Chapter 7
Soybean Breeding for Rust Resistance

Jacqueline Siqueira Glasenapp and Osvaldo Toshiyuki Hamawaki

Abstract Among several pathogens that can impair soybean (*Glycine max* L. Merrill) production from the beginning of the planting to the harvest, *Phakopsora pachyrhizi*, an extremely aggressive biotrophic fungus that causes the disease known as soybean rust (SBR), is undoubtedly one of the most feared by soybean producers. Genetic resistance seems to be the most efficient available tool to control the disease and reduce environmental impacts. In the last decades, seven Rpp (resistance to *P. pachyrhizi*) loci were discovered and introduced into new soybean varieties. Nevertheless, some currently available rust-resistant cultivars have been showing no durable resistance. Twelve Uruguayan *P. pachyrhizi* isolates from Uruguay were able to overcome the resistance of many Rpp genes. All isolates were extremely virulent on soybean differentials with Rpp1, Rpp3, and Rpp4 genes. Furthermore, with the emergence and rapid spread of new *P. pachyrhizi* strains, some populations of this pathogen have increased their tolerance to fungicides. In this context, the development of high yield with cultivars that have durable resistance to SBR is necessary to safeguard soybean stability and food production. Even when resistance is not the immune-type, fungicides are more efficient when their use is associated with tolerant cultivars. Main phenotypic parameters used for the development of rust resistance/tolerance include SBR severity, which represents the proportion of leaf area affected; the progress of the disease, demonstrated by the progress of the rust toward the upper portion of the plant; and resistance reaction types. Evaluations of severity are usually assisted by standard area diagrams (SAD), which are systems of reference composed of images with different severity proportions for visual estimates of the leaf's area affected. The area under the disease progress curve (AUDPC) is used to resume disease progress over time. It allows combining multiple observations of the progress of the disease across years and locations. Besides, lesions of different colors are formed on infected leaves depending upon the type of resistance. Soybean lines that present reddish-brown lesions on

J. S. Glasenapp (✉)
Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE, USA

O. T. Hamawaki
Universidade Federal de Uberlândia, Instituto de Ciências Agrárias, Uberlândia, MG, Brazil

S. H. Wani et al. (eds.), *Soybean Improvement*, https://doi.org/10.1007/978-3-031-12232-3_7

the leaves are more efficient in reducing the multiplication of the fungus. Susceptible cultivars have brown lesions with abundant sporulation, as light brown types, characteristics of susceptible plants, and reddish-brown is the type of lesion typical of resistant plants. Regarding the development of genetic rust resistance, presently, soybean improvement programs can employ tools for the development of high-yielding soybean varieties with two, three, or even four pyramided Rpp genes. Gene pyramiding can be applied to accelerate the development of new durable resistant cultivars, especially when associated with marker-assisted selection (MAS), which helps in the identification of the best progenies in each generation, while the different genes of resistance are pyramided into a single genotype.

Keywords Soybean · Soybean rust · Gene pyramiding · Marker assisted selection · Rpp genes

7.1 Introduction

Several pathogens can impair soybean (*Glycine max* L. Merrill) production from the beginning of the planting to the harvest. Estimates of annual decreases in soybean yield caused by diseases in 28 states in the USA, and Ontario, east-central Canada, pointed that soybean cyst nematode (*Heterodera glycines*) caused more than double losses than any other disease (https://crop-protection-network.s3.amazonaws.com/Publications/cpn-1018-soybean-disease-loss-stimates-from-the-united-states-and-ontario-canada.pdf). Seedling diseases, charcoal rot (*Macrophomina phaseolina*), and sudden death syndrome (*Fusarium virguliforme*) were other diseases that lead to more losses in descending order.

Allen et al. (2017) reported estimates of $60.66 average losses per acre caused by diseases across US states and Ontario Province in east-central Canada from 2010 to 2014. Remarkably, soybean, the yield of eight leading producing countries in the world, was decreased by 59.9 million metric tons (to) due to diseases just in 2006. Major yield losses were occasioned by soybean rust (*Phakopsora pachyrhizi*), followed by cyst nematode, brown spot, seedling diseases, anthracnose, and charcoal rot in decreasing order of total yield loss (Wrather et al. 2010).

Although there are two soybean rust species, *P. pachyrhizi* is more aggressive and of most economic significance (Bonde et al. 2006). It stands out among diseases that can affect soybean yield because of its destructive potential. *Phakopsora meibomian* is less aggressive and limited to some countries in North America and South America continents. *P. pachyrhizi* is a widespread species and can be found in countries such as Nigeria, Indonesia, and Brazil and in the continents like South America, Europe, North America, Oceania, and Africa (CABI 2020).

In Brazil, although dozens of pests and diseases are favored by the tropical climate and threaten soybean yield, soybean rust (SBR) caused by *P. pachyrhizi* is

undoubtedly one of the most feared by producers. Because of its high dissemination capacity and adaptation to adversity, SBR represents one of the biggest challenges for soybean production. The disease was first reported in Brazil in the 2001/2002 harvest (Jaccoud Filho et al. 2001; Yorinori et al. 2001). Two years later, it was found present in most of the country's soy-producing regions (Yorinori et al. 2005). The rapid spreading of the fungus was facilitated by the wind and the presence of soybeans in the field for most of the year when considering all the producing regions of Brazil (Hartman et al. 2015; Godoy et al. 2016a).

Currently, SBR management and control strategies are mostly limited to fungicides, cultural practices, and cultivars with genes of resistance. However, fungicides costs are high. In Brazil, 96% of soybean producers cost with fungicides in the 2016/2017 harvest was destined for rust control (https://www.cepea.esalq.usp.br/br). Alternately, genetic resistance can reduce production costs and environmental damage by decreasing the amount and frequency of fungicide needed to control the disease. Nevertheless, some currently available rust-resistant cultivars have been presenting no durable resistance, and farmers often ended up having only fungicides as an option to manage the fungus.

Despite the development of new SBR-resistant cultivars which is extremely important for the suitability of the crop and has a profound impact on farmers' production and profitability, the overall impact of SBR in soybean cultivars carrying specific resistance genes is associated with the variation of the pathogen and its degree of virulence. Even if the resistance is not the immune type, fungicides are more efficient when used associated with tolerant cultivars. Although the complete control of the disease cannot be guaranteed by the adoption of only one single measure, it can be achieved by a set of measures as the planting of resistant/tolerant cultivars, employment of appropriate husbandry, and application of fungicides at the correct stages of plant growth and disease development. Also, tolerant/resistant cultivars may show all their yield potential when cultivated obeying its adaptation range, sowing season, and adoption of a vacuum and chemical control. Even if over the years these materials may lose some resistance, they still better coexist with the disease in the field than non-resistant cultivars and produce higher yields.

This chapter generally describes the genetic mechanisms of rust resistance and the main aspects and parameters used for the development of resistance/tolerance. Updates and more detailed information about the distribution and survey status of SBR can be found in the USDA-National Invasive Species Information Center website. Estimates of soybean yield reductions caused by SBR estimated from 1996 through 2014 are available at the University of Illinois College of Agriculture website. It produces annual estimates of reductions in soybean yield caused by diseases and pathogens in the major producer states in the USA. Successful examples of rust-resistant soybeans are the cultivars Inox®, developed by TMG (Tropical Breeding & Genetics) and BRSGO, BRSMS Bacuri, and BRS 511, developed by Embrapa (Empresa Brasileira de Pesquisa Agropecuaria).

7.2 The Genetic Variability of *P. pachyrhizi* Populations

Bromfield and Hartwig reported the first occurrence of genetic variability in *P. pachyrhizi* populations in tests carried out in 1980. Susceptibility to an isolate from Taiwan and resistance to an isolate from India were observed in the PI 200492 and PI 462312 cultivars, respectively. Besides, Hartwig and Bromfield (1983) also verified that the SBR genes of resistance Rpp1, Rpp2, and Rpp3 do not possess the same spectrum protection against different isolates of the rust.

The analysis of samples of the fungus collected in the South, Southeast, and Midwest of Brazil revealed significant genetic variability between populations, which could put the vertical genetic resistance conferred by one unique gene in jeopardy (Tschurtschenthaler et al. 2012). Data from molecular markers demonstrated that the genetic diversity of *P. pachyrhizi* is small in large geographical areas and large in local populations (Murithi et al. 2016; Akamatsu et al. 2013; Yamanaka et al. 2010; Twizeyimana et al. 2009). Besides, the virulence of fungus can vary over time, and resistant genotypes' responses may differ (Bromfield 1984); furthermore, differences between new and old isolates were observed (Bonde et al. 2006).

Significant variation in fungi was unveiled through the comparison of pathogenicity profiles of 59 *P. pachyrhizi* populations from Brazil, Argentina, and Paraguay (Akamatsu et al. 2013). Although the potential of infection of *P. pachyrhizi* differed according to geographic regions where samples were taken, the fungus spores presented a high capability of dispersion over large distances, which likely allowed the movement of virulent genotypes. It seems that the presence of resistance genes in the host did not directly reduce the frequency of virulence genes in populations (Murithi et al. 2016; Akamatsu et al. 2013; Yamanaka et al. 2010; Twizeyimana et al. 2009). Furthermore, Brazilian isolates showed greater virulence, which was reflected by the higher levels of sporulation observed in tests performed in four varieties carrying Rpp1 (Yamanaka et al. 2010).

7.3 Soybean Rust Infection and Development

In susceptible species, *P. pachyrhizi* infection is initiated by a uredospore that germinates and produces a germ tube that grows across the leaf surface and forms a structure known as appressoria. The epidermis penetration occurs through specialized hyphae (haustoria), which absorb the nutrients from the cytoplasm. SBR infection and symptoms can be observed at any stage of plant development, with a higher incidence from the closure of the canopy due to the emergence of a more favorable microclimate to infection (Isard et al. 2006). Symptoms usually start on the leaves in the lower third of the plant as numerous necrotic tiny spots surrounded by a chlorotic halo with variable sizes. Usually, lesions are gathered in clusters of pustules that present darker greenish to a greenish-gray color (Bock et al. 2016). Over time, the infected leaves turn yellow, dry, and fall off. The early defoliation prevents the

complete development of the grains with consequent reduction in productivity (Godoy et al. 2016b). The drop of leaves takes place when disease severity is nearly 80% of its upper limit (Kumudini et al. 2008). The progression of the disease during the formation and filling of pods is most prejudicial to yield (Kawuki et al. 2004).

7.4 Soybean Rust Evaluations

The first method for assessment and rating of SBR was based on severity, which represents the proportion of leaf area affected, and was developed to deal with the possibility of rust spread in US soybean crops (Bromfield 1984). In 1977, after the international soybean rust meeting held in Thailand, the International Working Group on Soybean Rust (IWGSR) proposed a new rating system to assess SBR. Accordingly, a final classification based on a scoring system of three-digit was suggested (Li et al. 2010). The first digit indicated the sampled leaf position in the crop canopy; the second digit was designated to represent the disease severity of the sampled leaf rated in comparison with a standard diagram; the third digit was related to the reaction type observed on the sampled leaf (Godoy et al. 2006). However, in 1984, Bromfield pointed that data obtained in such evaluations are qualitative in nature and cannot be subjected to appropriate statistical analysis. Consequently, this scale would have limited applications in the selection practices of breeding programs (Tukamuhabwa and Maphosa 2010).

Subsequently, other diagrammatic scales for estimations of SBR severity appropriate for statistical analysis were developed and used in comparison to actual diseased leaves, which improved the precision, accuracy, and reliability of the assessments of disease severity. These scales represented standardized systems with illustrated images of diseased plants with distinct severity levels. Modern trends in the development of new scales for estimations of SBR severity include sets of diagrams increasing in approximately linear increments, true color photos, and digital drawings, among others, and new statistical methods for evaluating the precision, accuracy, and reliability of the evaluations (Del Ponte et al. 2017).

Currently, main phenotypic parameters used for the development of rust resistance/tolerance include SBR severity; the progress of the disease, demonstrated by the progress of the rust toward the upper portion of the plant; and resistance reaction types.

7.5 Genetic Mechanisms of Rust Resistance

Qualitative and quantitative resistances are the main types of resistance to pathogens used for plant breeding purposes. Whereas qualitative resistance is primarily conferred by major dominant genes, which turn host-pathogen interactions

incompatible, quantitative resistance results from the accumulative effect of multiple genes of minor effects (Simko 2002; Ewing et al. 2000).

Different types of rust resistance are classified as race-specific, nonrace-specific, and nonhost. Race-specific resistance relies upon specific genetic interactions between host resistance (R) and pathogen avirulence (Avr) genes; however, such rust resistance genes might be effective against some but not every race of a pathogen (Jones et al. 2016). Nonrace-specific resistance is determined when a small number of major genes in the host confer resistance against all races of a pathogen or, in fewer cases, against multiple pathogens (Ellis et al. 2014; Krattinger and Keller 2016). Such resistance is known as quantitative and involves a partial resistant phenotype in which the pathogen development is slowed with no apparent immune system response. Although soybeans with this type of resistance do not necessarily dispense chemical control, they can delay the advance of the rust on the field when the climatic conditions are unfavorable to the use of fungicides (Periyannan et al. 2017). Nonhost resistance (NHR) is found in plant species that cannot be infected by a non-adapted pathogen (Bettgenhaeuser et al. 2014). It depends upon multiple protective mechanisms such as constitutive barriers and inducible reactions that are not easily overpowered by a pathogen (Nuernberger and Lipka 2005). Besides, NHR is known as the most durable and effective kind of resistance to disease; hence, related nonhost species are increasingly being utilized to identify new sources of resistance (Kawashima et al. 2016).

Plant resistance genes known as R genes have been extensively used to develop cultivars resistant to diseases. The introduction of R genes into new lines typically involves backcrossing or transformation. However, immunity mediated by R genes may not last long since many plant pathogen populations have the potential to adapt and overcome resistance. Alternatively, NHR has been acknowledged as the most durable broad-spectrum form of resistance against many pathogens in plants and has emerged as a new alternative for disease control (Lee et al. 2016).

Overall, NHR factors operate at preinvasive and postinvasive resistance phases. Preinvasive factors comprise physical or chemical barriers on the surface of the epidermis that restricts the establishment of nonhost pathogens (Fonseca and Mysore 2019). As regards, *Arabidopsis* NHR has been extensively used to study the genetic basis of resistance to several pathogens including *P. pachyrhizi* (Loehrer et al. 2008; Campe et al. 2014). Epidermal penetration resistance to *P. pachyrhizi* in *Arabidopsis* depends on the functional genes *pen1, pen2*, and *pen3* (Langenbach et al. 2013). Penetration and multiplication of nonhost pathogens are limited by postinvasive resistance through local and systemic immune response mechanisms (Fonseca and Mysore 2019). The functionality association of *pen2*, *pad4*, and *sag101* genes (Langenbach et al. 2013) controls the postinvasion resistance in mesophyll.

Comparisons of the genetic differential expression of *wild-type*, *pen2*, *pad4*, and *sag101* mutants after inoculation with *P. pachyrhizi* lead to the identification of a novel component of *Arabidopsis* mesophyll NHR to *P. pachyrhizi* named UDP-glucosyltransferase UGT84A2/bright trichomes 1 (BRT1) (Langenbach et al. 2013). BRT1 is a cytoplasmic enzyme induced in pen2 in the postinvasion resistance to

P. pachyrhizi. The *brt1* mutation did not affect NHR of wild-type plants, although silencing or mutation of BRT1 raised haustoria formation in pen2 mesophyll. Accordingly, BRT1 is essential for postinvasion NHR of *Arabidopsis* to *P. pachyrhizi* and can be useful in conferring durable soybean resistance to the rust.

In fact, Loehrer et al. (2008) results demonstrated that *Arabidopsis* can be useful in the investigation of mechanisms of NHR to SBR. Observed epidermal cell death of wild-type *Arabidopsis* was likely synchronized by the cumulus of H_2O_2 under P. *pachyrhizi* infection. However, even with cell death, the fungus hyphae grow, and the infection was terminated in the mesophyll boundaries. Such events are related to the expression of PDF1.2, which suggests that *P. pachyrhizi* mimics aspects of a necrotroph. *Arabidopsis* PEN mutants with defective penetration resistance exhibited expressive occupation of the mesophyll. *Pen3-1* double mutants in either jasmonic acid or salicylic acid signaling demonstrated the participation of both pathways in NHR of *Arabidopsis* to *P. pachyrhizi*. The expression of the AtNHL10 gene, expected in tissues going through hypersensitive response, was just triggered in infected pen3-1 mutants.

Following this, ten extra postinvasion-induced nonhost resistance genes (PINGs) were identified. These genes' transcription was co-regulated with BRT1 in *Arabidopsis* under *P. pachyrhizi* infection. Ping4, ping5, and ping9 genes enhanced *Arabidopsis* resistance to *P. pachyrhizi* and were fundamental postinvasion NHR response in *Arabidopsis*. Interestingly, ping7 overexpression in *Arabidopsis* enhanced resistance to *P. pachyrhizi* in transgenic lines when compared to control; however, silencing of ping7 did not increase susceptibility to the fungus, likely due to its functional redundancy in the GDSL-motif lipase/hydrolase gene family (Langenbach et al. 2016).

Besides, Kawashima et al. (2016) successfully cloned a rust resistance gene (CcRpp1) from pigeon pea (*Cajanus cajan*) that confers full resistance to *P. pachyrhizi* in soybean. Therefore, legume species related to soybeans such as pigeon pea, common bean, cowpea, and others could also provide new sources of resistance genes for the improvement of the crop.

7.6 Genes Conferring Rust Resistance to Soybean

Although the effectors or other determinants that collaborate to the recognition of *P. pachyrhizi* isolates in soybean are still unrecognized (Chander et al. 2019; Pedley et al. 2018), in the last decades, seven Rpp (resistance to *P. pachyrhizi*) loci named Rpp1 (Mc Lean and Byrth 1980), Rpp2 (Bromfield and Hartwig 1980), Rpp3, (Bromfield and Melching 1982), Rpp4 (Hartwig 1986), Rpp5 (Garcia et al. 2008), Rpp6 (Li et al. 2012), and Rpp7 (Childs et al. 2018b) were identified and mapped in the soybean genome. Rpp1 (Hyten et al. 2007), Rpp4 (Silva et al. 2008), and Rpp6 (Li et al. 2012) genes map to chromosome 18, while Rpp2 (Silva et al. 2008), Rpp3 (Hyten et al. 2009), Rpp5 (Garcia et al. 2008), and Rpp7 (Child et al.'s 2018b) map to chromosomes 16, 6, 3, and 19, respectively.

However, since it was verified that Rpp genes confer resistance to a limited number of *P. pachyrhizi* isolates, their ability for long-lasting resistance has been questioned (Miles et al. 2011; Hartman et al. 2005; Bromfield 1984). Rpp1 and Rpp6 genes still provided a good level of resistance to the SBR; however, varieties carrying Rpp2, Rpp3, Rpp4, and Rpp5 gens have shown incomplete resistance when compared to susceptible controls, with moderate levels of rust development (Walker et al. 2014). The resistance conferred by a single gene tended to be more easily broken, while the effectiveness of race-specific resistance to the fungus may be short-lived (Yorinori 2005; Hartman et al. 2005; Bromfield 1984).

7.7 Phenotypic Parameters Used for Rust Evaluations

7.7.1 Severity

With the possibility of rust spread in the soybean crops, different methods for the assessment of SBR severity were designed to overcome the statistical limitations of qualitative data analysis: as the percentage severity scale of 0–9, where 0 is no disease and 9 is 90% disease with defoliation (Walla 1979). In the mid-2000s, a standard area diagram (SAD) with a system of reference for visual estimates of SBR severity was developed (see Godoy et al. 2006). This SAD was made up of two-gray color images of soybean leaflets with six severity values (0.6%, 2%, 7%, 18%, 42%, and 78.5%) represented in one soybean leaflet diagram that was used in comparison to real diseased leaves. The scale lower and upper limits satisfied minimum and maximum proportions of the disease observed in the field. The estimation of the real intensity of the disease in the field and its representation in the scale was found highly precise. That enhanced the accuracy of evaluations by providing severity estimates near the true values (Godoy et al. 2006) and was extensively used for SBR severity evaluations in breeding programs and in experimental research (Beruski et al. 2020; Franceschi et al. 2020; Xavier et al. 2019; Lana et al. 2018; Rios et al. 2018).

However, Godoy et al.'s (2006) SAD was developed according to the now disproved assumption that increments between levels of severities should be based on the Weber-Fechner stimulus response law, which states that the logarithm of the stimulus is proportional to visual acuity (Franceschi et al. 2020; Bock et al. 2010; Nutter and Esker 2006). Besides, Godoy et al.'s (2006) SAD levels of severities from 18% to 78.5% presented a range where the error in the estimates was high (Franceschi et al. 2020).

As a result, Franceschi et al. (2020) developed a new SAD composed of six diagrams representing true colors with linear increments (c.15%) refined with four additional diagrams at low (<10%) severities, totaling ten diagrams (0.2%, 1%, 3%, 5%, 10%, 25%, 40%, 55%, 70%, and 84%). This new SAD significantly improved the accuracy (>0.95), reliability, and precision of the SBR estimates. The low

precision and a bias for underestimation with the increase in severity were the main problems with the SAD developed by Godoy et al. (2006). Franceschi et al.'s (2020) SAD was more efficient for detecting even the smallest differences in mean control than Godoy et al.'s (2006) SAD, which required double the sample size to achieve the same result.

The severity is currently the main data used to compare fungicide treatments and cultivars (Godoy et al. 2006; Lana et al. 2018; Hamawaki et al. 2019).

7.7.2 Disease Progress

Assessments of incidence, or appearance of the lesson, demonstrate the progress of the rust toward the upper portion of the plant. Plants can be visually divided into three parts and the incidence of the disease estimated using rankings or scores (Campbell and Madden 1990). In the identification of the resistant genotypes, field experiments are limited to areas of occurrence of the fungus; however, plants in the greenhouse can be artificially inoculated. Inoculums are usually locally collected and require at least 6 h of dew period at optimum temperatures for efficient infection of soybean leaves. Disease typically starts at a low portion of plants and gradually increases in incidence and/or severity over time. Evaluations of progress of disease on plants are usually performed several times during the pathogen infection (Godoy et al. 2016b).

The area under the disease progress curve (AUDPC) is used to resume disease intensity over time. It allows combining multiple observations of the progress of the disease across years and locations. The extent of disease is assessed in each evaluation using a scale based on severity, disease incidence, or an association of both (Simko and Piepho 2012).

Incidence and severity data obtained are commonly used in calculus of AUDPC and assess the quantitative resistance of soybean cultivars (Campbell and Madden 1990). AUDPC is usually determined by the trapezoidal method through the midpoint rule. That breaks up a disease progress curve into a series of trapezoids where the area of each one is calculated and then added up (Madden et al. 2017; Simko and Piepho 2012):

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} x\left(t_{i+1} + t_i\right)$$

where y_i is the assessment of the disease percentage or proportion at a time *ti* in the *i*th observation and *n* is the total number of observations.

The data for evaluation of the SBR range from the date when the disease is detected to advanced maturity stages of crop development as R6 or R7 stages. With the aid of a SAD, disease severity can be visually estimated in leaflets sampled in each of the three canopy heights and averaged at the plot level. Mean plot severity

can be calculated as a percentage of the severity values in lower, middle, and upper canopy heights (Franceschi et al. 2020; Lana et al. 2018; Glasenapp et al. 2015).

7.7.3 Resistance Reactions

SBR lesions are like those of bacterial pustules caused by *Xanthomonas campestris*, except by the presence of characteristic blisters like uredia on the abaxial surface of the leaves with a central pore that releases urediniospores (Yorinore 1994). These lesions grow gradually, turning from gray to tan, reddish-brown, or dark brown and assuming a polygonal shape restricted by leaf veins (Tschanz and Shanmugasundaram 1984).

Soybean lines that present reddish-brown lesions on the leaves are more efficient in reducing the multiplication of the fungus. Susceptible cultivars have brown lesions with abundant sporulation (Zambenedetti et al. 2007), and the color of lesions formed on the leaves due to the reaction to the infection by the fungus can be used to classify plant resistance, like light brown types, characteristics of susceptible plants, and reddish-brown typical of resistant plants.

Bromfield et al. (1980) reported three distinct infection types of SBR. The TAN type, found in susceptible plants, is a ten-colored lesion of about 0.4 mm^2, commonly with two to five uredia on the abaxial surface of the leaf. The RB type is characterized by a reddish-brown lesion of roughly 0.4 mm^2 often with zero to two uredia on the abaxial surface; this reaction type indicated host resistance associated with hypersensitivity. The zero types have no macroscopically visible evidence of lesions and indicated host immunity or near immunity. Over time, lesioned area and the number of uredia per lesion increase in both TAN and RB, but the rate of increase is slower for RB.

Additional levels of variation within RB and TAN infection types were observed by Bromfield (1984), and a new infection grade where 0 is no visible symptoms; 1 is RB lesions small, irregular, and lacking uredia; 2 is RB lesions with one or two sparsely sporulating uredia per lesion; 3 is RB lesions with three or more profusely sporulating uredia per lesion; 4 is TAN lesions with two to five uredia per lesions; and 5 is TAN lesions with more than five uredia per lesion was developed. Therefore, type 0 indicates host humanity; types 1, 2, and 3 host-specific resistance; and types 5 host susceptibility.

7.8 Final Observations

Even though we currently have 7 Rpp genes discovered and introduced into new soybean varieties, Stewart et al. (2019) obtained 12 Uruguayan *P. pachyrhizi* isolates able to overcome the resistance of many Rpp genes. Seven different pathotypes were observed, three of them were related to a unique virulence pattern, found

mainly on plants with Rpp3, Rpp4, and Rpp6 genes, and four showed identical virulence patterns with South America isolates. All isolates were extremely virulent on soybean differentials with Rpp1, Rpp3, and Rpp4 genes.

Furthermore, with the emergence of new strains of *P. pachyrhizi* and its rapid spread, some populations of the pathogen have increased its tolerance to fungicides. On March 8, 2017, the FRAC (Fungicide Resistance Action Committee) shared the partial results of monitoring of fungicides inhibitors of succinate dehydrogenase (ISDH, *carboxamidas*) carried out in Brazil. The statement reports reduced efficiency of these fungicides in areas with a high incidence of the fungus and a history of intensive use of ISDHs. *P. pachyrhizi* populations collected in 2015/2016 have shown a mutation in the C subunit at the I86F position. This very mutation was also observed in samples collected in 2016/2017, and its relevance to reducing sensitivity to ISDH is being investigated (http://www.frac-br.org/).

In this context, robust and durable resistant crop cultivars are necessary to safeguard soybean stability and food production. Although elucidation of defense mechanisms that contribute to the SBR resistance is fundamental for the development of new sources of resistance, the resistance genes already identified mapping to seven independent loci can be useful for the development of new materials. Efficient screening protocols were developed, and low-cost genotyping technology has been available for marker-assisted selection and backcrossing of Rpp loci. Therefore, nowadays soybean improvement programs can employ tools for the development of high-yielding soybean varieties with two, three, or even four pyramided Rpp genes (Childs et al. 2018a). Success strategies for developing new soybean cultivars with durable resistance could involve combinations of race-specific R and nonrace-specific genes. In this sense, gene pyramiding would effectively facilitate the addition of genes of interest into a single genetic background.

Gene pyramiding in association with marker-assisted selection (MAS) and other techniques can accelerate the development of durable resistant/tolerant lines by combining resistant genes into a single genetic background in a relatively short time (Dormatey et al. 2020). The strategy consists of concatenate genes of interest identified in multiple parents into a single genotype. Varieties with at least two pyramided Rpp genes tend to retain resistance characteristics for longer than varieties with a single gene (Maphosa et al. 2012; Yamanaka et al. 2010). For instance, soybean varieties with three genes of resistance (Rpp2, Rpp4, and Rpp5) pyramided are significantly more resistant to rust (Yamanaka et al. 2013; Lemos et al. 2011).

DNA markers can increase the speed of the pyramiding process by allowing genetic identification of the best progenies in each generation. However, the success of gene pyramiding depends upon a variety of important aspects such the number of genes intended to be transferred, the nature of germplasm, the number of genotypes selected in each breeding generation, and the distance between the target genes and flanking markers, among others (Joshi and Nayak 2010).

References

Akamatsu H, Yamanaka N, Yamaoka Y, Soares RM, Morel W, Ivancovich AJG, Bogado AN, Kao M, Yorinori JT, Suenaga K (2013) Pathogenic diversity of soybean rust in Argentina, Brazil, and Paraguay. J Plant Pathol 79:28–40

Allen TW, Bradley CA, Sisson AJ, Byamukama E, Chilvers MI, Coker CM, Collins AA, Damicone JP, Dorrance AE, Dufault NS et al (2017) Soybean yield loss estimates due to diseases in the United States and Ontario, Canada, from 2010 to 2014. Plant Health Prog 18(1):19–27

Beruski GC, Del Ponte EM, Pereira AB, Gleason ML, Câmara GMS, Juniorip A, Sentelhas PC (2020) Performance and profitability of rain-based thresholds for timing fungicide applications in Soybean Rust control. Plant Dis 104(10):2704–2712

Bettgenhaeuser J, Gilbert B, Ayliffe M, Moscou MJ (2014) Nonhost resistance to rust pathogens–a continuation of continua. Front Plant Sci 5:664

Bock CH, Poole G, Parker PE, Gottwald TR (2010) Plant disease severity estimated visually, by digital photography and image analysis, and by hyperspectral imaging. Crit Rev Plant Sci 29:59–107

Bock CH, Chiang KC, Del Ponte EM (2016) Accuracy of plant specimen disease severity estimates: concepts, history, methods, ramifications and challenges for the future. CAB Rev 11:1–21

Bonde M, Nester S, Austin C, Stone C, Frederick R, Hartman G, Miles M (2006) Evaluation of virulence of *Phakopsora pachyrhizi* and *Phakopsora meibomiae* isolates. Plant Dis 90:708–716

Bromfield KR (1984) Soybean rust. Soybean rust, Monograph No. 11. American Phytopathological Society, St. Paul

Bromfield K, Hartwig E (1980) Resistance to soybean rust and mode of inheritance. Crop Sci 20:254–255

Bromfield KR, Melching JS (1982) Sources of specific resistance to soybean rust. Phytopathology 72:706

Bromfield KR, Melching JS, Kingsolver CH (1980) Virulence and aggressiveness of *Phakopsora pachyrhizi* isolates causing soybean rust. Phytopathology 70(1):17–21

CABI (2020) *Phakopsora pachyrhizi*. In: Invasive Species Compendium. CAB International, Wallingford. www.cabi.org/isc

Campbell CL, Madden LV (1990) Introduction to plant disease epidemiology. John Wiley & Sons, New York, p 523p

Campe R, Loehrer M, Conrath U, Goellner K (2014) Phakopsora *pachyrhizi* induces defense marker genes to necrotrophs in *Arabidopsis thaliana*. Physiol Mol Plant Pathol 87:1–8

Chander S, Ortega-Beltran A, Bandyopadhyay R, Sheoran P, Ige GO, Vasconcelos M, Oliveira ALG (2019) Prospects for durable resistance against an old Soybean enemy: a four-decade journey from Rpp1 (Resistance to *Phakopsora pachyrhizi*) to Rpp7. Agronomy 9:348

Childs SP, Buck JW, Li Z (2018a) Breeding soybeans with resistance to soybean rust (*Phakopsora pachyrhizi*). Plant Breed 137:250–261

Childs SP, King ZR, Walker DR, Harris DK, Pedley KF, Buck JW, Boerma HR, Li Z (2018b) Discovery of a seventh Rpp soybean rust resistance locus in soybean accession PI 605823. Theor Appl Genet 131:27–41

Del Ponte EM, Pethybridge SJ, Bock CH, Michereff SJ, Machado FJ, Spolti P (2017) Standard area diagrams for aiding severity estimation: scientometrics, pathosystems, and methodological trends in the last 25 years. Phytopathology 107:1161–1174

Dormatey R, Sun C, Ali K, Coulter JA, Bi Z, Bai J (2020) Gene Pyramiding for Sustainable Crop Improvement against Biotic and Abiotic Stresses. Agronomy 10(9):1255

Ellis JG, Lagudah ES, Spielmeyer W, Dodds PN (2014) The past, present and future of breeding rust resistant wheat. Front Plant Sci 5:641

Ewing EE, Simko I, Smart CD, Bonierbale MW, Mizubuti ESG, May GD, Fry WE (2000) Genetic mapping from field tests of qualitative and quantitative resistance to *Phytophthora infestans* in a population derived from *Solanum tuberosum* and *Solanum berthaultii*. Mol Breed 6:25–36

Fonseca JP, Mysore KS (2019) Genes involved in nonhost disease resistance as a key to engineer durable resistance in crops. Plant Sci 279:108–116

Franceschi VT, Alves KS, Mazaro SM, Godoy CV, Duarte HS, del Ponte EM (2020) A new standard area diagram set for assessment of severity of soybean rust improves accuracy of estimates and optimizes resource use. Plant Pathol 69:495–505

Garcia A, Calvo ES, Kiihl RAD, Harada A, Hiromoto DM, Vieira LGE (2008) Molecular mapping of soybean rust (*Phakopsora pachyrhizi*) resistance genes: discovery of a novel locus and alleles. Theor Appl Genet 117:545–553

Glasenapp JS, Sediyama T, Cruz CD, Matsuo E, Brommonschenkel SH, RCT O, Hamawaki OT (2015) Diversity of agronomic and molecular traits of the soybean cultivars with different levels of *Phakopsora pachyrhizi* resistance. Biosci J 31(1):25–36

Godoy CV, Koga LJ, Canteri MG (2006) Diagrammatic scale for assessment of soybean rust severity. Fitopatol Bras 31(1):63–68

Godoy CV, Seixas CDS, Soares RM, Marcelino-Guimarães FC, Meyer MC, Costamilan LM (2016a) Asian soybean rust in Brazil: past, present, and future. Pesq Agrop Brasileira 51:407–421

Godoy CV, Almeida AMR, Costamilan LM, Meyer MC, Dias WP, Seixas CDS, Soares RM, Henning AA, Yorinori JT, Ferreira LP, Silva JFV (2016b) Doenças da soja. In: Amorim L, Rezende JAM, Bergamin Filho A, Camargo LEA (eds) Manual de fitopatologia: doenças das plantas cultivadas, vol 2, 5th edn. Agronômica Ceres, Ouro Fino, pp 657–675

Hamawaki RL, Hamawaki OT, Nogueira APO, Juliatti FC, Glasenapp JS, Hamawaki CDL (2019) New high-yielding conventional soybean adapted to the states of Goiás, Minas Gerais and Mato Grosso, Brazil. Acta Scientiarum Agron 41:39913

Hartman GL, Miles MR, Frederick RD (2005) Breeding for resistance to soybean rust. Plant Dis 89:664–666

Hartman GL, Sikora EJ, Rupe JC (2015) Rust. In: Hartman GL, Rupe JC, Sikora EJ, Domier LL, Davis JA, Steffey KL (eds) Compendium of soybean diseases, 5th edn. American Phytopathological Society, St. Paul, pp 56–59

Hartwig EE (1986) Identification of a fourth major gene conferring resistance to Soybean Rust. Crop Sci 26(6):1135–1136

Hartwig EE, Bromfield KR (1983) Relationships among three genes conferring specific resistance to rust in soybeans. Crop Sci 23:237–239

Hyten DL, Hartman GL, Nelson RL, Frederick RD, Concibido VC, Narvel JM, Cregan PB (2007) Map location of the Rpp1 locus that confers resistance to soybean rust in soybean. Crop Sci 47:837–838

Hyten DL, Smith JR, Frederick RD, Tucker ML, Song Q, Cregan PB (2009) Bulked segregant analysis using the GoldenGate assay to locate the Rpp3 Locus that confers resistance to Soybean Rust in Soybean. Crop Sci 49(1):265–271

Isard SA, Dufault NS, Miles MR, Hartman GL, Russo JM, Wolf ED, Morel W (2006) The effect of solar irradiance on the mortality of *Phakopsora pachyrhizi* urediniospores. Plant Dis 90:941–945

Jaccoud Filho DS, Hiar CP, Bona PF, Gasperin L (2001) Ocorrência da ferrugem da soja na Região dos Campos Gerais do Paraná. In: Reunião de Pesquisa de Soja da Região Central do Brasil, Embrapa Soja. Documentos, 157. Embrapa Soja, Londrina, pp 109–110

Jones JDG, Vance RE, Dangl JL (2016) Intracellular innate immune surveillance devices in plants and animals. Science 354(6316):aaf6395

Joshi RK, Nayak S (2010) Gene pyramiding-A broad spectrum technique for developing durable stress resistance in crops. Biotechnol Mol Biol Rev 5(3):51–60

Kawashima CG, Guimaraes GA, Nogueira SR, Maclean D, Cook DR, Steuernagel B et al (2016) A pigeonpea gene confers resistance to Asian soybean rust in soybean. Nat Biotechnol 34:661–665

Kawuki RS, Tukamuhabwa P, Adipala E (2004) Soybean rust severity, rate of rust development, and tolerance as influenced by maturity period and season. Crop Prot 23:447–455

Krattinger SG, Keller B (2016) Molecular genetics and evolution of disease resistance in cereals. New Phytol 212:320–332

Kumudini S, Godoy CV, Board JE, Omielan J, Tollenaar M (2008) Mechanisms involved in soybean rust-induced yield reduction. Crop Sci 48:2334–2342

Lana FD, Paul PA, Godoy CV, Utiamada CM, Silva LHCP, Siqueri FV, Forcelini CA, Jaccoud-Filho DS, Miguel-Wruck DS, Borges EP et al (2018) Meta-analytic modeling of the decline in performance of fungicides for managing soybean rust after a decade of use in Brazil. Plant Dis 102:807–817

Langenbach C, Campe R, Schaffrath U, Goellner K, Conrath U (2013) UDP-glucosyltransferase UGT84A2/BRT1 is required for Arabidopsis nonhost resistance to the Asian soybean rust pathogen *Phakopsora pachyrhizi*. New Phytol 198:536–545

Langenbach C, Schultheiss H, Rosendahl M, Tresch N, Conrath U, Goellner K (2016) Interspecies gene transfer provides soybean resistance to a fungal pathogen. Plant Biotechnol J 14:699–708

Lee S, Whitaker VM, Hutton SF (2016) Mini review: potential applications of non-host resistance for crop improvement. Front Plant Sci 7:997

Lemos NG, Braccini AL, Abdelnoor RV, Oliveira MCN, Suenaga K, Yamanaka N (2011) Characterization of genes Rpp2, Rpp4, and Rpp5 for resistance to soybean rust. Euphytica 182:53–64

Li X, Esker PD, Pan Z, Dias AP, Xue L, Yang XB (2010) The uniqueness of the Soybean Rust pathosystem: an improved understanding of the risk in different regions of the world. Plant Dis 94(7):796–806

Li S, Smith JR, Ray JR, Frederick RD (2012) Identification of a new soybean rust resistance gene in PI567102B. Theor Appl Genet 125:133–142

Loehrer M, Langenbach C, Goellner K, Conrath U, Schaffrath U (2008) Characterization of nonhost resistance of *Arabidopsis* to the Asian soybean rust. Mol Plant-Microbe Interact 21(11):1421–1430

Madden LV, Hughes G, van den Bosch F (2017) The study of plant disease epidemics. American Phytopathological Society, St. Paul, 421p

Maphosa M, Talwana H, Tukamuhabwa P (2012) Enhancing soybean rust resistance through Rpp2, Rpp3 and Rpp4 pair wise gene pyramiding. Afr J Agric Res 7:4271–4277

Mc Lean R, Byrth DE (1980) Inheritance of resistance to rust (*Phakopsora pachyrhizi*) in soybeans. Australas J Agri Res 31:951–956

Miles MR, Bonde MR, Nester SE, Berner DK, Frederick RD, Hartman GL (2011) Characterizing resistance to *Phakopsora pachyrhizi* in soybean. Plant Dis 95:577–581

Murithi HM, Beed F, Tukamuhabwa P, Thomma BPHJ, Joosten HAJ (2016) Soybean production in eastern and southern Africa and threat of yield loss due to soybean rust caused by *Phakopsora pachyrhizi*. Plant Pathol 65(2):176–188

Nuernberger T, Lipka V (2005) Non-host resistance in plants: new insights into an old phenomenon. Mol Plant Pathol 6:335–345

Nutter FW, Esker PD (2006) The role of psychophysics in phytopathology: the Weber–Fechner law revisited. Eur J Plant Pathol 114:199–213

Pedley KF, Pandey AK, Ruck A, Lincoln LM, Whitham SA, Graham MA (2018) Rpp1 encodes a ULP1-NBS-LRR protein that controls immunity to *Phakopsora pachyrhizi* in soybean. Mol Plant-Microbe Interact 32:120–133

Periyannan S, Milne RJ, Figueroa M, Lagudah ES, Dodds PN (2017) An overview of genetic rust resistance: From broad to specific mechanisms. PLoS Pathog 13(7):e1006380

Rios VS, Rios JA, Aucique-pérez CE, Silveira PR, Barrosav AV, Rodrigues FA (2018) Leaf gas exchange and chlorophyll a fluorescence in soybean leaves infected by *Phakopsora pachyrhizi*. J Plant Phytopathol 166(2):75–85

Silva DC, Yamanaka N, Brogin RL, Arias CA, Nepomuceno AL, di Mauro AO, Pereira SS, Nogueira LM, Passianotto AL, Abdelnoor RV (2008) Molecular mapping of two loci that confer resistance to Asian rust in soybean. Theor Appl Genet 117:57–63

Simko I (2002) Comparative analysis of quantitative trait loci for foliage resistance to *Phytophthora infestans* in tuber-bearing Solanum species. Am J Potato Res 79:125–132

Simko I, Piepho HP (2012) The area under the disease progress stairs: calculation, advantage, and application. Anal Theor Plant Pathol 102(4):381–389

Stewart S, Rodríguez M, Yamanaka N (2019) Pathotypic variation of *Phakopsora pachyrhizi* isolates from Uruguay. Trop Plant Pathol 44:309–317

Tschanz A, Shanmugasundaram S (1984) Soybean rust. In: Shibles R (ed) World soybean research conference III. Westview Press Inc., London, pp 562–567

Tschurtschenthaler NN, Vieira LSN, Nora TD, Schuster I (2012) Variabilidade genética de *Phakopsora pachyrhizi* avaliada por meio de marcadores microssatélites. Pesq Agrop Brasileira 47(2):181–186

Tukamuhabwa P, Maphosa M (2010) State of knowledge on breeding for durable resistance to soybean rust disease in the developing world, Plant production and prospection paper series 204. Food and Agriculture Organization of the United Nations

Twizeyimana M, Ojiambo S, Sonder K, Ikotun T, Hartman GL, Bandyopadhyay R (2009) Pathogenic variation of *Phakopsora pachyrhizi* infecting soybean in Nigeria. Phytopathology 99:353–361

Walker DR, Harris DK, King ZR, Li Z, Boerma HR, Buckley JB, Weaver DB, Sikora EJ, Shipe ER, Mueller JD et al (2014) Evaluation of soybean germplasm accessions for resistance to *Phakopsora pachyrhizi* populations in the southeastern United States, 2009–2012. Crop Sci 54:1673–1689

Walla JM (1979) Soybean disease atlas by the Southern Soybean disease workers. In: Sinclair JB (ed) Compendium of soybean diseases, 2nd edn. American Phytopathological Society, St. Paul

Wrather A, Shannon G, Balardin R, Carregal L, Escobar R, Gupta GK, Ma Z, Morel W, Ploper D, Tenuta A (2010) Effect of diseases on soybean yield in the top eight producing countries in 2006. Online. Plant Health Progress

Xavier WD, Silva JVS, Guimarães CM, Ferreira JLS, Turozi TA, Colodel S (2019) Use of copper-based pesticides to control fungal diseases of Soybean in Northern Brazil. J Exp Agri Int 33(2):1–10

Yamanaka N, Lemos NG, Akamatsu H, Yamaoka Y, Kato M, Silva DCG, Passianotto AL, Santos JVM, Benitez ER, Abdelnoor RV, Soares RM, Suenaga K (2010) Development of classification criteria for resistance to soybean rust and differences in virulence among Japanese and Brazilian rust populations. Trop Plant Pathol 35:153–162

Yamanaka N, Lemos NG, Uno M, Akamatsu H, Yamaoka Y, Abdelnoor RV, Braccini AL, Uenaga K (2013) Resistance to Asian soybean rust in soybean lines with the pyramided three Rpp genes. Crop Breed Appl Biotechnol 13:75–82

Yorinori TJ (1994) Fungal diseases. In: Tropical soybean: Improvement and Production. EMBRAPA-CNPOS (Ed), pp. 37–38. FAO, 1994. Plant Production and Protection No. 27

Yorinori JT, Morel W, Fernandez FTP (2001) Epidemia de ferrugem de soja no Paraguai e na Costa Oeste do Paraná, em 2001. In: Reunião de Pesquisa de Soja da Região Central Do Brasil, Embrapa Soja. Documentos, 157, vol 23. Embrapa Soja, Londrina, pp 117–118

Yorinori JT, Paiva WM, Frederick RD, Costamilan LM, Bertagnolli PF, Hartman GE, Godoy CV, Nunes JJ (2005) Epidemics of soybean rust (*Phakopsora pachyrhizi*) in Brazil and Paraguay from 2001–2003. Plant Dis 89:675–677

Zambenedetti EB, Alves E, Pozza EA, Araújo DV, Godoy CV (2007) Avaliação de parâmetros monocíclicos e da intensidade da ferrugem-asiática (*Phakopsora pachyrhizi*) em diferentes genótipos de soja e posições de copa. Summa Phytopathol 33:178–181