAST analysis to find candidate genes related to biotic stresses (Zhukov et al. 2015).

3.13.4 Protein or Metabolome Databases

PlantPReS (Plant stress proteome database: www.proteome.ir) was developed by Agricultural Biotechnology Research Institute of Iran (ABRII) which presently contains more than 35,086 entries from 577 articles which are manually curated and more than 10,600 unique proteins related to stress response (Mousavi et al. 2016). PSPDB: Plant Stress Protein Database was developed by inserting data which are manually curated proteins from UniProt. It involves experimentally validated plant proteins related to biotic and abiotic stresses. It is useful for predicting function of proteins related to stresses (Anil Kumar et al. 2014) (http://www.bioclues.org/pspdb/index.php). Functional analysis of protein can be done using InterPro. It classifies protein into families and predicts domains and other important sites by using predictive model which is known as signatures (Mitchell et al. 2019). Pfam is a protein family database used to study protein domains which provides information about protein function. Latest release of Pfam 33.1 contains 18,259 entries as per May 2020 data (El-Gebali et al. 2019). Protein Information Resource (PIR) provides resource for protein informatics which supports proteomic research. It maintains mainly three databases which are the Protein Sequence Database (PSD), the Non-redundant Reference (NREF) sequence database, and the integrated Protein Classification (iProClass) database (Wu et al. 2003). PROSITE database is used to analyze protein domain, families and functional sites. It contains patterns and profiles for protein families and domains which gives information like structure and function of proteins (Sigrist et al. 2013). RCSB PDB (Protein Data Bank) is a database which contains structure information for proteins that helps researchers to visualize 3D structures of experimentally determined proteins. Recently PDB has become more users friendly by developing high-speed NGL Viewer which helps to visualize 3D molecules in any web browser (Rose et al. 2017). Universal Protein Resource (UniProt) is used to analyze protein sequence and annotation data. It is collaboration between three major databases European Bioinformatics Institute (EMBL-EBI), the SIB Swiss Institute of Bioinformatics and the Protein Information Resource (PIR) (Magrane and Consortium 2011). KEGG (Kyoto Encyclopedia of Genes and Genomes) is the pathway database which contains cellular processes like cell cycle, signal transduction, metabolism, membrane transport represented in graphical format (Kanehisa and Goto 2000).

3.14 Social, Political and Regulatory Issues

3.14.1 Concerns and Compliance

Genetic engineering and genome editing in various crops have opened up possibilities of manipulating a plethora of traits which were difficult earlier to handle. Genome sequencing of pea has been published (Kreplak et al. 2019) and will help molecular

breeders to precisely edit the genes mainly dealing with biotic stresses. Biotic stress in pea is very complex which gets more complicated with climate change scenarios. Breeding of gene edited crops will be ascertained to the necessity which has arisen due to such situations, which will lead to more and more social, political and regulatory issues pertaining to utilization of genome edited crops. Gene editing techniques has its own advantages and drawbacks in crop breeding, which needs to be taken into account for better production of pea (Arora and Narula 2017). Gene editing has led to issuance of fresh guidelines of use and marketing of gene-edited crops or genetically modified organism (GMO) worldwide. As gene edited crops are more or less like naturally occurring mutants or artificially induced mutants, concern arises for better management of germplasm (Zong et al. 2019). As different countries have different regulatory mechanisms for GMOs, it is high time for evolving an integrated global regulatory mechanism for genome edited crops (Schmidt et al. 2020).

3.14.2 Intellectual Property Rights, Treaties and Conventions

Novel pea varieties developed needs to be registered and protected in international consortium for protection of breeder's rights. New crop mutants are now patented for various traits and are being registered under several regulatory mechanisms in different countries. Worldwide the food security with plant variety protection is overseen by International Union for the Protection of New Varieties of Plants (UPOV). UPOV oversees an international system of intellectual property (IP) rights that guards plant breeders' rights and reassure innovation in agriculture through the invention and development of novel varieties (Rivoire 2019). In India, plant varieties are registered under PPVFR Act 2001 (The Plant Variety and Farmers Rights Act), in which New variety, Extant variety, Farmer's variety and Essentially derived variety are the four varieties which get registered (PPVFR 2003). The authority has received around 1,200 applications for registration, with 284 new varieties application, 900 for existing varieties application and 9 farmers' variety applications. These includes pulse crops like garden pea, chick pea, pigeon pea, French bean, lentil, black gram, green gram; cereals like rice, maize, wheat, pearl millet, sorghum and other crops like cotton and jute (Kumaran and Sridharan 2009) (Fig. 3.9).

Farmer's rights towards their traditionally grown varieties and traditional knowledge are protected in India by Biodiversity Act 2002, which has been promulgated by National Biodiversity Authority (NBA), an autonomous and statutory body under Government of India. It provides equitable sharing of benefits arising from traditional biological resources not limited to plants only but other organisms as well (NBA 2002). NBA, India comes under multilateral United Nation Conventions on Biological Diversity (UNCBD) in which 196 countries are signatories. The national legislation has to be followed by all the parties—farmers, breeders and the marketing companies, which are very crucial for protecting farmer's rights. Participation of all the parties are essential to make sure the conservation and sustainable use of plant

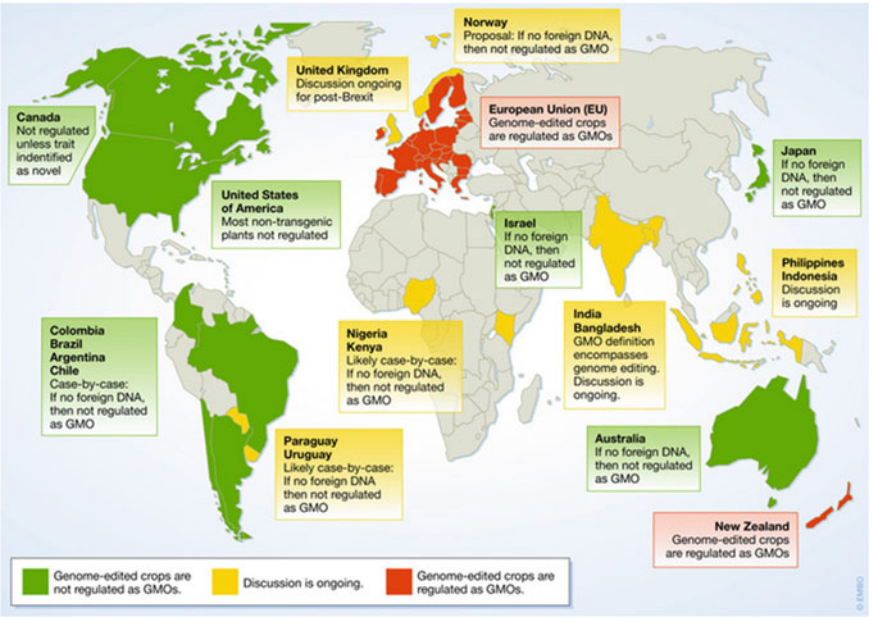

Fig. 3.9 Current situation of gene-edited crop regulations in various countries (Schmidt et al. 2020)

genetic resource are widely done and protected for sustainable development of the food and agriculture in the world (FAO 2009).

Mutual system of germplasm access and benefit sharing in food and forage crops has been established under the International treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). India is one of the signatory under ITPGRFA since 10th June, 2002. The main objective of the treaty are the protection and sustainable use of plant genetic resources for food and agriculture and the equal and equitable distribution of benefits resulting from their use for sustainable agriculture and food security, in accordance to UNCBD. ITPGRFA protected legumes covers about 29 food crops and 35 forage crop are referred in the treaty. Eight genera of pulse crops are included under these, namely *Cajanus, Cicer, Lathyrus, Lens, Phaseolus, Vigna, Vicia and Pisum* (Kochhar 2008).

3.15 Future Perspectives

Global warming and increasing human population has put a doublewhammy on the whole humanity but with the advent of modern biotechnological tools and improvement in the sector of artificial intelligence (AI) in agriculture, we could overcome these challenges faced today by the growers. Legumes are considered to be an affordable plant-based source for proteins, mainly among vegetarians. Pulse crops are

considered to be environmental friendly due to soil fertility enrichment due to its symbiotic nitrogen fixation capabilities. Importance of pulses has grown a lot as United Nations designated 2006 as the International year of Pulses, declaring the critical role of legumes in improving global food security. Various gaps and opportunities in pulse genetics research and relevance of pulse crop improvement in face of challenges like anthropogenic and climate change has to be addressed in future (Sahruzaini et al. 2020).

Lowering crop productivity and the increasing attacks of diseases and pest has made us to think more deeply with the help of precision agriculture. AI in agriculture is now being utilized in the area of weather forecasting and predictions, soil health (temperature and pH), water utility for healthy crop production, and detection of pest and diseases and application of pesticides and herbicides for maintaining better quality of crop produce (Talaviya et al. 2020). Utilization of drones for continuously monitoring the disease and pest spread helps by utilizing better IPM strategy in early phases.

Advent of novel software and hardware development in the field of AI will help the future farmers to be more climate change resilient. New-age Agri-entrepreneurs are making the most of AI and machine learning and taking the precision agriculture to new level. The number of modern age farmers becoming more tech-savvy will enhance the crop efficiency, crop productivity, and speeding up the agricultural finance for better outcome from agriculture (Faggella 2020). Agriculture Data analytics is an emerging area which helps growers to address the farm management issues by implementing improved IPM strategies for sustainable agriculture development (Coble et al. 2018).

Machine learning (ML) and deep learning is a new age concept for improving crop productivity by utilizing robotics through conventional learning process. It includes set of attributes or characteristics which are uploaded to software for analyzing the data for particular trait in crops or policy decisions for better crop management (Mcqueen et al. 1995; Raj et al. 2015; Rana and Miller 2019). Modern robotics for monitoring and analyzing the crop data for improved crop productivity will be new norm in the future farms which will effectively tackle both abiotic and biotic stress challenges (Kamilaris and Prenafeta-Boldú 2018).

Various types of morpho-physiological traits for both abiotic and biotic stress tolerance together were observed in different crop varieties including pea (Pandey et al. 2017). Combined adverse effect on crop productivity was observed due to abiotic stress circumstances like drought, salinity and extreme temperatures impacting the incidence and spread of microbes and insects (Scherm and Coakley 2003). Effects of combined stress from both abiotic and biotic stresses are mostly deleterious for crop survival itself, but sometimes genetic response curtailing both stresses are observed (Atkinson et al. 2013). Introduction of new species in non-conventional production areas are good for climate change responses but long-term repercussions in agronomy and ecology is expected (Peters et al. 2014).

Speed breeding is another recent advancement in the crop improvement research, mainly in case of shortening the breeding cycle and accelerating crop research through rapid advancement in generation time. Methods by speed breeding could

be achieved are, increasing the daily exposure of plants to light, early seed harvest, speedup cycle of seed to seed, thereby generation time is decreased for day-neutral or long-day crops. Crops like wheat, barley, chickpea, pea, and quinoa were raised under speed breeding conditions and enhanced productivity and increased generation cycles were achieved. Inclusion of next generation sequencing, genome editing and genetic selection, with speed breeding will speed-up the crop improvement rate (Watson et al. 2018; Ghosh et al. 2018).

Overall the impact of climate change has aggravated the infection levels of various pathogens. A combined effort from the conventional breeding with utilizing the modern biotech breeding tools along with nanotechnology and speed breeding will help modern day molecular breeder to design climate resilient pea varieties biotic stress ready.

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