Chapter 10
Charcoal Rot Resistance in Soybean: Current Understanding and Future Perspectives

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10.1 Introduction

Soybean (*Glycine max* L.) is the foremost source of protein (40%) and oil (20%) (Talukdar et al. 2009). It is a multipurpose crop having been used for human consumption, protein feed ingredient and industrial applications. Soybean production is challenged by various forms of biotic and abiotic stresses. Charcoal rot is the second most economical disease of soybean after brown spot (*Septoria glycines*) (Wrather et al. 2001).

Soybean charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid. is an economically significant disease throughout the world. In addition to soybean, this pathogen has a wide range of hostage including some economical crops like sorghum (*Sorghum bicolor* (L.) Moench) and maize (*Zea mays,* L.) (Mengistu et al. 2007). Charcoal rot disease in soybeans was first observed in the United States in 1949 (Young 1949). Severity of the disease increases with the increase in soil and air temperature (28–35 °C) (Mengistu et al. 2014). Under limited soil moisture conditions, synergistic yield losses occur due to both environmental stress and charcoal rot disease (Mengistu et al. 2011a). Confounding effects of drought make it difficult to estimate the yield loss per se by charcoal rot disease incidence. Under irrigated conditions, about 6–33% yield loss in susceptible cultivars is attributed to charcoal rot disease, indicating the importance of the disease even under irrigated conditions (Mengistu et al. 2011b, 2018).

Under field condition, infection is carried by microsclerotia present in the soil or through conidia present on infected plant tissue or debris to the host through rain splashes (Dhingra and Sinclair 1978). A reddish-brown discolouration at the emerging portion of the hypocotyls may be seen in the infected seedlings (Smith and

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Wyllie 1999). Root infection is evident through discolouration at the soil line and above (Smith and Wyllie 1999). Under hot and dry conditions, discoloured area turns dark brown to black and the infected seedlings may die. Under wet and cool conditions, infected seedlings survive and carry latent infection through the reproductive stages (Smith and Wyllie 1999). Infection often starts with the biotrophic phase with no visible symptoms and changes in environmental conditions, and plant stress and maturation can promote necrotic phase of infection (Twizeyimana et al. 2012). This phase is evident from visible symptoms such as yellowing, wilting and flagging of leaves due to blockage of water and nutrient transportation to the leaves and shoots by mechanical plugging of root vascular tissue by the fungus and necrosis caused by phytotoxins and enzymatic action (Smith and Wyllie 1999; Luna et al. 2017). A light grey or silvery discolouration develops on epidermal and subepidermal tissues of the taproot and lower part of the stem after flowering. Development of microsclerotia becomes so numerous that it gives a greyish-black colour to the stem pith tissues resembling a sprinkling of finely powdered charcoal (Smith and Wyllie 1999). Reddish-brown discolouration of the taproot vascular tissues is evident which later progresses to the vascular and pith tissues of the stem. Black streaks in the woody portion of the crown are observed in the split open taproot (Smith and Wyllie 1999).

10.2   Screening of Soybean Germplasm for Charcoal Rot Resistance

Host plant resistance is the only feasible method to prevent soybean yield losses by charcoal rot disease (Smith and Carvil 1997; Smith and Wyllie 1999; Silva et al. 2019). Lack of reliable, repeatable and efficient screening systems against charcoal rot is hindering the progress towards development of resistant soybean varieties (Mengistu et al. 2007).

There are six screening methods mainly used for soybean charcoal rot disease assessment: colony-forming unit index (CFUI); root stem severity (RSS); percent height of stem discolouration (PHSD); foliar symptoms (FS) taken at R7 stage; foliar symptoms taken at R1, R3, R5 and R7 and calculating the AUDPC; and cut-stem inoculation method (Mengistu et al. 2007; Twizeyimana et al. 2012).

Briefly, in the CFUI method, the lower stem and root portion of genotypes under study are excised just below the cotyledonary node at R7 stage. Ten such samples were taken from each plot. Thoroughly washed samples were ground and each 0.005 g ground sample was treated with 100 mL of 0.525% NaOCl for 3 min. The triturate was washed thoroughly with sterile distilled water and transferred to a 100 ml autoclaved selective media containing PDA, rifampicin (100 mg L^{-1}) and Tergitol (0.1 mL). Three days after incubation, CFU were counted and CFU per gram was estimated. A CFUI was developed for each genotype by dividing the CFU of the genotype with the CFU of the genotype producing the highest CFU (Mengistu et al. 2007). The genotypes were then classified in percentage based on this CFUI as

resistant (0 to <10), moderately resistant (10 to ≤30), moderately susceptible (>30 to 60) and susceptible (>60) (Schmitt and Shannon 1992; Mengistu et al. 2007).

Disease severity estimation by root stem severity (RSS) is done at R7 stage (Fehr et al. 1971) using the scale (1–5) established by Paris et al. (2006): 1 = no discolouration and no microsclerotia visible; 2 = no discolouration of vascular tissue, with very few microsclerotia visible in the pith, vascular tissue, or under the epidermis; 3 = partially discoloured vascular tissue, with microsclerotia partially covering the tissue; 4 = discoloured vascular tissue, with numerous microsclerotia visible in the tissue under the outer epidermis, in stem and in root sections; and 5 = vascular tissue with numerous microsclerotia producing a dark colour inside and outside of the stem and root tissue.

Percent height of stem discolouration (PHSD) is based on microsclerotial stem discolouration at R7 stage. Length of internal vascular necrosis above the ground level divided by plant height × 100 is percent height of vascular discolouration due to charcoal rot (Mengistu et al. 2007). Foliar symptoms (FS) at R7 stage for disease estimation is done by using Horsfall-Barratt scale (James 1974). The symptom is generally necrosis of soybean leaves at R7 stage (Mengistu et al. 2007). On the scale of 0 to 11 used for FS, 0 = no symptoms; 1 = 0 to 3%, 2 = 3 to 6%, 3 = 6 to 12%, 4 = 12 to 25%, 5 = 25 to 50%, 6 = 50 to 75%, 7 = 75 to 87%, 8 = 87 to 94%, 9 = 94 to 97%, 10 = 97 to 100% and 11 = 100%. Using this scale, the genotypes were classified into four categories: resistant (zero), moderately resistant (>0 and <5), moderately susceptible (≥5 and <8) and susceptible (≥8). Foliar symptoms taken at R1, R3, R5 and R7 were used to calculate the AUDP. In this method, foliar symptoms were recorded on weekly basis from the beginning of the foliar symptoms up to R7 stage (Mengistu et al. 2007). The percentage of affected plants in each plot and the infection intensity was rated and the foliar symptoms over time were used to calculate AUDPC (Tooley and Grau 1984; Mengistu et al. 2007).

In brief, in the cut-stem inoculation technique (Twizeyimana et al. 2012), the soybean plants were grown to V2 stage (Fehr et al. 1971) and a sharp laser blade was used to cut the stem 25 mm (or 40 mm, Coser et al. 2017) above the unifoliate node. The open end of a 10–200 μL pipette tip was pushed into actively growing margins of fungal culture growing on PDA medium, and a circular disk of mycelia plug along with agar was obtained. The fungal mycelium was immediately placed on open end cut stem and pressed to ensure the mycelia is embedded into the stem. Disease ratings are based on the length of stem necrosis. Measurements are recorded 3 days after inoculation and followed for every 3 days until 13–15 days after inoculation. The linear stem necrosis measured over time was used to calculate AUDPC.

Colony-forming unit index (CFUI) has been reported to be a reliable method of rating host compatibility between soybean genotypes and *M. phaseolina* (Paris et al. 2006; Mengistu et al. 2007; Smith and Carvil 1997). Except for CFUI, there was significant genotype by year interaction for other disease assessment methods such as root and stem severity (RSS), percent height of internal stem discolouration (PHSD) and foliar symptoms (FS) (Mengistu et al. 2007). Though it is time consuming, the CFUI method of disease assessment is considered to be the good measure of disease resistance across environments and is recommended when more

accurate and precise classification across the genotypes is needed for genetics of host plant resistance (Schmitt and Shannon 1992; Mengistu et al. 2007). Owing to its rapidity, compromising the accuracy, the root and stem severity (RSS) method of disease assessment is considered to be suitable for breeding programs where there is a need to screen a large number of breeding lines against *M. phaseolina* (Mengistu et al. 2007).

Disease incidence measured by CFUI and RSS is based on extent of colonisation by the fungus. However, since severity of charcoal rot incidence is much influenced on environmental factors like temperature and rainfall and plant maturity, they must be taken into consideration while interpreting field studies to screen soybean genotypes of different maturity groups for their resistance to charcoal rot (Pawlowski et al. 2015). Genotypes can be better screened for resistance at specific growth stages than at specific times after sowing (Pearson et al. 1984). Disease progress is slow throughout the vegetative and reproductive growth stages and is more at R7 stage since the population density of *M. phaseolina* is increased rapidly from R6 to R7 stage (Mengistu et al. 2011b; Mengistu et al. 2018). Dry matter accumulation is ceased at R7 stage (Ritchie et al. 1989), and at R8 stage saprophytic action of the fungus negates the differences among the genotypic reactions to it (Mengistu et al. 2018). Therefore, R7 stage is critical for identification of resistant sources for charcoal rot (Mengistu et al. 2018).

Till date, most of the studies on evaluating resistance to *M. phaseolina* are based on field screening by either inoculating the field plots or relying on the disease incidence history of the field. Variability among soil characteristics, soil microflora and their interaction with *M. phaseolina* and other genotypes by environment interactions may result in inconsistent results between field screening experiments. Field screening relying on field inoculum may not measure the true disease reaction of a genotype because of non-uniform concentration and non-random distribution of inoculums in the field plot. Furthermore, differences in plant maturity duration lead to confounding results of field evaluation usually done at R7 stage (Mengistu et al. 2011b). In such cases, genotypes under study may not reach R7 stage at a time failing to screen all the genotypes under identical environmental conditions. Screening under controlled or semi-controlled conditions will overcome most of the limitations of field evaluation. Cut-stem inoculation technique developed by Twizeyimana et al. 2012 is a screening technique under controlled environmental conditions for more precise comparison of partial resistance to charcoal rot across maturity groups (Pawlowski et al. 2015). Unlike in field evaluation, a uniform amount of inoculum can be applied to the genotypes for infection which minimises the diseases from escaping and experimental error and improves screening precision. Length of necrosis in this technique is a direct measure of disease level considering which will improve the precision of disease evaluation over indirect disease measures such as CFUI (Twizeyimana et al. 2012). The results of cut-stem inoculation technique and CFU index ratings showed similar ranks for the genotypes screened (Twizeyimana et al. 2012; Pawlowski et al. 2015) indicating the efficiency and effectiveness of

cut-stem inoculation technique in identifying the potential sources of charcoal rot resistance (Pawlowski et al. 2015).

M. phaseolina is a pycnidia-producing fungus (Smith and Wyllie 1999). Pycnidia production is common on garden beans and jute beans and occasional on soybean (Smith and Wyllie 1999). Ma et al. (2010) developed a screening technique using conidial suspension. Pycnidia production is not common in most of the culture media (Ma et al. 2010). Out of seven semi-defined media tested for pycnidia production, they found greater pycnidia and conidia production in peanut butter extract-saturated filter paper placed over soynut butter extract agar (PESEA). Conidia from the most aggressive isolate (pine tree, AR) were used to test their infectivity on the soybean radicles. Conidial suspension significantly differentiated susceptible genotype LS98-0358 from the moderately resistant genotype DT97-4290. Lesion length produced on LS98-0358 is significantly higher than that of DT97-4290 (Ma et al. 2010). Based on these results, Ma et al. (2010) concluded that PESEA can be used to produce conidia for inoculum for high-throughput evaluation of soybean genotypes for resistance.

Reznikov et al. (2019) developed an in vitro method of soybean root infection by *M. phaseolina* to evaluate charcoal rot disease reaction in soybean germplasm. Soybean seeds were surface sterilised for 1 min with 5% (v/v) NaClO followed by 70% (v/v) ethanol for 30 seconds and rinsed with sterile distilled water thrice, each for 1 min. Disinfected seeds were placed on a petri dish with a layer of filter paper soaked with 15 mL of distilled water. Seeds in the petri dish were incubated for 48 h in darkness at 28 °C to induce germination. Five germinated healthy seedlings were placed in autoclaved flasks having 3 cm layer of cotton and filter paper soaked with 50 mL of sterile distilled water. Three toothpick pieces each of 2 cm long, colonised with an isolate derived from a single microsclerotium, were added to each flask. The flasks having inoculated seedlings were kept in a growth chamber under a 16-h light (600 µE m-2s-1)/8-h dark regime and a temperature of 30 °C. Root disease severity was evaluated after every 48 h for 12 days using images captured with a digital camera. The length of necrosis in the root system of each infected seedling was measured with an image processing program and the disease severity was measured in terms of percentage of necrosis in the root system. The area under disease progress curve (AUDPC) was calculated using disease severity data (Madden et al. 2007). In addition, the in vitro assay as measured by AUDPC correlated with CFU/g (square root transformed) with a Spearman's rank correlation coefficient $r = 0.62$ ($P = 0.0004$), and the AUDPC data is correlated with the field disease severity data (ln-transformed) with a rank correlation coefficient $r = 0.59$ ($P = 0.0009$) thus validating the in vitro phenotyping method to screen genotypes for disease reaction.

So far, no soybean genotype having a high level of resistance to *M. phaseolina* has been identified (Mengistu et al. 2018), and investigation of disease reactions in available soybean germplasm is not extensive (Coser et al. 2017). Those reported genotypes showing moderate resistance to charcoal rot are presented in Table 10.1. Pedigree information of some of the reported moderately resistant sources is presented in Table 10.2.

Table 10.1 Soybean genotypes identified as moderately resistant to charcoal rot using different screening techniques

S. no	Genotypes	Screening	Reference
1	Asgrow 4715, DeltaPineLand 3478, Hamilton and Jackson II	Field screening based on CFU	Smith and Carvil (1997)
2	DT97-4290	Field screening based on RSS and CFU	Paris et al. (2006)
3	DT98-7553, DT99-17483 and DT99-17554	Field screening based on CFU	Mengistu et al. (2007) and Mengistu et al. (2011a)
4	DT99-16864	Field screening based on RSS and CFU	Mengistu et al. (2007), Mengistu et al. (2011a) and Gillen et al. (2016)
5	DG3905, Manokin	Field screening based on CFU	Mengistu et al. (2011a)
6	PI 594302, PI 567562A, PI 506764 and PI 567334	Field screening based on CFU	Mengistu et al. (2013)
7	PI 548302, PI 548414 and PI 548178	Percentage of seedling survival 8 days after inoculation	Pawlowski et al. (2015)
8	PI 548302 and PI 548414	Cut-stem inoculation technique	Pawlowski et al. (2015)
8	PI379559D	Field screening through RSS	Coser et al. (2017)
9	PI567241	Cut-stem inoculation technique	Coser et al. (2017)
10	Y 227-1	Field screening based on CFU	Smith et al. (2018)
11	Munasqa RR	In vitro phenotyping through root infection	Reznikov et al. (2019)

Table 10.2 Pedigree information of some of the moderately resistant sources

S. no	Genotype	Pedigree	Reference
1	DT97-4290	Asgrow 'A5979' 3 Delta Pine 'DP3478'	Paris et al. (2006)
2	DT99-16864	S59-60′ × 'Bolivar'	Gillen et al. (2016)
3	JTN-4307	S97-1688 (PI 633736) × V94-0198	Arelli et al. (2017)
4	Y227-1	SS93-6181 × DT97-4290	Smith et al. (2018)

10.3 Factors Effecting Charcoal Rot Incidence

Charcoal rot disease incidence is influenced by climatic and other factors. Studies on effect of drought, maturity and soybean cyst nematode on charcoal rot disease severity are reviewed in the following.

10.3.1 Drought

Charcoal rot disease is likely to become more predominant owing to climate change scenarios of increased heat and drought stress (Saleh et al. 2010). Increased air and soil temperature and limited soil moisture aggravate the disease (Smith et al. 2018; Mengistu et al. 2011b, 2018; Gary et al. 1991; Pearson et al. 1984; Smith and Wyllie 1999). Low soil moisture will enhance the growth and survival of the pathogen (Short et al. 1980). Drought is a common stress for rain-fed soybean. Drought stress in soybean is more often due to limited rainfall but may also happen due to poor root growth. Research is underway to develop cultivars having resistance to both charcoal rot and drought (Mengistu et al. 2011a). Wrather et al. (2008) studied the disease reaction in seven drought-tolerant soybean genotypes and suggested that not all drought-tolerant genotypes necessarily are resistant to charcoal rot but some drought-tolerant genotypes may resist root colonisation by the fungus. Mengistu et al. (2018) reported that the relationship between drought tolerance as measured by stress tolerance index (STI) and charcoal rot resistance as measured by colony-forming units (CFU) is very weak and found that not all drought-tolerant genotypes under his study exhibited charcoal rot resistance. Therefore, no stronger relationship between drought tolerance and charcoal rot resistance has been reported yet. However, different, diverse drought-tolerant genotypes in a more stressful environment might produce a stronger relationship between the two traits (Mengistu et al. 2018).

Resistance to *M. phaseolina* is associated with drought tolerance in common bean (*Phaseolus vulgaris*) (Pastor-Corrales and Abawi 1988). Drought aggravates the disease in other crops like sorghum (*Sorghum bicolor* (L.) Moench) and sunflower (*Helianthus annuus* L) (Manici et al. 1995; Gary et al. 1991). In case of cowpea (*Vigna unguiculata* (L) Walp.), Muchero et al. (2011) identified three resistance governing quantitative trait loci (QTL), *Mac-4, Mac-5* and *Mac-9*, co-located with seedling drought-tolerant QTLs *Dro-5*, *Dro-10* and *Dro-7* (Muchero et al. 2009), respectively. In each case, the *M. phaseolina*-resistant haplotype corresponded with the seedling drought-tolerant haplotype. On the contrary, in crops such as sorghum reports of no interdependence of non-senescence drought tolerance and charcoal rot resistance are available (Tenkouano et al. 1993).

10.3.2 Maturity

Mengistu et al. (2018) studied the effect of maturity on charcoal rot disease severity. They evaluated six genotypes of MG IV and seven genotypes of MG V for disease severity as measured by AUDP and CFU and found that there is no significant difference in either measure under irrigated or non-irrigated environments.

Confounding effects of genetic backgrounds can be eliminated by using near-isogenic lines (NILs). The true effect of maturity on charcoal rot disease severity as

measured by CFU was first studied by Mengistu et al. (2014). Two sets of NILs, one set with 9 isogenic lines in the background of 'Clark' (Johnson 1958) and the other set with 7 isogenic lines in the background of 'Horosoy' (Weiss and Stevenson 1955), were used to assess disease severity in terms of CFU. Isogenic lines in each set have maturity differences due to different maturity gene combinations but are otherwise genetically homogenous. Field experiment was conducted on two soil types, sandy loam and clay for 2 years, and CFU was estimated for each isogenic line at its physiological maturity. Regression analysis investigating the relationship between maturity and diseases severity indicated that there was no significant relationship between maturity and disease severity.

In other related crops like cowpea (*Vigna unguiculata* (L) Walp.), Muchero et al. (2011) identified resistance governing QTLs *Mac-6* and *Mac-7* co-located with maturity-related senescence QTLs *Mat-2* and *Mat-1*, respectively, suggesting the association between earliness and susceptibility to *M. phaseolina*. Such studies can be done in soybean in evaluating the relationship between charcoal rot resistance and the reported genes governing early maturity.

10.3.3 *Soybean Cyst Nematode (*Heterodera glycines*)*

Interaction between soybean cyst nematode (*Heterodera glycines*) and charcoal rot has been documented long ago (Todd et al. 1987; Meyer et al. 1974), and it was reported that nematode infection increases the colonisation of soybean roots by *M. phaseolina.* Disruption of vascular tissues owing to the nematode infection results in host susceptibility to moisture stress which in turn aggravates the fungal colonisation (Radwan et al. 2014). While SCN is a biotroph, *M. phaseolina* is a necrotroph. Both diseases have different resistant pathways. Hypersensitive reaction induced by the host in response to SCN infection will help *M. phaseolina* to infect the host roots. Understanding the mechanisms underlying the molecular interactions between these two pathogens is essential to design a breeding program to control both the diseases (Radwan et al. 2014). On contrary, some studies have shown no interaction between the two organisms under field conditions (Francl et al. 1988).

10.4 Host Specialisation

M. phaseolina is a generalist clonal plant pathogen (Saleh et al. 2010; Radwan et al. 2014). Host specialisation is very less in this pathogen (Su et al. 2001; Saleh et al. 2010; Zveibil et al. 2012). Few studies have reported host specialisation with host species like corn (Su et al. 2001). Cloud and Rupe (1991) reported host specialisation of *M. phaseolina* with soybean but not with sorghum. They used one isolate each of soybean and sorghum in a cross-inoculation experiment and found that

soybean root infection was significantly greater when inoculated with soybean isolate than with the sorghum isolate, whereas no significant differences in colonisation of sorghum roots were observed when inoculated with either isolate. Su et al. (2001) conducted a cross-inoculation experiment to confirm differential colonisation of soybean roots by isolates from different host species. They used 7 isolates of soybean, 9 isolates of sorghum, 9 isolates of cotton and 6 isolates of corn in their study and came out with the conclusion that soybean root colonisation was significantly more by corn isolates than the isolates from any other hosts. Therefore, no extensive study has been done to confirm the specialisation of *M. phaseolina* with soybean.

10.5   Host-Pathogen Interaction (HPI)

The existence of host-pathogen-specific interactions among soybean genotypes and *M. phaseolina* isolates was for the first time demonstrated by Reznikov et al. (2019). When seven *M. phaseolina* isolates (Mp15, Mp17, Mp18, Mp32, Mp37, Mp42 and Mp48) were used to infect four soybean genotypes (DM 6.2i RR, CRIA 4, DT 97-4290 and Munasqa RR) under both field conditions and in vitro conditions, significant genotype × isolate interactions for both in vitro assay (as measured by AUDPC) ($P = 0.0277$) and field conditions (as measured by CFU) ($P = 0.0025$) were observed. Under field study, the lowest value of CFU/g was observed for the combination Munasqa RR × MP 15 (33.3) and the highest value of CFU/g was observed for the combination DM6.2iRR × MP15 (2366.7). Under in vitro conditions, the lowest value of AUDPC was noticed for the combination Munasqa RR × MP 17 (4.21) and the highest value of AUDPC was noticed for the combination DM6.2iRR × MP37 (233.3). Till date, no study on molecular interactions between soybean and *M. phaseolina* has been done (Radwan et al. 2014). Gene expression profiling of *M. phaseolina*-infected roots of *Medicago truncatula* identified genes involved in jasmonic acid and ethylene pathways that are important for plant defence against necrotrophic fungi. Also, genes involved in auxin homeostasis, polar auxin transport and auxin signalling were found to be regulated by the infection process (Mah et al. 2012). Differential expression of auxin-related genes suggested that the host susceptibility may be partially due to suppression of auxin response in the host by the pathogen (Mah et al. 2012). Such transcriptomic studies must be done in soybean to identify the molecular basis of host-pathogen interaction.

10.6   Genome of *Macrophomina phaseolina*

Genome of *M. phaseolina* was sequenced in 2012 (Islam et al. 2012). Genomes of *M. phaseolina* and *Fusarium oxysporum* Schltdl. have shared many syntenic regions suggesting the similarities in both the pathogens with the pathways to infect hosts

(Islam et al. 2012). A large number of shared genes and syntenic regions observed with the comparative genomics between *M. phaseolina* and *Fusarium oxysporum* may reflect the common infection strategies in the two phytopathogens having a broad host range. To penetrate into the host tissue, the pathogen degrades the host cell wall polysaccharides and lignocelluloses by producing abundant secreted oxidases, peroxidases and hydrolytic enzymes. To overcome the plant defence mechanism, it encodes a significantly higher number of P450s, MFS-type membrane transporters, glycosidases, transposases and secondary metabolites than any other fungi. Being a wide host range pathogen, its genome has several host-pathogen interaction genes including those encoding for adhesion, signal transduction, cell wall breakdown and purin and patulin biosynthesis (Islam et al. 2012). Loss of function mutations in the avirulence gene may result in gain of virulence further, in development of new races (Kang et al. 2001). Wide virulence potential of a fungal genome is often associated with transposon-mediated deletion or inactivation of PAMP (pathogen-associated molecular patterns) encoding genes whose products trigger the host plant's adaptive immune system (Kang et al. 2001; Islam et al. 2012). In this respect, *M. phaseolina* genome comprises 3.98% transposable elements; most of them are DNA transposases (Islam et al. 2012) having potential in evolving virulence and resulting in development of new races.

10.7 Breeding for Charcoal Rot Resistance

Breeding for resistance is the most effective way to combat soybean yield losses due to charcoal rot disease. Insufficient information regarding genetic mechanisms to charcoal rot resistance (CR) is hindering the progress in resistance breeding (Coser et al. 2017). Identification of transgressive lines in breeding and mapping populations indicates the potential for selecting novel resistance forms in the population (Muchero et al. 2011). Till date, no breeding program is expended in combining drought tolerance and charcoal rot resistance in soybean (Mengistu et al. 2018). For environments where both charcoal rot and drought are the problems, selection criterion for breeding programs should include both charcoal rot resistance and drought tolerance. In such case, for screening of segregating population, charcoal rot infestation of the soil and drought stress must be properly measured and strategically employed (Mengistu et al. 2018). Simultaneous screening for drought tolerance and charcoal rot resistance can be done in an infested field under non-irrigated conditions, but screening alone for one trait may not necessarily select for the other (Mengistu et al. 2018). Developing varieties having high yielding potential, moderate resistance to CR and some level of drought tolerance would be the optimum selection criterion to maximise the farmer's produce with limited soil moisture (Mengistu et al. 2018). In order to target yield and resistance, screening under irrigated conditions is recommended (Mengistu et al. 2011b).

Yield is the major criterion in any breeding program. No extensive study has been done in correlating soybean charcoal rot resistance with its yield. Mengistu

et al. (2018) attempted to study the effect of CFU at R7 on seed yield of 13 soybean genotypes over 3 years (2011 and 2012) under both irrigated and non-irrigated conditions. Regression of seed yield on CFU at R7 stage indicated that a significant ($P \leq 0.05$) negative relationship (i.e., as CFU increases, seed yield decreased) between CFU at R7 and seed yield was found only in 2012 non-irrigated environment and a significant ($P \leq 0.10$) negative linear relationship between the two traits was found in other two environments (2011 irrigated, 2012 irrigated) environment. A pooled (global) slope calculated for the six independent regressions (3 years × 2 irrigation environments) indicated a yield loss of 11.5 kg/h for every 1000 CUs at R7. These results indicate that there is a potential relationship between CFU at R7 and seed yield in at least some environments. On the other hand, Smith et al. (2018) studied the effect of charcoal rot on yield losses of six soybean genotypes in an infested plot under irrigated environment over 3 years (2011–13). Regression analysis indicated that only one genotype (LG03-4561-14) showed negative linear relationship between CFU and yield loss in two [2011 ($r^2 = 0.43$; $P = 0.0403$) and 2013 ($r^2 = 0.71$; $P = 0.0023$)] of the three environments. This is the first study to demonstrate the negative linear relationship between CFU and yield loss for a particular genotype. None of the other five genotypes showed significant ($P \leq 0.05$) linear relationship, thus concluding that not all soybean genotypes that were colonised by *M. phaseolina* show yield penalty. Therefore, it seems that no consistent and significant negative linear relationship between CFU and seed yield has been reported and the trends in relationship cannot be generalised across all the genotypes and environments. Tolerance is defined as the condition where the yield of a susceptible genotype is not affected significantly by the colonisation of *M. phaseolina* (Smith et al. 2018). Smith et al. (2018) in his study considered LG03-4561-14 to be intolerant to colonisation by *M. phaseolina*, whereas the other five genotypes are considered to be tolerant to colonisation with different threshold CFU levels. Therefore, it is understood that those genotypes which are susceptible may be tolerant and need not show yield losses (Smith et al. 2018).

In crops like sorghum, there are reports indicating no significant relationship (Williams et al. 2009) or with a perfect positive correlation (i.e., as lesion length increases, yield decreased) (Das and Prabhakar Indira 2008; Bandara et al. 2015) of lesion length (a measure for resistance) with yield and yield-attributing traits. In such cases, screening of genotypes exclusively for resistance is not appropriate to assess the yield under disease pressure (Bandara et al. 2015). Keeping the yield in view, an improved method of screening using *resistance-tolerance index* (*Index*$_{RT}$) (Bandara et al. 2015) was developed by considering yield-related plant tolerance indicators under disease pressure along with plant resistance against the pathogen. In order to take the tolerance into consideration to breed for high yielding under disease pressure, such indices should be developed and employed in soybean for selection of high-yielding lines under disease pressure.

Recombinant inbred lines (RILs) are a kind of mapping population developed for high-resolution mapping of QTL. It has an added advantage of recovering transgressive lines having higher resistance levels than the resistant parent (Muchero et al. 2011). In a common bean (*Phaseolus vulgaris* L.) breeding program, using a RIL

population derived from two parental lines, BAT 477 (resistant to charcoal rot and drought)/Pinto UI-114 (susceptible to charcoal rot and drought), Garcia-Olivares et al. (2012) identified RILs having stable, high yield and resistance to charcoal rot and drought in charcoal-rot-infected field under rain-fed or terminal heat stress conditions. Such populations should be developed in soybean to recover transgressive lines having improved resistance and yield.

Possible association between high levels of phenolic compounds, sugars and boron in seeds with charcoal rot resistance is demonstrated by Bellaloui et al. (2012). Selection of seeds having high levels of these substances in breeding populations may complement charcoal rot resistance breeding.

10.8   Genetics of Charcoal Rot Resistance and QTL Mapping

Understanding the mode of inheritance and heritability of trait are important in any breeding program. No extensive studies have been done to know whether charcoal rot resistance in soybean is a monogenic or oligogenic trait with high heritability or a polygenic trait with low heritability (Silva et al. 2019). Broad-sense heritability for charcoal rot resistance in soybean is reported as 0.06 by Coser et al. (2017) and 0.45 by Silva et al. (2019) indicating the significant influence of environment on disease reaction and necessitating the need for multilocation evaluation of advanced-generation inbred lines such as RILs in a replicated trial for selection of stable sources of resistance. Talukdar et al. (2009) demonstrated the polygenic gene action for charcoal rot resistance through a continuous distribution of disease reaction, ranging from highly susceptible through moderately resistant to highly resistant. Normal distribution for disease reaction as expressed in terms of length of necrosis is reported by Silva et al. (2019). These studies indicate that charcoal rot resistance in soybean is controlled by multiple loci. Correspondingly, in case of sorghum, inheritance of CR was studied in F2 and backcross populations of the cross 1202A × CSV-5 and concluded that the gene action was polygenic in nature having a major role played by epistatic interaction in inheritance (Rao and Shinde 1985). On the contrary, epistatic gene action is reported in crops like common beans (*Phaseolus vulgaris* L.). Inheritance of charcoal rot resistance in common beans was studied by Olaya et al. (1996) in F2 population of the cross BAT-477 (R)/A--70(S) and observed the disease reaction segregated in 9:7 ratio. Results suggested that the resistance in BAT-477 was governed by two dominant complementary genes. Bulked segregant analysis (BSA) identified two unlinked RAPD (random amplification of polymorphic DNA) markers linked to the resistance. From the F2 population of the cross BATT-477/Pinto UI-114, Hernández-Delgado et al. (2009) concluded that the charcoal rot resistance in BATT 477 was governed by two dominant genes with double recessive epistasis. One possible QTL was found on LG1 in BATT 477.

QTL mapping of charcoal rot resistance is done in a soybean accession PI 567562A (Silva et al. 2019). An F2:3 family (*N* = 140) derived from the cross PI

567562A (R)/ PI 567437 (S) was used to identify genomic regions conditioning charcoal rot resistance. Three QTLs governing resistance against *M. phaseolina* were identified, one QTL on chromosome 15 and two QTL on chromosome 16. QTL on chromosome 15 was mapped within a confidence interval of 1209 kb between SNPs (single-nucleotide polymorphism) *Gm15_01842053* and *Gm15_03051337* (LOD = 5.25; R^2 = 29.4%). On chromosome 16, the first QTL was mapped in a 1533-kb interval between SNPs, *Gm16_28961127* and *Gm16_30493887* (LOD = 4.32; R^2 = 25.4%). The second QTL on chromosome 16 was mapped into 1105-kb interval between SNPs, Gm16_35973543 and *Gm16_37078478* (LOD = 3.6; R^2 = 8.84%). Resistant alleles for all the three QTLs were contributed by the resistant parent PI 567562A. This is the first report of QTL mapping in a bi-parental mapping population.

10.9   Genomics of Charcoal Rot Resistance

Breeding for polygenic resistance is challenging. Genome-wide association studies (GWAS) provide an insight into the genetic architecture of any trait and provides parental choice in QTL mapping (Korte and Farlow 2013). GWAS is very effective in identifying genetic variants underpinning complex traits such as disease resistance (Iquira et al. 2015). In soybean, GWAS is being used for identifying genes governing several forms of biotic stresses including Sclerotina stem rot (Bastien et al. 2014; Iquira et al. 2015; Zhao et al. 2015; Moellers et al. 2017; Wei et al. 2017), Phytophthora root rot (Sun et al. 2014; Schneider et al. 2016; Qin et al. 2017), sudden death syndrome (Wen et al. 2014; Bao et al. 2015; Zhang et al. 2015), soybean cyst nematode (Bao et al. 2014; Vuong et al. 2015; Zhang et al. 2016), tobacco ring spot virus (Chang et al. 2016), soybean aphid (Hanson et al. 2018) and charcoal rot (Coser et al. 2017; Vinholes et al. 2019). Coser et al. (2017) attempted to decipher the genetic architecture of charcoal rot resistance and to identify the genes responsible for resistance. Both field screening and glasshouse screening was done in a diverse collection of 459 plant introductions of the USDA soybean germplasm core collection. Five significant SNPs and putative candidate genes governing biotic and abiotic stress response were identified in field screening, while in glasshouse screening, eight loci associated with eight candidate gene families controlling the functions of plant defence response were identified. Intriguingly, no commonality of genes or markers has been identified between field and glasshouse screenings indicating the complexity of the mechanism underlying the resistance to CR across different environments (Coser et al. 2017).

Vinholes et al. (2019) attempted to identify genomic regions conferring CR in a soybean association mapping panel through GWAS, using SNP markers and haplotype information. An association mapping panel (Contreras-Soto et al. 2017) containing 169 core Brazilian varieties used by farmers from 1991 to 2010 was used for field evaluation of CR. Phenotyping was based on percent mortality. The evaluation of plant mortality is started 4 weeks after sowing when infection symptoms began

to appear. The number of dead plants within each plot was counted every 7 days for 12 weeks. The data was expressed in terms of percentage of mortality. Through genome-wide association analysis, six SNPs were identified for association with CR in soybean (Vinholes et al. 2019). Two haplotypes, of three SNP markers *Gm08_44422211_T_C*, *Gm08_18909193_A_G* and *Gm19_34320762_A_C,*were identified where genotypes having haplotype TAC had lesser mortality percentage than genotypes possessing haplotype CGA (Vinholes et al. 2019).

Developments in genomics since the last decade allowed the use of new breeding strategies for crop improvement. Understanding complex biological systems in legumes is facilitated by comparative genomics using model plants such as *Medicago truncatula* and *Lotus japonicas* (Li et al. 2015). Candidate genes for CR were identified in cowpea based on annotated genic SNPs and comparative genomic studies with soybean and *Medicago truncatula*. Out of nine QTLs identified for resistance based on plant mortality in field and glasshouse experiments, QTL peak of the major QTL *Mac-2* is co-located with a SNP marker derived from a gene inhibiting pectin esterase (Muchero et al. 2011). Comparative genome analysis of the QTL *Mac-2* revealed that the corresponding soybean genomic region on chromosome 8 had a pectin esterase inhibitor gene and two copies of a gene encoding pectin esterase. Another major QTL *Mac-1* coincided with a MATE efflux family protein encoding gene which was highly syntenic to homeologous regions on chromosomes 10 and 20 of soybean (*Glycine max* L*)*. The syntenic region on chromosome 10 harboured nine copies of the MATE efflux family protein gene, whereas the syntenic region on chromosome 20 carried three copies of the same gene. Three resistance QTLs, *Mac-4, Mac-5 and Mac-9*, were syntenic to the soybean genomic regions harbouring osmotic-stress-responsive genes such as heat shock, calcium sensing and sodium hypersensitive genes (Muchero et al. 2011).

10.10   Conclusion and Future Perspectives

Macrophomina phaseolina (Tassi) Goid is a generalist plant pathogen having a wide host range. Economic importance of this pathogen is likely to increase with increase in heat and drought stress under climate change scenarios. Quality and quantity of inoculums and standardisation and repeatability of screening technique are crucial for determining the disease reaction of individual plants in breeding and mapping populations (Ma et al. 2010). Reliable, repeatable and high-throughput screening methods have to be developed for speeding of development of resistant cultivars. Though greenhouse screening is robust, correlation between resistance observed in field and glasshouse conditions is not consistent in some cases (Coser et al. 2017). Several factors such as environmental conditions, growth stages, resistance mechanisms, amount and distribution of inoculums and plant part inoculated must be considered while standardising and correlating different screening techniques. In spite of several limitations, field screening which represents the ideal crop environments must be still considered for disease evaluation. A glasshouse screening technique

that positively and consistently correlates with field screening experiment must be developed. Such technique can prescreen the genotypes prior to a detailed investigation in the field (Coser et al. 2017). An extensive investigation into genome-based host-pathogen interaction (Islam et al. 2012) will be effective in designing breeding strategies for disease control. Charcoal rot resistance is a quantitative trait (Talukdar et al. 2009; Silva et al. 2019). Due to its quantitative nature, information on marker trait association can be well applied in genomic selection rather than marker-assisted selection (MAS). MAS are ineffective in improving polygenic traits. Since many small-effect loci are controlling the CR, genomic selection would be suggested (Coser et al. 2017). Since large-scale precise phenotyping for disease reaction is challenging and heritability of the trait is considerably low, genomic selection can be effective. Precise phenotyping data and marker effects are used to develop a prediction model in a training population. Such prediction models can be applied in 'testing population' to indirect prediction of the phenotypes using only marker genotype. Breeding must aim at high yielding potential, moderate resistance and at least some drought tolerance to maximise the produce even under low soil moisture regimes (Mengistu et al. 2018). Broad-sense heritability of charcoal rot resistance is low (Coser et al. 2017; Silva et al. 2019) and influenced much by environmental factors. In such cases, selection of stable sources of resistance and identification of stable QTL and epistatic QTL interactions can only be done under multilocation environments in replicated trials. Soybean has a narrow germplasm. In such cases, populations like MAGIC (multiparent advanced-generation intercross)-derived RILs resulting from three generations of recombination events among eight diverse parents can have a broad genetic base (Shivakumar et al. 2018). Nested association mapping populations, where a charcoal-rot-tolerant genotype is used as common parent, may be utilised for identifying genetic background effect on QTLs conditioning charcoal rot resistance. Such populations are needed to be developed for soybean improvement against charcoal rot disease for fine mapping of QTL or candidate genes governing resistance and for selection of lines having resistance to charcoal rot. Since charcoal rot disease aggravates under drought conditions, parents selected for MAGIC RIL development must include both charcoal-rot-resistant genotypes and drought-tolerant genotypes. The resultant advanced inbred lines can have both charcoal rot resistance and drought tolerance.

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