Chapter 8
Molecular Breeding for Resistance to Economically Important Diseases of Pulses

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Abbreviations

AFLP	Amplified Fragment Length Polymorphism
BC	Backcross
BSA	Bulked Segregant Analysis
DAF	DNA Amplification Fingerprinting
DArT	Diversity Arrays Technologies
DHL	Doubled Haploid Lines
ELISA	Enzyme-Linked Immunosorbent Assay
EST	Expressed Sequence Tags
GWAS	Genome-Wide Association Studies
ICRISAT	International Crops Research Institute for the Semi-Arid Topics
InDel	Insertion-Deletion
ISSR	Inter Simple Sequence Repeat
MAB	Marker-Assisted Breeding
MABC	Marker-Assisted Backcrossing
MAGIC Population	Multiparent Advanced Generation Intercross Population
MAS	Marker-Assisted Selection

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NAM Population	Nested Association Mapping Population
NIL	Near-Isogenic Lines
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RGC	Resistance Gene Candidates
RIL	Recombinant Inbred Lines
SCAR	Sequence-Characterized Amplified Region
SNP	Single Nucleotide Polymorphism
SRAP	Sequence-Related Amplified Polymorphism
SSR	Simple Sequence Repeat
STMS	Sequence-Tagged Microsatellite Sites
STS	Sequence-Tagged Sites
TRAP	Target Region Amplification Polymorphism

8.1 Introduction

Plants are continuously threatened by various pathogens in the environment. In natural condition, some of the wild plants have intrinsic resistance power which helps them to resist such attack for survival (Tanksley and McCouch 1997; Maxted and Kell 2009). Whenever plants got domesticated and further improved for yield attributing traits by humans, they gradually lost the resistance and hence became vulnerable to pathogen attacks (Warschefsky et al. 2014). Even though they contain certain resistance genes, the genetic resistance can also be overcome by the newly evolved strains of pathogen. This continuous co-evolution phenomenon between crop plants and their pathogen demands sustainable plant breeding efforts to generate newer crop varieties or to pyramid resistance genes in well-adapted varieties (Mundt 2014). Another concern is the expected increase in climatic variability (IPCC 2012), which could enhance the occurrence of pathogens in a given locality. Host plant resistance is generally the most favorable control method for environmental, economical, and social reasons (Mundt 2014). Conventional plant breeding method has helped till now to tackle this problem. But demand for newer resistant crop varieties has to be made within a short time frame. Molecular breeding or marker-assisted breeding (MAB) has ample potential to ease such problem and tackle it in a more efficient manner within a shorter time period than conventional breeding (Varshney et al. 2014a, b). Moreover, the selection of resistant plants can easily be achieved without the disease occurrence in the field in MAB. Marker-assisted gene pyramiding is a method of choice for gene stacking within an adapted variety without the need of screening against multiple races of pathogen in different environments.

Pulses are important food crops that balance our diet and are the main principal protein source for the semi-arid topical region of the world. In farming system, pulses are very important crop for restoration of soil fertility and maintenance of

soil health (FAO 2016). Among the major pulses grown in the world, chickpea (*Cicer arietinum* L.), pigeon pea [*Cajanus cajan* (L.) Millsp.], mung bean [*Vigna radiata* (L.) Wilczek], black gram [*Vigna mungo* (L.) Hepper], cowpea [*Vigna unguiculata* (L.) Walp.], lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.), and common bean (*Phaseolus vulgaris* L.) are the important sources of protein for humans. Based on their climatic condition for growth, they are divided into tropical pulse crops (pigeon pea, mung bean, urd bean, cowpea, common bean, etc.) and temperate pulse crops (chickpea, lentil, pea, etc.). These pulses are damaged by several plant pathogens that include virus, bacteria, fungus, and pathogenic weed species. Of them, yellow vein mosaic virus is a common problem for tropical legumes like mung bean, urd bean, and cowpea. Both chickpea and lentil are heavily damaged by *Ascochyta* blight. *Fusarium* wilt is a common problem for both chickpea and pigeon pea. Sterility mosaic by a virus is an endemic problem in pigeon pea cultivation in subtropics. To rescue the pulse production from these plant pathogens, the development of resistant cultivars in the above pulse crops is an immediate need. Recent genome sequencing initiative in major pulse crop has generated immense marker data and molecular breeding or genomics platform. The usage of those has helped to generate fewer improved varieties and has great scope in future toward the development of disease-resistant cultivars of pulses. We will discuss here on recent developments and progress on molecular breeding for disease resistance in these pulse crops.

8.2 Development of Molecular Markers in Pulse Crops

DNA-based markers have shown great promises in expediting plant breeding methods. At the present time, exploitation of genetic markers is not a dream to a traditional plant breeder since it is used worldwide in all major cereal crops as a component of plant breeding because of the availability of a large amount of basic genetic and genomic resources (Gupta et al. 2010). In the past few years, major emphasis has also been given to develop similar kind of genomic resources for improving productivity and developing resistance for diseases of pulse crops (Varshney et al. 2009). The use of molecular marker technology can give real output in terms of high-yielding genotypes in pulses because high phenotypic instability for important traits makes them difficult for improvement through conventional breeding methods.

8.2.1 Establishment of Mapping Population

In genetics and breeding, mapping populations are the tools used to identify the genetic loci controlling measurable phenotypic traits. Mapping population is a group of individuals on which genetic analysis is carried out. The decisions on the selection of parents and mating design used for the development of a mapping

population depend mainly on the objectives of the study. The parents of mapping populations must have sufficient variation for the traits of interest at both the DNA sequence and the phenotype level. Mapping population may comprise F_2, backcross (BC), recombinant inbred lines (RIL), doubled haploid lines (DHL), F_2 derived F_3 (F_2:F_3) populations, and near-isogenic lines (NILs). F_2, backcross, and recombinant inbred are the three primary types of mapping populations used for molecular mapping of any traits. In other cases, DHLs are also the products of one meiotic cycle and hence comparable to F_2 in terms of recombination information. DHLs are permanent mapping population and hence can be replicated and evaluated over locations and years and maintained without any genetic change like in RIL. It provides opportunity to induce homozygosity in single generation and instant production of homozygous lines. Since it involves in vitro techniques, relatively more technical skills are required in comparison with the development of other mapping populations. Till now, suitable culturing methods, organogenesis, and haploid production methods are not available for most of the pulse crops, and hence, successful production of DHLs in this crop is not reported much.

A mapping population is essential to develop tightly linked molecular markers for disease resistance gene in any crops. To develop a mapping population, two diverse genotypes should be crossed to each other, and at the same time, they should not be too genetically distant so as to a) cause sterility of the progenies and/or b) show very high levels of segregation distortion during linkage analysis. Thus, wide hybridizations (interspecific or intraspecific crosses) are needed. For example, a chickpea RIL population was made by crossing *Cicer arietinum* x *Cicer reticulatum* (Ratnaparkhe et al. 1998; Winter et al. 2000; Cobos et al. 2005). Similarly *Cicer arietinum* and *Cicer echinospermum* were crossed to produce a mapping population for identification of marker for *Ascochyta* blight resistance (Collard et al. 2003). Eujayl et al. (1998) used an RIL population to identify molecular markers linked to the single dominant gene conditioning *Fusarium* vascular wilt resistance, while Gupta et al. (2008) has developed an RIL population in black gram by crossing a cultivated black gram (*Vigna mungo*) variety with *Vigna mungo* var. *silvestris* for the development of first linkage map in this crop. Sometimes two morphologically distinct genotypes can also be crossed to produce a population like in Desi × Kabuli (Iruela et al. 2006; Tar'an et al. 2007) or Desi × Desi (Radhika et al. 2007) crosses in chickpea. Among the various mapping populations, F_2 population (Bohra et al. 2012), BC population (Skiba et al. 2004; Kongjaimun et al. 2012), and RIL population (Dhole and Reddy 2013; Bhadauria et al. 2017) were much used in pulses. Pulses are generally self-pollinated crops, and thus, artificial hybridization is needed to develop above kind of population for genetic and QTL mapping. The usage of association mapping population is another way to reveal high resolution markers for *Aphanomyces* root rot disease resistance in pea (Dasgroux et al. 2016), *Fusarium* root rot in pigeon pea (Patil et al. 2017), and anthracnose and angular leaf spot resistance in common bean (Perseguini et al. 2016). In a recent example, high resolution mapping for *Ascochyta* blight resistance in pea was achieved by using a heteroge-

neous inbred family's population (Jha et al. 2017). The use of MAGIC and NAM population is another way to capture panoramic view of genetic factors affecting the disease resistance in different pulse cops (Jha et al. 2017).

8.2.2 Development of Genetic Maps

A genetic map, or linkage map, is a map of the frequencies of recombination that occurs between tested markers on homologous chromosomes during meiosis. Recombination frequency between two markers is proportional to the distance separating the markers. The greater is the frequency of recombination, the greater is the distance between two genetic markers. Thus, a genetic map is a depiction of recombination events and frequencies, rather than a physical map. An appropriate mapping population, a suitable marker system, and the software for analyses of data are the key requirements for constructing a genetic linkage map. Genetic map construction requires (i) selection of the most appropriate mapping population(s), (ii) calculation of pairwise recombination frequencies using the population, (iii) establishment of linkage groups and estimation of map distances, and (iv) determination of map order.

Genetic maps are the prerequisite for the identification of linked markers or QTLs for a particular disease resistance in any crops except in association mapping. Projects on development of genetic maps of pulses had started during the 1990s. The first genetic linkage map on pulse crop was made during the 1990s. Havey and Muehlbauer (1989) developed a genetic linkage map of lentil followed by the genetic map of garden pea (Weeden and Wolko 1990). Later, an integrated genetic map was made in chickpea by Simon and Muehlbauer (1997). These maps were later improved by incorporation of new markers in them although the genetic linkage map targeted for tagging of disease resistance gene was started later. Genetic maps targeting for *Ascochyta* blight in chickpea were developed in different years by several scientists (Lichtenzveig et al. 2006; Tar'an et al. 2007; Sabbavarapu et al. 2013). Similarly, transcriptome sequencing studies of lentil have generated an EST database which has delivered large numbers of EST-derived SSR and SNP markers (Kaur et al. 2014). These sequences-derived marker systems have been used to construct dense genetic linkage maps and to identify QTLs for disease resistance in the past few years (Kaur et al. 2014). Further sequence-linked genetic markers facilitated the identification of bridging loci between population specific genetic maps and subsequent integration to produce high-density consensus maps in lentil (Sudheesh et al. 2016). Molecular maps were also developed in cowpea by using various markers like RFLP (Young 1999), AFLP and RAPD (Ouedraogo et al. 2002), SNP (Xu et al. 2011; Muchero et al. 2009; Lucas et al. 2011), and SSR (Anadrgie et al. 2011; Kongjaimum et al. 2012) either in F_2 or RIL populations.

8.2.3 Screening for Disease Resistance

Screening of plants for a particular disease resistance could be achieved by field screening, green house screening, laboratory screening, and bioassay techniques. Since in actual field situation different strains/races of plant pathogens are present, it is wise to screen disease resistance of plant based on multi-environment field screening. It gives an idea about the reaction of plant genotypes to a particular disease in a particular environment, and often, plants with horizontal resistance against a particular disease got isolated through this technique. Thus, plant breeders mostly follow this screening technique in disease resistance breeding scheme. In actual field conditions, a disease was evaluated based on various disease scales (depending on the plant and its type of disease). The disease scale was normally developed by the plant pathologist, and it was based on the percent disease incidence of plant (Reddy et al. 1994). For uniform pathogen distribution around field, infector row (for aerial pathogens) or sick plots (for soil-borne pathogens) must be there in the field-based screening (Rana et al. 2013). Eujayl et al. (1998) demonstrated the use of sick plot technique for screening of mapping population of lentil against *Fusarium* wilt disease toward the study of its genetics and marker development.

But often, field-based disease screening gives error-prone result due to complex interaction of host, pathogen, and environment. The actual susceptible plant may escape the disease symptoms, and hence, interpretation may be wrong. In sick plots or field, there will be always a risk that multiple soil-borne diseases could be present at the same time and interfere with the disease assessment. To meet out this problem, one can go for screening for disease resistance under controlled conditions, i.e., greenhouse or laboratory conditions (Infantino et al. 2006). In such cases, green house screening or laboratory screening or bioassay-based technique is followed. To do so, disease inoculum must be mass multiplied in the laboratory, and certain amount of inoculum should be either injected or sprayed to the plants in congenial weather condition inside the green house or in the laboratory. A mini-dome technique (Chen and Muehlbauer 2003) was used to measure pathogenic variation of different isolates of *Didymella rabiei* for *Ascochyta* blight disease of chickpea by spraying 2×10^5 pycnidiospores ml^{-1} over the plants (Chen et al. 2004), whereas "cloth chamber screening technique" was followed to screen different accession of wild *Cicer* species against *Ascochyta* blight (Gurha et al. 2003). Some of the obligate pathogens may not be culturable, and thus, they cannot be mass multiplied easily. In such cases, a disease should be maintained in susceptible host throughout the year, and infector rows should be maintained in the green house or laboratory condition for spreading of the disease. Another easy protocol called excised/detached leaf technique was also used in mung bean (Reddy et al. 1987) and pea (Warkentin et al. 1995; Fondevilla et al. 2006) for screening powdery mildew resistance in laboratory condition.

In the case of plant virus, different bioassay techniques along with controlled growth facilities are involved in screening for virus resistance in plants. Along with typical symptoms, the resistance screening for virus particle may also involve

ELISA, PCR, and real-time PCR technique to determine the titer value of the virus particle inside the infected plant tissue. Moreover, artificial inoculation based on agro-inoculation technique has been widely used to screen MYMV resistance in mung bean and other pulses (Mandal et al. 1997). In some viral diseases, it is important to inoculate the test plant with the vector for spreading the disease. Such a situation demands "infector hedge row" and "leaf stapling technique" which was followed to screen genotypes resistant to sterility mosaic disease in pigeon pea (Nene and Reddy 1976). The use of hardcore molecular technique toward screening of disease resistance in pulse crop is no longer a dream now. Ghosh et al. (2017) used loop-mediated isothermal amplification (LAMP) assay that targets fungal specific 5.8 S rDNA and partial ITS (internal transcribed spacer) region for visual detection of *Rhizoctonia bataticola* causing dry root rot of chickpea.

8.2.4 Identification of Molecular Markers for Important Disease Resistance in Major Legume Crops

In general, identification of molecular markers for any disease resistance demands development of a segregating mapping population for disease reaction, genotyping of the population with molecular markers, and analysis toward marker identification. Using these approaches, different markers were identified in pulse crops for different disease resistance (Table 8.1). The details of those will be covered "Successful Examples in Tropical/Temperate Pulse Crops" in this chapter.

8.3 Exploitation of Linked Molecular Markers in Marker-Assisted Breeding

8.3.1 Example for MAS in Chickpea

Sequence-tagged microsatellite markers have been used for MAS to isolate near-isogenic lines in chickpea. The SSR markers tightly linked to *foc5* (TA59) and $foc0_1$ (TR59, TS35) were used to assist selection of resistant and susceptible genotypes toward the development of NILs in chickpea (Castro et al. 2010; Jendoubi et al. 2016). MABC lines resistant to *Fusarium* (*foc1* and *foc3*) and *Ascochyta* blight were developed in the genetic background of C 214 cultivar at ICRISAT (Varshney et al. 2014). Similarly, MABC lines resistant to *foc4* were developed in the genetic background of JG 74, Phule G12, and Annigeri 1 at various agricultural universities in India. Another set of MABC lines resistant to *foc2* has been developed in the background of Pusa 256 at Indian Institute of Pulses Research, Kanpur, India (Varshney et al. 2013; Saxena et al. 2016).

Table 8.1 List of identified QTLs and linked markers for various diseases of pulse crops

S. no.	Crop (s)	Trait(s)	QTLs/genes	Type of marker(s)	Reference(s)
1	**Chickpea (*Cicer arietinum* L.)**				
	1.1	*Ascochyta* blight	QTL-1, QTL-2	RAPD and ISSR	Santra et al. (2000)
			QTLs	RAPD	Millan et al. (2003)
			Ar19	RAPD	Rakshit et al. (2003)
			QTLar2b	SSR	Udupa and Baum (2003)
			Ar19	STMS	Cho et al. (2004)
			QTLar1, QTLar2	SSR	Iruela et al. (2006)
			QTL	SSR	Tar'an et al. (2007)
			QTL_{AR3}	SSR	Iruela et al. (2007, 2009)
			QTL2	SSR	Kottapalli et al. (2009)
			QTL	SSR	Anbessa et al. (2009)
			QTL	STMS	Aryamanesh et al. (2010)
			AB-Q-SR-4-1	SSR	Sabbavarapu et al. (2013)
			QTLs	SNP	Daba et al. (2016)
	1.2	*Fusarium* wilt	*Foc3*	SSR	Sharma et al. (2004, 2005)
			TR59	STMS	Cobos et al. (2005)
			QTL_{foc02}, QTL_{foc5}	SSR	Cobos et al. (2009)
			Foc1, foc2, Foc3	SSR	Gowda et al. (2009)
			FW-Q-APR – 6-2	SSR	Sabbavarapu et al. (2013)
			Foc-1	STMS	Barman et al. (2014)
			QTL (GSSR 18-TC14801)	SSR	Jingade and Ravikumar (2015)
			QTL_{CaLG02}	SSR and SNP	Garg et al. (2018)

(continued)

Table 8.1 (continued)

S. no.	Crop (s)	Trait(s)	QTLs/genes	Type of marker(s)	Reference(s)
2	**Pea (*Pisum sativum* L.)**				
	2.1	Powdery mildew	*er*	SCAR	Janila and Sharma (2004)
			er	RFLP	Dirlewanger et al. (1994)
			er (Sc-OPO-18$_{1200}$)	RAPD/SCAR	Tiwari et al. (1998)
			er (OPD-10$_{650}$)	RAPD	Timmerman et al. (1994)
			er (PSMPSAD60, PSMPSAA374e, PSMPA5, PSMPSAA369, PSMPSAD51	SSR	Ek et al. (2005)
			er1-6	SNP	Sun et al. (2016)
			er-1	STMS	Frew et al. (2002)
	2.2	*Ascochyta* blight	QTLs	SSR, RAPD, and CAPS	Miranda (2012)
			QTLs	STS	Timmerman-Vaughan et al. (2004)
			Asc2.1, Asc4.2, Asc4.3 and Asc7.1	Candidate defense-related sequences	Timmerman-Vaughan et al. (2016)
			QTL abIII-1 and abI-IV-2	SNP	Jha et al. (2016)
			abI-IV-2.1 and abI-IV-2.2	SNP	Jha et al. (2017)
			QTLs	SSR	Tar'an et al. (2003a)
			QTLs	SNP	Jha et al. (2015)
			MpII.1, MpIII.5, MpV.2 and MpV.3	SNP	Carrillo et al. (2014)
	2.3	*Fusarium* wilt	*Fw*	RAPD	Dirlewanger et al. (1994)
			Fw	RAPD, AFLP	McClendon et al. (2002)
			Fnp	SSR and RAPD	McPhee et al. (2012)
			Fw_Trap_480, Fw_ Trap_340, and Fw_Trap_220	SCAR	Kwon et al. (2013)
	2.4	Pea common mosaic virus	*mo*	RFLP	Dirlewanger et al. (1994)
	2.5	Pea rust	*Up1*	RAPD	Barilli et al. (2010)
	2.6	Pea seed-borne mosaic virus	*Sbm-1*	STS	Frew et al. (2002)

(continued)

Table 8.1 (continued)

S. no.	Crop (s)	Trait(s)	QTLs/genes	Type of marker(s)	Reference(s)
3	**Lentil (*Lens culinaris*)**				
	3.1	*Ascochyta* blight	QTL	RAPD	Ford et al. (1999)
			Ra/2	RAPD, SCAR	Chowdhery et al. (2001)
			QTL1 QTL 2	RAPD, ISSR, RFLP, AFLP	Tar'an et al. (2003a)
			QTL-1-5 QTL-6-8	RAPD, ISSR, AFLP	Rubeena et al. (2006)
			QTL	RAPD, AFLP, SSR	Tullu et al. (2006)
			QTL 1	EST-SSR/SSR, ISSR, RAPD, ITAP	Gupta et al. (2012)
			AB_IH1 *AB_IH1.2* *AB_NF1*	Genomic DNA-derived SSR, –EST-SSR, SNP	Sudheesh et al. (2016)
	3.2	*Fusarium* wilt	*fw*	RAPD	Eujayl et al. (1998)
			fw	AFLP, SSR	Hamwieh et al. (2005)
	3.3	Anthracnose	*LCt-2*	AFLP, RAPD	Tullu et al. (2003)
			LCt-2, OP-P4$_{400}$	AFLP, RAPD	Tullu et al. (2006)
4	**Common bean (*Phaseolus vulgaris*)**				
	4.1	Common bacterial blight	QTL	RAPD, SCAR, STS, SSR, RFLP	Tar'an et al. (2001)
			QTLs	SSR, SCAR	Zhu et al. (2016)
			QTL	RFLP	Lopez et al. (2003)
	4.2	Bean common mosaic virus	QTL-I	RAPD	Jung et al. (1996)
			QTLs	RAPD	Miklas et al. (1996)
				SCAR	Melotto et al. (1996)
	4.3	Anthracnose	*Are gene*	SCAR	Adam-Blondon et al. (1994)
			QTLs/genes	SSR	Choudhary et al. (2018)
			QTLs/genes	CAPS, SCAR, RAPD	Boersma et al. (2013)
			QTLs	SNP, SSR	Perseguini et al. (2016)
	4.4	White mold	QTLs	RAPD, AFLP	Kolkman and Kelly (2003)

(continued)

Table 8.1 (continued)

S. no.	Crop (s)	Trait(s)	QTLs/genes	Type of marker(s)	Reference(s)
			QTLs	SSR, AFLP, and SRAP	Lara et al. (2014)
			WM1.1, WM2.2, WM3.1, WM5.4, WM6.2, WM7.1, WM7.4, WM7.5, and WM8.3	SNP	Vasconcellos et al. (2017)
			WM2.2, WM8.3, and WM7.3	SRAP and RAPD	Soule et al. (2011)
	4.5	*Fusarium* wilt	*PvPR1, PvPR2*	RAPD	Schneider et al. (2000)
			QTLs	SNP	Hagerty et al. (2015)
			QTLs	RAPD	Fall et al. (2001)
	4.6	Root rot	QTLs	SNP	Hagerty et al. (2015)
			QTLs	SSR	Kamfwa et al. (2013)
			QTLs	RAPD	Schneider et al. (2000)
	4.7	Angular leaf spot	QTL	SSR	Teixeira et al. (2005)
			QTL ALS11^{AS}	SNP, SSR	Bassi et al. (2017)
			ALS10.1^{DG,UC}, ALS5.2	SSR	Oblessuc et al. (2012)
			ALS	SSR	Teixeira et al. (2005)
			ALS4.1$^{GS,\ UC}$	SSR, Tm markers	Keller et al. (2015)
			QTLs	SNP, SSR	Perseguini et al. (2016)
			ALS	RFLP	Lopez et al. (2003)
	4.8	Rust	*Ur-3*	SNP, SSR	Hurtado-Gonzales et al. (2017)
			Ur-13	SCAR	Mienie et al. (2005)
			Ur-7	RAPD	Park et al. (2004)
	4.9	Powdery mildew	*PWM2^{AS} and PWM11^{AS}*	SNP, SSR	Bassi et al. (2017)
5	**Mung bean (*Vigna radiata* L.)**				
	5.1	Powdery mildew	*qPMR-1 and qPMR-2*	SSR	Kasettranan et al. (2010)
			QTLs	RFLP	Humphry et al. (2003)

(continued)

Table 8.1 (continued)

S. no.	Crop (s)	Trait(s)	QTLs/genes	Type of marker(s)	Reference(s)
			QTLs	RAPD, CAP, AFLP	Chen et al. (2007)
	5.2	Mung bean Yellow mosaic India virus	*qYMIV1, qYMIV2, qYMIV3, qYMIV4, and qYMIV5*	SSR	Kitsanachandee et al. (2013)
			OPB07-SCAR_583 (MYMVR-583)	SCAR	Dhole and Reddy (2013)
	5.3	*Cercospora* leaf spot	*qCLS*	SSR	Chankaew et al. (2011)
6	**Black gram (*Vigna mungo* L. Hepper)**				
	6.1	Yellow mosaic virus	Monogenic	STS-RGA	Basak et al. (2004)
	6.2	Mung bean Yellow mosaic India virus	QTL	SSR and RGH markers	Anjum et al. (2010)
	6.3	Powdery mildew	QTL	SSR and RGH markers	Anjum et al. (2010)
7	**Faba bean**				
	7.1	Faba bean rust	*Uvf-1*	RAPD	Avila et al. (2003)
	7.2	*Ascochyta* blight	QTL-1, QTL-2, QTL-3, QTL-4,	SNP, EST-SSR	Kaur et al. (2014)
			Af-1, Af-2, Af-3,	SSR	Atienza et al. (2016)
			Af-1, Af-2	RAPDs, isozymes, ESTs, SCAR, SSRs, STSs, and intron-spanning markers	Díaz-Ruiz et al. (2009)
8	**Cowpea [*Vigna unguiculata* (L.) Walp.]**				
	8.1	Cowpea rust	*Ruv1, Ruv2, Ruv3,*	SNP	Wu et al. (2017)
			QTLs	SSRs	Uma et al. (2016)
	8.2	Cowpea bacterial blight	*CoBB-1, CoBB-2*	SNP	Agbicodo et al. (2010)
	8.3	Cowpea golden mosaic virus	QTLs	AFLP	Rodrigues et al. (2012)
	8.4	Fusarium wilt resistance (*Fot race 3*)	QTLs	SNP	Pottorff et al. (2012)
	8.5	*Fusarium* wilt resistance (*Fot race 4*)	QTLs	SNP	Pottorff et al. (2014)

(continued)

Table 8.1 (continued)

S. no.	Crop (s)	Trait(s)	QTLs/genes	Type of marker(s)	Reference(s)
9	**Pigeon pea (*Cajanus cajan* L. Millsp.)**				
	9.1	Sterility mosaic disease	*qSMD3 qSMD4 qSMD5 qSMD6*	SSR	Gnanesh et al. (2011)
			C.cajan_01839	SNP	Singh et al. (2016a, b)
			CcLG11	SNP	Saxena et al. (2017b)
	9.2	*Fusarium* wilt	*Fw Gene*	RAPD	Kotresh et al. (2006)
			C.cajan_03203	SNP	Singh et al. (2016a, b)
			qFW11.1, qFW11.2 and qFW11.3	SNP	Saxena et al. (2017a)
10	**Lathyrus (*Lathyrus sativus* L.)**				
	10.1	*Ascochyta* blight	QTL	RAPD, STMS	Skiba et al. (2004)

8.3.2 Examples of MAS in Common Bean

Most of the breeding programs for common bean improvement in the world attempted to bring resistance against bean common mosaic virus (BCMV) in most of the released cultivars. Melotto et al. (1996) has developed a SCAR marker SW13 which was found linked to the dominant BCMV resistance I gene in this crop. This SW13 SCAR was much used in various breeding programs to introduce dominant resistance in common bean (Miklas et al. 2006). Similarly SR2 SCAR has been very useful for bringing in bean golden yellow mosaic virus resistance in this plant (Blair et al. 2007; Beebe 2012). A marker SU91 is reported to be linked to a QTL for common bacterial blight (CBB) resistance on linkage group B8. The marker BC420 is linked to another QTL for CBB resistance in B6 linkage group (Miklas et al. 2000; Pedraza et al. 1997; Yu et al. 2000). O'Boyle et al. (2007) demonstrated the usage of those SCAR markers SU91 and BC420 for the successful isolation of CBB resistant lines from 93 $F_{3:4}$ single plant selections. Various resistant common bean germplasm like advanced cranberry, pinto, great northern, and snap bean with resistance to CBB have been developed in the USA using MAS approach (Miklas et al. 2006). In the recent past, three major rust resistance genes, *Ur-5*, *Ur-11*, and *Ur-14*, were pyramided into a high yielding common bean variety "Carioca" through marker-assisted backcrossing method. This improved varieties used to be most consumed in Brazil and representing around 70% of their internal market (Souza et al. 2014).

8.3.3 MAS in Cowpea

Striga, a parasitic weed of cowpea, is important in African countries. Different QTLs conferring *Striga* resistance were identified by using AFLP and SCAR markers (Ouédraogo et al. 2002; Boukar et al. 2004). The large numbers of molecular markers developed for this resistance trait have been used for marker-based backcrossing incorporating foreground and background selection for improved version of local cultivars. At International Institute of Tropical Agriculture, IT93K-452-1 and IT89KD-288 were officially released varieties that are being improved for *Striga* resistance through MAS (Boukar et al. 2016).

8.4 Successful Examples in Tropical Pulse Crops

8.4.1 Mung Bean and Black Gram

Mung bean (*Vigna radiata* (L.) Wilczek) and black gram (*V. mungo* (L.) Hepper) are important legume crops widely cultivated in Indian subcontinent. Low productivity is a major concern in these crops. Of the various agronomic factors, biotic stresses are also responsible for this low productivity. Among biotic stresses, yellow mosaic disease (YMD) caused by mung bean yellow mosaic virus (genus *Begomovirus*, family *Geminiviridae*), powdery mildew (PM) caused by fungus *Erysiphe polygoni* DC., and *Cercospora* leaf spot (CLS) caused by *Cercospora canescens* Illis & Martin are the most important diseases which reduced seed yield considerably depending on the stage at which plant gets infected (Khattak et al. 2000; Pandey et al. 2009). Pathogens of all three diseases are obligate parasites and hence cannot be grown and maintained on the artificial media. In this case, marker-assisted selection will be very useful for development of resistant varieties to diseases like YMD and PM in both mung bean and black gram. Genomic resources are required for tagging the disease resistance genes and their transfer through marker-assisted selection. Until recently genomic resources were very scarce in these neglected pulse crops. The estimated genome size of mung bean and black gram is 579 Mbp (0.60 pg/IC) and 574 Mbp (0.59 pg/IC), respectively (Arumuganathan and Earle 1991). After the availability of mung bean SSR markers, the gene tagging and linkage analysis has started (Kumar et al. 2002a, b; Miyagi et al. 2004; Gwag et al. 2006), which was further strengthened after the availability of 100 Mb genome sequence information of mung bean (Tangphatsornruang et al. 2009). With the availability of draft genome sequence of mung bean, there is an enough scope for acceleration of marker-assisted breeding program in both mung bean and black gram (Kang et al. 2014). Recently, the 993 genic-SSR markers were designed successfully in black gram from immature seed transcriptome (Souframanien and Reddy 2015).

Yellow Mosaic Disease (YMD) In the case of YMD, the virus is not transmitted by sap or seed but transmitted only by insect vector whitefly (*Bemisia tabaci*). Hence, it cannot be created artificially, and screening entirely depends on field screening at hot spot by infector row method. The two different strains, i.e., MYMV and MYMIV, are reported in Indian subcontinents (Hussain et al. 2004; Pant et al. 2001; Ilyas et al. 2010), which leads to further complications in screening for virus resistance. Resistance to YMD in mung bean was reported to be controlled by a single recessive gene (Malik et al. 1986; Reddy and Singh 1995; Saleem et al. 1998; Basak et al. 2004; Reddy 2009), a dominant gene (Sandhu et al. 1985), two recessive genes (Verma and Singh 1988; Pal et al. 1991; Ammavasai et al. 2004), and complementary recessive genes (Shukla and Pandya 1985). In black gram, YMD resistance is reported to be governed by single recessive gene (Souframanien and Gopalakrishna 2006; Kundagrami et al. 2009) and two recessive genes (Verma and Singh 1986). The RAPD markers linked to YMD resistance gene were identified in mung bean (Selvi et al. 2006; Dhole and Reddy 2013) and further converted to SCAR markers (MYMVR-583) for better reproducibility in MAS (Dhole and Reddy 2013). In black gram, ISSR marker linked to YMD resistance was developed into SCAR marker and validated in different resistant black gram genotypes (Souframanien and Gopalakrishna 2006). The resistant gene analog (RGA) markers YR4 and CYR1 were found associated with resistance to YMD in black gram (Maiti et al. 2011). Before the availability of SSR markers in these crops, the markers from cowpea, azuki bean, and common bean were found to be useful in both mung bean and black gram (Gupta and Gopalakrishna 2009; Gupta and Gopalakrishna 2010). The cowpea SSR marker CEDG180 was found to be associated with YMD resistance in black gram (Gupta et al. 2013). For MYMIV resistance, three QTLs, i.e., *qYMIV1*, *qYMIV2*, and *qYMIV3* in India and two QTLs, i.e., *qYMIV4* and *qYMIV5* in Pakistan were identified through composite interval mapping of mung bean (Kitsanachandee et al. 2013). AFLP and SSR markers were used for identification of four major QTLs for MYMIV resistance (Chen et al. 2013). Three markers, ISSR 811_{1357}, YMV1-FR, and CEDG180 were found to discriminate the YMV resistant and susceptible black gram genotypes which can be used for MAS (Gupta et al. 2015).

Powdery Mildew The second most important disease of mung bean and black gram is powdery mildew which can be screened in field as well as in laboratory conditions by using excised leaf technique (Reddy et al. 1987). Three independent dominant genes (Pm_1, Pm_2, *and* Pm_3) governing resistance reaction to powdery mildew disease were identified in mung bean at Bhabha Atomic Research Centre, Mumbai, India (Reddy 2007; Reddy 2009). The RFLP markers were the first markers used in mung bean for identification of linkage between a major powdery mildew resistance locus and the marker (Humphry et al. 2003), while two QTLs, i.e., *qPMR-1* and *qPMR-2*, for powdery mildew resistance were reported in mung bean (Kasettranan et al. 2010). The SSR markers DMBSSR 130 and VM 27 were found to be associated with powdery mildew-resistant plants in F_2 population of black gram (Savithramma and Ramakrishnan 2016).

Cercospora **Leaf Spot (CLS)** It is the third most important disease of mung bean and black gram mainly confined to rainy season (June to September) in India. Field screening at hot spot and that to humid climate is the only method of screening genotypes for CLS. Single dominant gene conferring resistance to *Cercospora* leaf spot disease was identified (Chankaew et al. 2011). Very few studies were carried out on tagging of *Cercospora* resistance gene in mung bean and black gram. Seven SSR markers, i.e., CEDC031, CEDG044, CEDG084, CEDG117, CEDG305, VR108, and VR393, were found to be associated with CLS resistance in F_2 and BC_1F_1 population of mung bean (Chankaew et al. 2011).

8.4.2 Cowpea

Cowpea (*Vigna unguiculata* L. Walp.) is a very important crop cultivated worldwide in each continent. It is used for both vegetable and grain purposes and is a rich source of protein and minerals for humans and livestock. Major yield constraints of cowpea include diseases caused by bacteria, viruses, and fungi. The most important diseases of cowpea are bacterial blight (*Xanthomonas axonopodis* pv. *vignicola* (Xav)) and bacterial pustule (*Xanthomonas* sp.) followed by viral diseases like bean common mosaic virus (BCMV), cowpea aphid-borne mosaic virus (CABMV), cowpea mosaic virus (CPMV), southern bean mosaic virus (SBMV), cowpea mottle virus (CPMoV), cucumber mosaic virus (CMV), and cowpea golden mosaic virus (CGMV). In fungal diseases, anthracnose and brown blotch (*Colletotrichum* sp.), charcoal rot (*Macrophomina phaseolina*), *Cercospora* leaf spot (*Cercospora canescens*), and *Fusarium* wilt are commonly appearing in cowpea. Growing of disease-resistant varieties is the only solution to combat yield losses in cowpea. The development of multiple disease-resistant varieties is a prime breeding objective in cowpea which is the host for so many diseases. Marker-assisted backcrossing and selection can boost the gene pyramiding for resistance to multiple diseases and save time and effort for disease screening. The development of tightly linked molecular markers with disease-resistant gene depends on genomic information available in the target crop. Cowpea is having the chromosome number $2n = 22$ with a genome size of 620 Mb (Varshney et al. 2009). The first attempt to sequence cowpea genome includes sequencing for about 97% of all known cowpea genes by using Illumina paired-end technology on GAII, and then they were assembled together with Sanger BAC-end sequences and "gene-space" sequences (Timko et al. 2008) using SOAPdenovo (Luo et al. 2012). Before the availability of cowpea SSR and SNP markers, RFLP (Fatokun et al. 1993), AFLP (Fang et al. 2007), DAF (Simon et al. 2007), and RAPD (Zannou et al. 2008) markers were used for genetic diversity studies and linkage mapping in cowpea. Molecular maps were developed in cowpea by using various F_2 and RIL populations, and markers like RFLP (Young 1999), AFLP and RAPD (Ouedraogo et al. 2002), SNP (Xu et al. 2011; Muchero et al. 2009; Lucas et al. 2011), and SSR (Anadrgie et al. 2011; Kongjaimum et al. 2012) were used. In cowpea, bacterial blight resistance gene candidate (RGC) loci were

reported to be placed on various locations of LG3, LG5, and LG9 on the integrated cowpea map constructed by using RFLP markers (Kelly et al. 2003). QTLs CoBB-1, CoBB-2, and CoBB-3 represent RGC loci and are present on linkage groups LG3, LG5, and LG9, respectively, on SNP marker-based cowpea genetic map (Agbicodo et al. 2010). A QTL for cowpea yellow mosaic virus (CYMV) resistance was identified and validated using SSR markers (Gioi et al. 2012). Cowpea genetic map showed that blackeye cowpea mosaic potyvirus (B1CMV) and southern bean mosaic virus (SBMV) resistance was mapped to LG8 and LG6, respectively, and resistance to cowpea mosaic virus (CPMV) and cowpea severe mosaic virus (CPSMV) was mapped to opposite ends of LG3, while the CPSMV resistance was mapped near a locus conferring resistance to *Fusarium* wilt (Ouédraogo et al. 2002). Three QTLs were reported for cowpea golden mosaic virus resistance by using AFLP markers in F_2 population (Rodrigues et al. 2012). Nine QTLs for resistance to *Macrophomina* were identified to be located on various linkage groups (Muchero et al. 2010; Muchero et al. 2011). QTLs conferring *Fusarium* wilt resistance against race 3 was found to be located on LG6 and race 4 was on LG8, LG 9, LG3, respectively (Pottorff et al. 2014).

8.4.3 Pigeon Pea

Pigeon pea (*Cajanus cajan* (L.) Millsp.) is an important pulse crop in India that is the largest producer and consumer in the world. The productivity of this crop is severely affected by some major diseases like *Fusarium* wilt (*Fusarium udum* Butler), sterility mosaic disease (SMD) caused by pigeon pea sterility mosaic virus (PPSMV), and *Phytophthora* blight (*Phytophthora drechsleri* f. sp. *cajani*). Other diseases, viz., *Alternaria* blight, sudden death, and root rots, are appearing recently due to climatic changes (Sharma and Ghosh 2016). *Fusarium* wilt (FW) is the most important and destructive disease in Indian Subcontinent (Saxena 2008). Due to five different variants of *Fusarium udum* (Tiwari and Dhar 2011), precise phenotyping at field level becomes difficult for genetic studies. Hence, different reports were found on genetics of FW resistance. A single dominant gene, two duplicate dominant genes, two complementary genes, and multiple genes governing the resistance reaction to FW disease are reported in various studies (Saxena and Sharma 1990; Okiror 2002; Singh et al. 2016a, b). Recently an association-based mapping approach has detected significant association of the SSR marker HASSR18 (accounting for 5–6% phenotypic variation due to wilt resistance across the years) with the genetic resistance against *Fusarium* wilt variant 2 in pigeon pea (Patil et al. 2017).

The PPSMV is transmitted by an eriophyid mite (*Aceria cajani* Channabasavanna) and hence spread rapidly, which leads to epidemics under congenial conditions. Development of varieties resistant to PPSMV becomes very difficult through conventional breeding because pigeon pea is a long-duration and often cross-pollinated crop, and phenotyping is tedious due to the existence of three different strains of

PPSMV in India (Kulkarni et al. 2003) and disease spread depends on mite population. Moreover, it becomes difficult to transfer resistant genes from wild relatives due to linkage drag. In the absence of efficient screening method, phenotyping is based mainly on symptoms which may vary depending on time and stage at which infection occurs. Hence, reports on genetics of resistant gene lead to different results. PSMD resistance was reported to be controlled by single gene (Ganapathy et al. 2009; Murugesan et al. 1997; Srinivas et al. 1997), oligo-genes (Gnanesh et al. 2011; Nagaraj et al. 2004; Sharma et al. 1984), and two genes with inhibitory gene action (Daspute et al. 2014). While four QTLs for Patancheru PSMD isolate and two QTLs for Bangalore PSMD isolate were also identified (Gnanesh et al. 2011). Short-duration pigeon pea varieties are very important for multiple cropping and to avoid terminal drought. Another disease, *Phytophthora* blight, is found to be more severe in short-duration cultivars as compared to long- or medium-duration genotypes (Ratnaparkhe and Gupta 2007). Looking at the losses due to different diseases, the development of disease-resistant varieties is the best means to resolve these problems, but lack of efficient and reliable screening methods limits the use of conventional breeding methods. Recent advances in genomics of pigeon pea pave the way for marker-assisted disease-resistant breeding for pyramiding the resistance genes for different diseases. Earlier, very limited information was available as far as the genomic resources are concerned. With the availability of draft genome sequence in pigeon pea (genome size = 833.07 Mb), this crop became rich for genomic resources (Varshney et al. 2012). Thus, a large number of SSR markers are now available, viz., 3072 SSRs from 88,860 BESs (Bohra et al. 2011), 3583 SSR markers from ESTs (Raju et al. 2010), and 309,052 SSRs from scanning the draft genome sequence of pigeon pea (Varshney et al. 2012). In addition to this, 10,000 SNPs are also available in pigeon pea research community (Varshney et al. 2013). These markers are going to be very useful for saturating the genetic maps with plenty of molecular markers and tagging QTL/genes for important traits like disease resistance. The genetic maps were developed by using an interspecific population and SSR markers (Bohra et al. 2011), DArT-based paternal and maternal-specific genetic maps (Yang et al. 2011), and a dense genetic map with SNP makers (Saxena et al. 2012). Consensus genetic maps have been developed by using SSR markers in six intraspecific populations (Bohra et al. 2012). Several markers linked to resistance genes were reported for these diseases which can be utilized for marker-assisted selection and gene pyramiding for multiple disease resistance. Different types of markers were used and identified to be linked with *Fusarium* wilt (FW) resistance, viz., two RAPD markers ($OPM03_{704}$ and $OPAC11_{500}$) (Kotresh et al. 2006), six SSRs (ASSR-1, ASSR-23, ASSR-148, ASSR-229, ASSR-363, and ASSR-366) (Singh et al. 2016a, b), and five SSR markers (PFW 26, PFW 31, PFW 38, PFW56, and PFW70) (Khalekar et al. 2014), while three important QTLs (qFW11.1, qFW11.2, and qFW11.3) were reported by using SNPs (Saxena et al. 2017a). Two genes, i.e., *C. cajan*_01839 for SMD resistance and *C. cajan*_03203 for FW resistance, were identified through SNP mapping (Singh et al. 2016a, b). For SMD, mapping was attempted by using AFLP markers, and four markers, E-CAA/M-GTG_{150}, E-CAA/M-GTG_{60}, E-CAG/M-GCC_{120}, and E-CAG/M-GCC_{150}, were identified

which were found to be linked with the SMD resistance gene at the distance of 5.7, 4.8, 5.2, and 20.7 cM from the resistance loci (Ganapathy et al. 2009). A single coupling phase short decamer random DNA marker ($IABTPPN7_{414}$) and a repulsion phase marker ($IABTPPN7_{983}$) were reported to be co-segregating with PSMD reaction (Daspute and Fakrudin 2015). Six QTLs (qSMD1, qSMD2, qSMD3, qSMD4, qSMD5, and qSMD6) linked to SMD were identified by using SSR markers in two different populations (Gnanesh et al. 2011). Another 10 QTLs including three major QTLs associated with SMD resistance were identified in three different populations (Saxena et al. 2017b). So far, reported linked markers are not utilized successfully to transfer the resistance genes toward the development of disease-resistant pigeon pea varieties. Validation of these markers across different genetic background is equally important as far as the application of these markers for breeding program is concerned. The tightly linked markers to disease resistance genes should be developed using multi-parent mapping populations (MAGIC) with very precise phenotyping so that it can be applicable across the pigeon pea populations. In the near future, with the availability of throughput approaches and tightly linked markers, the MABC will become very much possible for the resistance breeding to serious diseases like FW and SMD in pigeon pea.

8.4.4 Common Bean

Common bean (*Phaseolus vulgaris* L.) has often been termed as nutritional powerhouse for human diet (Broughton et al. 2003). It is used as food in the form of tender pods, fresh seeds, and dry beans. It originated in Central America and has two centers of domestication (Mesoamerican and Andean) with well-defined types in each gene pool (Singh et al. 1991). The crop used to hamper by different foliar and root diseases like angular leaf spot by *Pseudocercospora griseola* Sacc., common bacterial blight by *Xanthomonas axonopodis* pv. *phaseoli*, bean common mosaic virus, bean common mosaic necrosis virus, anthracnose by *Colletotrichum lindemuthianum*, root rots by *Fusarium solani* and *Rhizoctonia solani*, and rust by *Uromyces fabae*. Genetic resistance against bean common mosaic virus (BCMV) is conditioned by four different recessive loci, bc-1, bc-2, bc-3, and bc-u, along with a dominant gene I in *P. vulgaris*. Although mechanism of resistance of recessive and dominant gene is different, breeders want to pyramid them together for developing durable resistance. Melotto et al. (1996) have developed a SCAR marker (SW13) which was found linked to the dominant BCMV resistance I gene in this crop. Similarly SR2 SCAR has been very useful for bringing in bean golden yellow mosaic virus resistance in the plant (Blair et al. 2007; Beebe 2012). Anthracnose resistance in common bean is also conditioned by multiallelic Co-1 locus. Of these various alleles, Co-4 has been much used in breeding program due to the availability of a tightly linked SCAR marker SB 114 (Miklas et al. 2006).

Common bacterial blight (CBB) disease is caused by *Xanthomonas axonopodis* pv. *phaseoli*. Genetic resistance against this pathogen is quantitatively inherited,

and thus, different QTLs were identified in different linkage group of common bean (Jung et al. 1996; Bai et al. 1997; Miklas et al. 2000). Two dominant SCAR markers, SU91 and BC420, were independently developed from resistant tepary bean germplasm. The marker SU91 is reported to be linked to a QTL for CBB resistance on linkage group B8. The marker BC420 is linked to another QTL in B6 linkage group (Miklas et al. 2000; Pedraza et al. 1997; Yu et al. 2000). Resistance to angular leaf spot (ALS) disease (*Pseudocercospora griseola* Sacc.) is controlled by either dominant or recessive genes in common bean. These genes are independent as different types of molecular markers were identified for each resistance and they were placed in different chromosomes. The resistance gene *Phg-1 in* Andean cultivar AND 277 was mapped to chromosome 1 (Goncalves-Vidigal et al. 2011). Another major resistance locus on linkage group Pv04 was identified in other Andean accession G5686 (Mahuku et al. 2009). This locus was later confirmed and named ALS4.1$^{GS,\ UC}$ (Oblessuc et al. 2012). In addition, Mahuku et al. (2009) reported two complementary resistance genes in G5686 on Pv09 (ALS9.1^{GS}) and Pv04 (ALS4.2^{GS}). Further QTL studies also supported a more quantitative nature of ALS resistance (Lopez et al. 2003; Teixeira et al. 2005; Mahuku et al. 2011; Oblessuc et al. 2012). A major QTL explaining 75.3% of ALS resistance in the G5686 × Sprite population was validated, mapped to 418 kbp on chromosome Pv04, and tagged with two closely linked SNP markers (Marker50 and 4M437). These findings have enough potential to be used in MAS. ALS4.1$^{GS,\ UC}$ defines a region of 36 genes including 11 STPKs, which are likely candidates for the resistance gene. Additionally, three minor QTLs were identified (Keller et al. 2015). Bean rust is distributed around the world, but it effectively causes major production problems in humid tropical and subtropical areas (Souza et al. 2014). Several RAPD markers associated with genes conferring resistance to rust in common bean have been identified, and some of them were converted into SCAR markers to increase the reproducibility of the markers (Souza et al. 2007; Souza et al. 2008).

8.5 Successful Examples in Temperate Pulse Crops

8.5.1 Chickpea

Chickpea (*Cicer arietinum* L.) is the second most important pulse crop in the world after common bean. It is mainly used as a dietary protein source in Mediterranean region, India, Pakistan, and North Africa. The main biotic constraints for increasing yield in these countries are the susceptibility of the crop to foliar diseases like *Ascochyta* blight and *Fusarium* wilt. In addition, dry root rot (DRR) along with *Fusarium* wilt has emerged as a highly devastating root disease in central and southern India in context with climate change. A recent report described the identification of two flanking SSR markers for a dominant DRR resistance gene in chickpea (Talekar et al. 2017). There are eight different *Fusarium oxysporum* f. sp. *ciceri*

races that are present globally. Of these, the presence of resistance gene against *foc 1A* or *foc 1B/C* can protect the chickpea plant from early wilting, while resistance genes against *foc 0*, *foc 2*, *foc 3*, *foc 4*, and *foc 5* impart complete resistance over all the growing stages of the plant (Sharma et al. 2005; Sharma and Muehlbauer 2007). Marker information on all these different *Fusarium* wilt resistance genes of chickpea is summarized in Table 8.2. Most of the markers identified earlier were of RAPD, ISSR, or AFLP markers, but their usage in MAS is limited due to problem of reproducibility. Thus, most of the recent works were focused on the usage of SSR and SNP markers for this cause. A molecular map based on intraspecific cross (Kabuli-Desi cross) was developed and used to tag genes for resistance to *Fusarium* wilt. Two SCAR markers and two RAPD markers (Mayer et al. 1997) were found associated with resistance to race 1 and one ISSR marker with resistance to race 4 (Ratnaparkhe et al. 1998). The genes for resistance to races 4 and 5 were found to be linked and located close to one STMS and one SCAR marker (Winter et al. 2000). Recently eight QTLs were found associated with *Ascochyta* blight resistance in chickpea. Of them, a cluster of QTLs were found in chromosome 8 at a map interval of 8.5 cM (Daba et al. 2016). Li et al. (2017) identified 100 kb region in chromosome 4 that is significantly associated with *Ascochyta* blight in chickpea through genome-wide association mapping in Australian breeding population. Chetukuri et al. (2011) identified three QTLs for *Botrytis* gray mold disease of chickpea. Of these, QTL 3 (flanked by TA 159 and TA 118) in linkage group 3 explained 48% of the phenotypic variation due to botrytis grey mold disease reaction. Two sequence-tagged microsatellite sites (STMS) markers, TA18 and TA180 (3.9 cM apart), were identified as the flanking markers for rust resistance gene in chickpea (Madrid et al. 2007). These findings could be the starting point for a marker-assisted selection (MAS) program for rust resistance in chickpea.

Using traditional bi-parental populations, several QTLs for AB resistance have been identified on linkage groups LG2 (Udupa and Baum 2003; Cho et al. 2004), LG3 (Tar'an et al. 2007), LG4 (Lichtenzveig et al. 2006; Tar'an et al. 2007; Sabbavarapu et al. 2013; Stephens et al. 2014), LG5 (Sabbavarapu et al. 2013), LG6 (Tar'an et al. 2007; Sabbavarapu et al. 2013), and LG8 (Lichtenzveig et al. 2006). One major QTL has been repeatedly reported in a similar region of LG4 across several studies and therefore makes this locus a good candidate region for improving AB resistance in chickpea (Lichtenzveig et al. 2006; Tar'an et al. 2007; Sabbavarapu et al. 2013; Stephens et al. 2014).

8.5.2 Lentil

Lentil (*Lens culinaris* Medik) is a self-pollinating diploid ($2n = 2x = 14$) grain legume. It is cultivated globally and is valued for its quality protein and mineral content (particularly Fe content). Its production is limited by many biotic stresses including infection by the pathogen causing *Ascochyta* blight (*Ascochyta lentis* Vassilievsky), *Fusarium* wilt (*Fusarium oxysporum* f. sp. *lentis*), anthracnose

Table 8.2 Detailed information of linked markers, mapping population, and types of resistance genes for *Fusarium* wilt disease of chickpea

Foc genes	Nature of markers	Name of markers	Mapping population	Linkage group	References
foc 0	RAPD	$OPJ20_{600}$	RIL (CA2139 × JG 62)	LG 2	Rubio et al. (2003)
	RAPD and SSR	$OPJ20_{600}$ TR 59	RIL (CA2139 × JG 62)	LG 3	Cobos et al. (2005)
foc 0_2	SSR	–	RIL (CA2139 × JG 62) (CA 2156 × JG 62)	LG 2	Halila et al. (2009)
Foc 1A	RAPD	UBC 170	RIL (WR315 × C104)	LG 2	Tullu (1996)
	SCAR	CS 27 $CS27_{700}$			Mayer et al. (1997)
foc 1	SSR	TA 110	RIL JG 62 × Vijay	LG 2	Sant (2001)
	SSR	H3A12 TA 110	RIL (Vijay × JG 62)	LG 2	Gowda et al. (2009)
	SSR	QTLs: FW-Q-APR-6-2 FW-Q-APR-6-1	$F_{2:3}$ (C214 × WR 315)	LG 6	Sabbawarapu et al. (2013)
	SSR	TA 37 TA 200 TA 2	RIL (WR315 × C 104)	LG 2	Barman et al. (2014)
	SSR	QTLs: Wilt-1 (30 DAS) Wilt-2 (60 DAS)	RIL (JG 62 × WR 315)	LG 2	Patil et al. (2014)
	SSR and SNP	Five QTLs	RIL (JG 62 × ICCV05530	LG 2 LG 4 LG 6	Garg et al. (2018)
Foc 1 (H_2 locus)	SSR	QTL in between GSSR 18 and TC 14801	RIL (K 850 × WR 315)	LG 1	Jingade and Ravikumar (2015)
foc 2	SSR	TA 96 TA 27 TA 19	RIL (WR 315 × C 104)	LG 2	Sharma and Muehlbauer (2005)
	SSR	TA 96 H3A12	RIL (Vijay × JG 62)	LG 2	Gowda et al. (2009)
foc 3	SSR and STS	TA 96 TA 27 CS 27A (STS)	RIL (WR 315 × C 104)	LG 2	Sharma et al. (2004)
	SSR	H1B06y TA 194	RIL (Vijay × JG 62)	LG 2	Gowda et al. (2009)
	SSR and SNP	Two QTLs	RIL (JG 62 × ICCV05530	LG 2 LG 4	Garg et al. (2018)

(continued)

Table 8.2 (continued)

Foc genes	Nature of markers	Name of markers	Mapping population	Linkage group	References
foc 4	ISSR	UBC 855_{500}	RIL (ICC 4958 × *C. reticulatum* (PI489777))	LG 2	Ratnaparkhe et al. (1998)
	RAPD	UBC 170_{500} CS 27_{700}	RIL (C104 × WR 315)	LG2	Tullu et al. (1998)
	RAPD	CS 27_{700}	F_2 and F_3 (JG 62 × Surutato 77)	LG2	Tullu et al. (1999)
	SCAR or ASAP	$CS27_{700}$	RIL (ICC 4958 × *C. reticulatum* (PI489777))	LG2	Tekeoglu et al. (2000)
	SSR and AFLP	CS 27 TA 96 EAAMCTA12	RIL (ICC 4958 × *C. reticulatum* (PI489777))	LG 2	Winter et al. (2000)
	DAF	R 2609-1	RIL (*C. arietinum* × *C. reticulatum*)	LG 2	Benko-Iseppon et al. (2003)
foc 5	SSR and AFLP	ECAMCTA07	RIL (ICC 4958 × *C. reticulatum* (PI489777))	LG 2	Winter et al. (2000)
	SCAR or ASAP	$CS27_{700}$	RIL (ICC 4958 × *C. reticulatum* (PI489777))	LG2	Tekeoglu et al. (2000)
	SSR	QTL_AR3 TA110 TA 89	RIL	LG 2	Iruela et al. (2007)

(*Colletotrichum truncatum*), stemphylium blight (*Stemphylium botryosum*), rust (*Uromyces viciae-fabae*), botrytis gray mold (*Botrytis cinerea* and *B. fabae*), and white mold (*Sclerotinia sclerotiorum*). A SRAP marker F7XEM4a was identified for rust resistance gene in lentil by Saha et al. (2010a). This marker was placed 7.9 cM from the rust resistance gene. Later, an SSR marker Gllc 527 was identified to be linked to rust-resistant locus at a genetic distance of 5.9 cM in lentil (Dikshit et al. 2016). Toward the marker development on stemphylium blight resistance, two SRAP markers, ME5XR10 and ME4XR16c, were identified to be significantly associated with the QTLs for disease resistance in lentil (Saha et al. 2010b). Taran et al. (2003) developed RAPD ($OPE06_{1250}$ and UBC 704_{700}) and AFLP markers ($EMCTTACA_{350}$, $EMCTTAGG_{375}$, and $EMCTAAAG_{175}$) which were linked to LCt-2 locus for disease resistance against *Colletotrichum truncatum* (causal organism of anthracnose disease). In another study, a QTL (explained 41% of the variation in the

reaction to *Ascochyta* blight) was identified on the linkage group 6. This QTL was localized between an AFLP marker (ctcaccB) and LCt2 (Tullu et al. 2006). Eujayl et al. (1998) used an RIL population to identify molecular markers linked to the single dominant gene conditioning *Fusarium* vascular wilt resistance. On the other hand, resistance to *Fusarium* vascular wilt was mapped on linkage group 6, and this resistance gene was found flanked by a microsatellite marker SSR59-2B and an AFLP marker p17m30710 at distances of 8.0 cM and 3.5 cM, respectively (Hamwieh et al. 2005).

Among various diseases in lentil, *Ascochyta* blight is the most economically concerned in the majority of lentil-producing regions of the world. From 1999 onward, various molecular markers were identified for this disease resistance in lentil. Ford et al. (1999) identified two RAPD markers (RB18 and RV01) for a dominant *Ascochyta* blight disease resistance gene *AbR1*. Andrahennadi (1994) reported that a recessive gene *ral2* conditioned the resistance against *A. lentis* in cv. Indianhead. Later, this finding was confirmed by Choudhury et al. (2001) who have developed two RAPD markers ($UBC227_{1290}$ and $OPD10_{870}$) that are linked to *ral2* in lentil. Very recently genomic DNA-derived SSRs and SNP markers were developed based on the seedling (at 14 days) disease reaction and QTL analysis. Of the four QTLs identified by the authors, an SNP marker (SNP_20005010) was consistently found in two different mapping populations (Sudheesh et al. 2016). These particular SNP markers along with other flanking markers identified in the above QTL study showed promise for marker-assisted selection in the future. An international sequencing effort for lentil cultivar "CDC Redberry" is presently undergoing in full swing. The availability of an improved and well-annotated genome sequence assembly will allow development of more markers for *Ascochyta* blight resistance in the future. Till now, the utilization of these markers in MAS is very limited in lentil. In the past, Taran et al. (2003) used markers linked to *ral2* (UBC 227_{1290}), to *Ab*R1 ($RB18_{680}$), and to the major gene for resistance to anthracnose ($OPO6_{1250}$) to isolate RILs which were resistant to the disease.

8.5.3 Pea

Pea (*Pisum sativum* L.) is an important legume mainly grown as spring crop in temperate regions. It is the cheap source of high-quality vegetable proteins both for human food and animal feed and is able to fix atmospheric nitrogen symbiotically, improves soil fertility, and reduces the need for nitrogen fertilizers (Sun et al. 2015; Ghafoor and McPhee 2012). However, pea frequently suffers from various diseases throughout its lifecycle which severely affects its yield and seed quality. *Ascochyta* blight, *Fusarium* wilt, downy and powdery mildew, bacterial blight, root rot and damping off, etc., are the major diseases that occur in pea.

Powdery Mildew Powdery mildew disease is caused by *Erysiphe pisi* which reduces the pea yield up to 25–50%. Several pea germplasm lines had been identified

and characterized for resistance to *E. pisi* and their resistance genes. Two recessive genes (*er1* and *er2*) and one dominant gene (*Er*3) have been identified for resistance to powdery mildew in pea germplasm (Fondevilla et al. 2007). Genetic analyses of resistance to *E. pisi* indicated that gene *er1* is the most commonly present in all resistant pea genotypes whereas *er2* is found in only few resistant individuals. The newly identified dominant gene *Er3* is now characterized and transferred into cultivated pea for powdery mildew resistance (Sun et al. 2015; Tiwari et al. 1997). To aid MAS in pea breeding programs, several studies have been carried out to identify the genomic regions associated with *er1* locus by RFLP, RAPD, SCAR, and SSR markers (Shrivastava et al. 2012). Sarala (1993) and Timmerman et al. (1994) stated that the *er1* gene was present on pea linkage group (LG) VI based on their linkage study by using both morphological and molecular markers. Dirlewanger et al. (1994) found the position of *er1* gene at 9.8 cM distance from RFLP marker p236, whereas Timmerman et al. (1994) found that the RAPD marker, $OPD10_{650}$, was positioned at 2.1 cM from *er1* gene. Janila and Sharma (2004) converted the RAPD marker ($OPD10_{650}$) into a SCAR marker, which was mapped at a distance of 3.4 cM from *er1* gene. Three SSR markers, viz., PSMPSAD60, PSMPSAA374, and PSMPA5, were developed by Ek et al. (2005) which are linked with *er1* gene at a distance of 10.4, 11.6, and 14.9 cM, respectively. According to Tonguc and Weeden (2010), the *er1* locus is positioned between two markers, BC210 and BA9. They found that *er1* was 8.2 cM away from the marker BC210, and further they confirmed the presence of *er1* locus on LG VI of the genetic map of pea. The efficacy of MAS for powdery mildew was investigated by Nisar and Ghafoor (2011) in the F_2 population of the hybrid Fallon (*er1*)/11760-3(*ER1*) with RAPD marker $OPB18_{430}$ which is linked to *er1* gene at 11.2 cM distance. Recently, Sun et al. (2016) discovered a novel *er1* allele designated as *er1–6*, conferring powdery mildew resistance in Chinese pea. They found that resistance effect of *er1–6* was consistent with those of *er1–2* allele through transcript analysis.

Marker-assisted breeding for powdery mildew resistance in pea was performed by Rakshit et al. (2001) using an RAPD marker OPD 10_{650} which was linked to powdery mildew resistance locus at 3.6 cM. However, Tiwari et al. (1998) did not find $OPD10_{650}$ to be useful for MAS in progeny derived from a cross of the resistant cultivar Highlight (*er1*) and the susceptible cultivar Radley. Since *er1* is a recessive gene, therefore, introgression of *er1* requires a generation of selfing after every backcross generation to obtain homozygous resistant BC_nF_2 parents for the next backcross cycle. Marker-assisted selection provides an ideal strategy for transferring *er1* gene into superior cultivars having powdery mildew susceptibility (Ghafoor and McPhee 2012). Thus, several marker-trait associations for powdery mildew resistance have been identified with varying degrees of linkage which needs to reconfirm the marker-trait association for use in MAS-based breeding pea for powdery mildew resistance in the future.

Ascochyta Blight Ascochyta blight or black spot is the most destructive disease of field peas, and it is distributed throughout the world (Bretag et al. 2006). The disease *Ascochyta* blight in pea is caused by a complex of three fungal pathogens, commonly

referred to the *Ascochyta* complex, including *Ascochyta pinodes* L.K. Jones (teleomorph: *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr.), *Phoma medicaginis* var. *pinodella* (L.K. Jones) Morgan-Jones & K.B. Burch, *Ascochyta pisi* Lib. (teleomorph: *Didymella pisi* sp. nov.), and *Phoma koolunga* Davidson et al. sp. nov. (Davidson et al. 2009; Liu et al. 2013). It reduces the grain yield up to 10–40% and causes damage on the leaves, stems, and roots limiting proper plant metabolism and also reduces grain quality (Liu et al. 2016). Among various management strategies, genetic resistance is the reasonably and ecologically sound approach to control *Ascochyta* blight in field pea (Fondevilla et al. 2011). Several linkage maps have been developed in pea using AFLP, RAPD, SSR, STS, and EST-SSR markers for the identification of genomic regions associated with *Ascochyta* blight resistance (Prioul et al. 2004; Fondevilla et al. 2008). Scientists are continuously working on *Ascochyta* blight resistance in pea and found more than 30 QTLs associated with *Ascochyta* blight resistance on all the seven linkage groups (LGs) (Prioul et al. 2004; Tar'an et al. 2003a, b; Timmerman-Vaughan et al. 2002, 2004). Timmerman-Vaughan et al. (2002, 2004) reported 19 QTLs for AB resistance on LGs I, II, III, IV, V, and VII and Group A in two pea mapping populations, whereas Tar'an et al. (2003a) identified three QTLs on LGs II, IV, and VI. Prioul et al. (2004) reported six QTLs on LGs III, V, VI, and VII and 10 QTLs on LGs II, III, V, and VII under controlled and field conditions, respectively. In *P. sativum* ssp. *syriacum*, six QTLs were reported on LGs II, III, IV, and V by Fondevilla et al. (2008), whereas three additional QTLs were identified by Fondevilla et al. (2011) on LGs III and VI. Carrillo et al. (2014) identified four new QTLs on LGs II, III, and V controlling cellular mechanisms involved in *Ascochyta* blight resistance in *P. sativum* ssp. *syriacum*. Fondevilla et al. (2011) indicated that QTLs MpIII.1, MpIII.3, and MpIII.2 detected in *P. sativum* ssp. *syriacum* corresponded to the QTLs mpIII-1, mpIII-3, and mpIII-5 identified in *P. sativum* by Prioul et al. (2004). Co-localization of QTLs for disease resistance with candidate genes including RGAs (resistance gene analogs), PsDof1 (a putative transcription factor), and DRR230-b (a pea defensin) involved in defense responses to *P. pinodes* was reported in pea (Timmerman-Vaughan et al. 2002, 2016; Prioul-Gervais et al. 2007). Further, Jha et al. (2015) reported significant association of SNPs detected within candidate genes *PsDof1* (PsDof1p308) and *RGA-G3A* (RGA-G3Ap103) with *Ascochyta* blight scores. Most recently, nine QTLs were identified for *Ascochyta* blight resistance in an interspecific pea population (PR-19) developed from a cross between Alfetta (*P. sativum*) and wild pea accession P651 (*P. fulvum*) (Jha et al. 2016). QTLs abI-IV-2 and abIII-1 were further fine mapped in RIL-based HIF populations through SNP-based GBS by Jha et al. (2017). They found two new QTLs, abI-IV-2.1 and abI-IV-2.2 within abI-IV-2 QTL for *Ascochyta* blight resistance, and these QTLs were individually explained 5.5 to 14% of the total phenotypic variation.

Fusarium **Wilt** Fusarium wilt (*Fusarium oxysporum* f. sp. *pisi* (*Fop*)) of pea is one of the most widespread diseases worldwide and causes a vascular wilt resulting in significant crop losses. Based on the differential pathogenicity on pea genotypes, mainly four races, viz., *Fop*1, *Fop* 2, *Fop* 5, and *Fop* 6, of *Fusarium oxysporum* f. sp. *pisi*

were identified (Kraft and Pfleger 2001). According to McClendon et al. (2002), resistance to most *Fop* races are governed by single gene. Resistance to *Fusarium* wilt race 1 was reported as a single gene, *Fw*, located on linkage group III. Resistance to *Fop* race 2 was postulated to be qualitative and was assigned a single gene (*Fnw*) called *Fusarium* near wilt. The major locus *Fnw* has now been mapped to LG IV of pea and named *Fnw4.1* (McPhee et al. 2012). Two other significant minor QTLs, viz., *Fnw 3.1* and *Fnw 3.2*, on LG III for *Fop* race 2 have been also identified by McPhee et al. (2012). Gene *Fwf* conferring resistance to *Fop* race 5 has been placed on LG II (McClendon et al. 2002; Okubara et al. 2005). The genetics of resistance to *Fop* race 6 is not clear, but few scientists believed that it is governed by single dominant gene (Haglund and Kraft 2001).

McClendon et al. (2002) identified one AFLP marker, ACG: CAT_222 at 1.4 cM away from Fw locus. A RAPD maker, Y15_1050 (4.6 cM from *Fw*) was developed into a dominant 999 base-pair (bp) SCAR marker which identified a Y15 allele linked in coupling phase to susceptibility (McClendon et al. 2002; Okubara et al. 2005). Later on, Loridon et al. (2005) mapped the *Fw* locus on the pea SSR consensus map between AA5-235 (3.3 cM) and AD134-213 (2.5 cM). Kwon et al. (2013) successfully developed SCAR markers tightly linked to *Fw* in pea using the TRAP marker technology in conjunction with BSA. They described the production of three useful SCAR markers linked to *Fop* race 1 resistance in pea. Using a combination of two SCARs, Fw_Trap_480 and Fw_Trap_220, in a multiplex PCR, the accuracy for marker-assisted selection was improved later (Kwon et al. 2013).

8.6 Major Bottlenecks

Since the inception of molecular markers in crop plants, several genetic linkage maps were developed in pulses. Many markers for disease resistance are available in common bean, lentil, chickpea, and some tropical legumes. But, most of them are RAPD, SCAR, or AFLP markers. Report on SSR markers in these pulses has started appearing since last 10 years. Availability of high-resolution genetic linkage map in pulse crops is lacking. Information on genome sequences, expression databases, and genomics platform are available for most of these major pulse crops in this decade. With this advent, the development of high-resolution maps of major pulse crops like pigeon pea, chickpea, lentil, etc. is needed. Availability of reference genome sequences in pulses triggers adoption of re-sequencing and GWAS approach in some pulses. Such re-sequencing approaches have ample scope for the development of breeder-friendly markers (like InDel, STMS, and SNP markers). The usage of these new markers for the development of high-resolution maps is of immediate need. Moreover, such markers could be better utilized in tagging disease resistance genes through bi-parental mapping. The generation of high-resolution bi-parental mapping population in some of the pulse crops (like lentil and chickpea) is cumbersome due to their inherent low pod setting per artificial cross. To avoid this problem,

future thrust should be given on GWAS approaches utilizing available global germplasm, mini-core collection, diversity panels, MAGIC population, etc. Another important bottleneck in disease resistance breeding is the frequent evolution of pathogen races and breakdown of genetic resistance. To overcome such unavoidable situations in the field, breeding efforts must be directed toward incorporation of horizontal resistance or bringing in recessive resistances which have broad-spectrum activity in the field (Ning et al. 2017; Ning and Wang 2018). Moreover, improved varieties in pulses should be pyramided with various disease resistances with the help of MAS in the future.

8.7 Conclusion and Perspective

The reproduction rate of pathogen is higher than its host. In nature, pathogen can generate variability through mutation, sexual recombination, heterokaryosis, and parasexual cycle. To keep the pace with this continuous load of pathogenic strains in the field, resistance breeding should be well focused for economical crops like pulses. Research should be focused on development of quick/fast disease screening protocol, rapid identification of resistant genotypes and molecular markers, and pyramiding of various disease resistance genes through marker-assisted selection procedure. At present, genomic pipelines in most of the major pulse crops have been generated (Varshney 2016). It is utmost need to develop complementary genomic pipelines in pathogen too. Generation of genomic pipelines and expression data in pathogen will help in genome-wide identification of effector repertoires. Such effectors can be used for effector-mediated screening of germplasm for disease resistance through agro-infection or virus-mediated infection in plants. This "effectoromics" approach will be a potent contributor in modern disease resistance breeding for pulse crops (Vleeshouwers and Oliver 2014). Although enough markers were developed in pulses for various disease resistance traits, their exploitation in field remains elusive due to the problem in reproducibility, unreliability, and larger map distance between the marker and the targeted resistance genes. In the era of genomics technologies, reliable marker-trait association should be established through GWAS in diversity panel or in MAGIC or NAM populations. NGS technologies along with the above approaches will help to develop various SNP markers within a close proximity to candidate gene or within gene itself. Such developments will trigger high-throughput germplasm screening, MAS, and pyramiding of different resistances through the usage of various SNP platforms in the future.

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