DISCOVERY, CHARACTERIZATION, AND UTILIZATION OF *RPP* GENES FOR RESISTANCE AGAINST SOYBEAN RUST

by

SILAS PAUL CHILDS

(Under the Direction of Zenglu Li)

ABSTRACT

Soybean rust is a disease of soybean caused by *Phakopsora pachyrhizi* Syd. & P. Syd and *Rpp* genes can help protect soybean from this disease. In this study, a novel *Rpp* gene was mapped to a 154 kb interval on chromosome 19 in PI 605823 and named *Rpp7*. In addition, five germplasm accessions were identified which had unique reaction phenotypes to nine diverse *P. pachyrhizi* isolates and may carry novel *Rpp* alleles. Four new sources of resistance were mapped to either the *Rpp3* or *Rpp6* locus using bulked segregant analysis. Eleven accessions with a putatively novel *Rpp3* allele were identified with a KASP™ marker developed within an *Rpp3* candidate gene region. In addition, 11 breeding lines with stacked *Rpp1* and *Rpp3* loci were selected with molecular markers, and the introgression sizes of *Rpp* loci were estimated in 13 breeding lines or NILs using the SoySNP50K iSelect Beadchips.

INDEX WORDS: *Glycine max*, *Phakopsora pachyrhizi* resistance gene (*Rpp*), Soybean rust (SBR), Plant introduction (PI), Germplasm screening, Linkage mapping, Haplotype analysis, Bulked segregant analysis (BSA), Single nucleotide polymorphism (SNP), KASP, Introgression size, Backcrossing, *Rpp7*

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DEDICATION

I dedicate this work to my uncle Wesley Bicha who inspired me to pursue an extraordinary life and helped me overcome my fear of writing; and to my friend Whitney Garton with whom I planned this adventure and dreamed of a better future, and who has stood by me at every step. We made it!

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CHAPTER 1

LITERATURE REVIEW

Soybean introduction

Soybean [*Glycine max* (L.) Merrell] is an internationally important crop, with over 320 million tonnes produced globally in 2015 (ASA, 2016). More than 90% of global production occurs in North and South America, and the USA is the world's top producer, where soybean is planted on approximately 33.4 million ha (ASA, 2016). Since soybean contains approximately 20% oil and 40% protein, it is typically crushed for vegetable oil extraction and the remaining meal is used as a high-protein source for animal feed (Shurtleff and Aoyagi, 2007). Farm-gate value averages US \$294 – \$514 tonne^{-1} depending on market conditions (ASA, 2016), and average yields in the USA for 2015 were 3.23 tonnes ha^{-1} (NASS, 2016).

Soybean is a dicotyledonous plant in the family Fabaceae and the subfamily Papilionideae (Shurtleff and Aoyagi, 2004). It was domesticated in China at least 4,000 years ago from a plant that was originally vining and bore small darkly-colored seeds (Shurtleff and Aoyagi, 2004; Singh, 2010).

Soybean is a diploid organism with 20 pairs of chromosomes (Chr) (2n=2x=40). It has a genome size of approximately 1.1 gigabases (Gb) that has been sequenced and annotated for reference (Schmutz et al., 2010). It is naturally self-pollinating, so modern cultivars are inbred lines rather than hybrids (Singh, 2010). Active breeding of soybean has been carried out since ca. 1900 and has been greatly helped by the collection of a large number (~20,000) of accessions (PIs) in the USA from countries near the soybean center of diversity. These accessions, in addition to wild relatives (*Glycine soja, G. tomentella,* etc.), provide a source of novel alleles that confer desirable characteristics (Singh, 2010; Singh and Nelson, 2015).

Soybean rust history, and crop damage

Soybean rust (SBR), caused by *Phakopsora pachyrhizi* Syd. & P. Syd., was first described on *Glycine soja* Siebold & Zucc. in 1902 in Japan, but received its current name after being found on jicama (*Pachyrhizus erosus* L. Urban) in 1913 in Taiwan (Bromfield, 1984). It spread to Australia and eastern Asian countries by 1934 (Bromfield, 1984). SBR had become an economic threat to soybean production in Japan by 1948 (Kitani and Inoue, 1960), but was not reported as an economic problem for the eastern Asian regions or countries of Taiwan, Thailand, Indonesia, or the Philippines until the 1960's (Bromfield, 1984). It became a problem in Australia, China, and India beginning in the 1970's (Bromfield, 1984). SBR was observed in Africa in 1975 (Bromfield, 1984), but did not begin to spread through the continent until the late 1990's (Sconyers et al., 2006). SBR was then observed in Hawaii in 1994 (Killgore and Heu, 1994), South America (Paraguay) in 2001 (Yorinori et al., 2005), and North America (Louisiana) in 2004 (Schneider et al., 2005). Once established in the American continents, SBR has been observed throughout all the soybean growing regions, including Brazil, Argentina, Colombia, Bolivia, 20 states of the USA, and Ontario, Canada (Sconyers et al., 2006; Sikora et al., 2014). Although reports of *P. pachyrhizi* in the Americas were noted by Bromfield and Hartwig (1980) in the early 1900's, these reports were most likely *P. meibomiae*, a less virulent American species (Goellner et al., 2010; Hartman, 2011).

SBR is of economic importance to soybean producers because it reduces seed size, seed weight, oil content, and total yield (Bromfield, 1984). Since *Phakopsora pachyrhizi* is endemic to eastern Asia, it is interesting to look at the historical disease losses observed in those locations. Losses due to SBR in Japan ranged from 15 – 40% in 1960 (Bromfield, 1984; Kitani and Inoue, 1960). In Taiwan, losses were 12 – 30% under average disease pressure in the late 1960's and up to 80% in severely-infected fields (Bromfield, 1984). Pod production, directly correlated with yield, was reduced by 10% on average and a maximum of 40% between treatments with and without fungicide in trials conducted at the Asian Vegetable Research and Development Center (AVRDC) in 1986-87 in Taiwan (Yang et al., 1991). Yield

losses between 10% and 30% were reported in China, depending on the region and weather conditions (Bromfield, 1984).

Since the largest soybean-growing region is now the Americas, the impact of SBR in this region is of special importance. In 2001, yield losses from SBR in Paraguay were up to 60% and average yield losses in Brazil were 30 – 75% in the same year (Yorinori et al., 2005). A study conducted in 2006-07 in Brazil showed average yield reduction between 37% and 67% depending on timing of disease development (Kumudini et al., 2008). In the USA (2006), yield losses of 27% in Attapulgus, GA and 35% in Quincy, FL were reported (Mueller et al., 2009). In a 2013 trial in Alabama, fungicide application for SBR increased yield 19 – 31%, depending on location, while two other locations showed no significant difference due to low disease pressure (AAES, 2015).

Economic losses due to SBR have been consistently high in soybean production areas of Brazil and Paraguay since the introduction of *P. pachyrhizi* in 2001 (Chakraborty et al., 2009). Yield losses of up to US $1.22 billion yr^{-1} were observed for the first three years (2001 – 2004) when fungicides labeled for SBR control were unavailable (Godoy et al., 2016). In subsequent years, fungicide applications costing approximately US $40/ha were applied 2-3 times per season, reducing yield losses to US $172 million yr^{-1} on average, but costing Brazilian growers US $1.77 billion yr^{-1} in fungicide expenses (Godoy et al., 2016).

In the USA, yield losses since the occurrence of SBR in 2004 have been limited. Likely this is the result of environmental conditions in many growing seasons that have not favored rapid reproduction of the rust pathogen, and because *P. pachyrhizi* is typically slow to spread from the southeastern USA to the major soybean production regions each season. Protective fungicides have begun to be deployed more widely, costing soybean growers $2.22 million annually in Georgia alone (2005 – 2013) (King et al., 2017). However, SBR may become a greater problem for U.S. growers in the future with rising global climate instability and the development of *P. pachyrhizi* populations tolerant to major fungicides (Rosa et al., 2015).

In addition to this Asian species of soybean rust, an "American" rust species exists, known as *Phakopsora meibomiae* Arthur (Goellner et al., 2010). This species is much less virulent on soybean, differs from *P. pachyrhizi* in telial morphology (Ono et al., 1992), shares only 80% DNA homology of the ribosomal internal transcribed spacer (ITS) region (Frederick et al., 2002), and has only 62% similarity between the intergenic regions of mitochondrial DNA (Stone et al., 2010). However, the similarity between the two species could easily result in misidentification of the virulent Asian species where both species coexist.

***Phakopsora pachyrhizi* morphology, life cycle, and host symptom development**

P. pachyrhizi is a hemicyclic rust, producing only uredinia and telia, while the other rust spore stages (pycnia, aecia, and basidia) have never been observed in the field (Bromfield, 1984; Hartman, 2011; Vittal et al., 2011). Urediniospores are the infective type of spore, produced in the active growing season. Teliospores are produced rarely in response to cold temperatures in autumn and have been observed on soybean in Asia (Bromfield, 1984) and on kudzu (*Pueraria spp*.) in the USA (Harmon et al., 2006). Basidiospores have been produced from teliospores under controlled conditions (Saksirirat and Hoppe, 1991), but are not known to germinate, since the alternate host is either extinct or has never been found (Hartman, 2011).

Urediniospores are ovate to globose in shape, yellowish-brown to hyaline in color, 18 × 23 µm in size, with a rough (echinulated) outer wall (Bromfield, 1984; Vittal et al., 2011). They are produced from pimple-like uredinia which form on both sides of the leaf, but are more common and larger on the abaxial side (Bromfield, 1984; Goellner et al., 2010). Uredinia are subepidermal, 100-200 µm in diameter, and contain short sporophores that produce urediniospores within the dome-shaped covering of paraphyses (Bromfield, 1984). An ostiole opens in the top of the uredinium to allow the escape of urediniospores (Goellner et al., 2010).

Urediniospores are carried by the wind and will germinate on either adaxial or abaxial leaf surfaces under appropriate environmental conditions (Bromfield, 1984). Germination produces a single

germ tube, that in turn forms an appressorium of a similar size as the original urediniospore (Goellner et al., 2010). The appressorium penetrates directly through the leaf epidermis, a tactic that is rare for rust fungi, which typically penetrate only through the stomata (Bromfield, 1984). Once inside the leaf lamina, the fungal hyphae quickly colonize the intracellular spaces and form intercellular haustoria within 12 to 36 hr after germination (Goellner et al., 2010). The fungal hyphae form into a dense mass (primordium) between the two layers of epidermis at the site of infection. This primordium gives rise to new uredinia approximately 9 to 14 d after infection, and these uredinia produce new urediniospores for approximately 3 wk (Bromfield, 1984; Sconyers et al., 2006).

Disease symptoms are visible 5 to 6 d after infection and first appear on the adaxial side of the leaf as chlorotic spots (Kumudini et al., 2010). Lesions are approximately 0.5 mm in diameter initially, but can enlarge to nearly 5 mm in diameter. The lesion color is typically light brown, often darkening with age, but may be reddish brown or other colors depending on the host-pathogen interaction (Bromfield, 1984; Rosa et al., 2015). The uredinia are a similar color as the lesions, but the masses of urediniospores often give the abaxial leaf lesions a tan appearance (Bromfield, 1984; Paul et al., 2015). Chlorosis often develops around the lesions over time, and heavily infected leaves may undergo severe chlorosis and premature senescence (Bromfield, 1984).

Host range and factors affecting disease severity

P. pachyrhizi has a wide host range, but is restricted to members of the subfamily Papilionideae of the Fabaceae family (Hartman, 2011). Of the 14,000 species in this subfamily, *P. pachyrhizi* is known to infect 152 species in 53 genera, based on artificial greenhouse inoculations (Hartman, 2011; Slaminko et al., 2008), although only 81 species were observed with sporulating uredinia (Slaminko et al., 2008). Slaminko et al., (2008) found 80 species to be hosts under North American field conditions with high disease pressure, and 53 species developed sporulating uredinia. The most common host in North America other than soybean is kudzu [*Pueraria montana* (Lour.) Merr. or *P. lobata* (Willd.) Ohwi], which is an invasive vine from southeastern Asia that is widespread across 3 million ha in the

southeastern USA (Jordan et al., 2010; Sikora and Delaney, 2016). This alternative host is considered the major source of overwintering inoculum for *P. pachyrhizi* in the USA (Jordan et al., 2010; Sikora et al., 2014).

Disease development from *P. pachyrhizi* urediniospores requires inoculum, favorable environmental conditions, and host susceptibility. Since *P. pachyrhizi* is an obligate biotroph, it requires a living host to reproduce. Urediniospores from *P. pachyrhizi* are the primary and secondary inoculum and are spread through wind currents. Large weather events, such as hurricanes, can carry the urediniospores across oceans. For example, Hurricane Ivan is thought to have brought *P. pachyrhizi* from South America to the USA in 2004 (Li et al., 2010). In addition, trade winds that blow east to west across the Atlantic Ocean may provide new inoculum yearly to South America from the African continent (Rocha et al., 2015). In the USA, *P. pachyrhizi* is believed to overwinter primarily on kudzu growing in the Gulf Coast region (Sikora et al., 2014), but the possibility that new windborne inoculum may be carried north from South America cannot be ruled out.

Urediniospore germination and infection requires moderate temperatures between 10 to 28.5°C (Bromfield, 1984), with an optimal temperature around 23°C (Rosa et al., 2015; Li et al., 2010) or night/day temperatures of 17/27°C, although this varies depending on the *P. pachyrhizi* isolate used (Bromfield, 1984). Disease is suppressed at temperatures greater than 30°C (Li et al., 2010), although sporulation has been observed in fields when temperatures exceeded 32°C (Sconyers et al., 2006). Urediniospores require at least 6 hr in free water from rain or dew to germinate on the host, although 10 to 18 hr of free water provide optimal infection, depending on temperature (Bromfield, 1984; Li et al., 2010). Urediniospores are sensitive to solar radiation, with studies suggesting nearly 100% loss of viability after exposure to sunlight for 2 d (Young et al., 2012). This has impact on the survivability of urediniospores traveling long distances in air currents and suggests that cloudiness and shorter day lengths would protect the inoculum (Li et al., 2010). Long day length may also directly reduce disease development, as SBR does not usually become a problem in long-day regions, such as in the USA, until

late August, even when temperature and humidity are at favorable levels (Li et al., 2010; Sconyers et al., 2006).

Infection by *P. pachyrhizi* causes yield loss in soybean in several ways. The SBR lesions directly reduce the green area of the leaf, but have been shown to reduce the photosynthetic capacity of the leaf to a greater degree than can be explained by the simple reduction in green area (Kumudini et al., 2008, 2010). The pathogen appears to disrupt photosystem II, reducing the overall photosynthate produced, and also lowering the harvest index by reducing dry matter accumulation in the seed (Kumudini et al., 2008). Leaf drop occurs when disease severity reaches approximately 80% of maximum (Kumudini et al., 2008), which further reduces the plant's photosynthetic capacity. Disease progression during the period of pod formation and pod filling, or approximately R5, is most detrimental to yield (Kawuki et al., 2004). Consequently, late-maturing cultivars may lose the most yield to SBR because the pod formation period is lengthy (Kawuki et al., 2004).

Cultural and chemical controls

In response to SBR disease pressure, cultural control methods have been tested and implemented in some areas. The establishment of a mandatory 60- to 90-day soybean-free period in Brazil was instituted in 2007 (Godoy et al., 2016). This window of time free of soybeans (typically July 1 to September 1) was supposed to reduce the pathogen inoculum present during the off-season and has been partially effective in delaying the onset of the disease (Godoy et al., 2016). In addition, some Brazilian states have prohibited late-planting (after December 31) to avoid the effect of high-inoculum pressure on young plants (Godoy et al., 2016). The harmful effect of late planting was observed in Nagaland, India where late planting (30 days difference) decreased yields by 926 kg ha^{-1} under high SBR pressure (Kumar et al., 2016).

Providing optimal soil nutrition may also play a role in minimizing SBR yield losses. Soil with cation exchange capacity ratios of calcium, magnesium, and potassium (Ca:Mg:K) were found to be optimal at 55:15:5, and making soil adjustments provided a 54% reduction in SBR severity compared to

poorly-adjusted soil (Gaspar et al., 2016). In addition, a study comparing five nitrogen (N) levels showed some increase in SBR severity when N was applied in excess but a slight decrease in SBR severity when moderate levels were applied (Ramos et al., 2016).

Fungicide has been widely deployed to protect soybean from *P. pachyrhizi* infection. In the Southeastern USA, the DMI fungicides tebuconazole and tetraconazole are recommended for SBR protection and cost US $24 to $37 ha^{-1} ($10 to $15 ac^{-1}) per application and provide protection for approximately 3 wk (Robert Kemerait, personal communication). Only 1 to 2 applications per season are typically required, and fungicide loss-of-effectiveness has not been observed in the USA (Clayton Hollier, personal communication).

In Brazil, over-use of demethylation inhibitor (DMI) fungicides, especially applied at lower concentrations than recommended, has led to widespread loss-of-effectiveness, with tebuconazole providing only 18% control in 2015 (Godoy et al., 2016) (Leon Sun, personal communication). Tank mixes with DMI and Qo inhibitor (Qol) modes of action are now commonly applied, but they have also lost effectiveness, providing only 40% control in 2015 (Godoy et al., 2016). Older, broad-spectrum fungicides such as mancozeb and chlorothalonil are sometimes applied to counter the emerging resistant *P. pachyrhizi* strains (Godoy et al., 2016) (Leon Sun, personal communication).

Other non-traditional chemicals have been suggested as treatments for SBR. For example, “shale water” a by-product of petrochemical extraction, induced systemic acquired resistance (SAR) after seed treatment and foliar application, reducing SBR severity by nearly 90% in field trials (Mehta et al., 2015).

Resistance and tolerance to *P. pachyrhizi*

Host resistance to *P. pachyrhizi* has been observed in several landraces of soybean (Bromfield, 1984), wild *Glycine* relatives of soybean (Burdon and Marshal, 1981; Singh and Nelson, 2015), kudzu (Jordan et al., 2010), and other hosts (Slaminko et al., 2008). A susceptible reaction (TAN) typically creates a lesion with light-brown coloration, 2 to 5 uredinia, and abundant sporulation (Bromfield, 1984). Resistance to *P. pachyrhizi*, or *Rpp,* genes provide host resistance in soybean and drive two major types

of resistant reactions – immune or red-brown. An immune, or IM, reaction lacks macroscopic lesions and has no uredinia or sporulation, although slight discoloration may be microscopically visible around the site of infection (Bromfield, 1984; Jordan et al., 2010; Kumudini et al., 2010; Miles et al., 2011). A red-brown, or RB, reaction creates a lesion with reddish-brown coloration, 0 to 2 uredinia, and minimal to no sporulation (Bromfield, 1984; Paul et al., 2015). The RB reaction is considered incomplete resistance because the pathogen can still grow and reproduce in a limited manner (Miles et al., 2011). Within the RB and TAN reactions, considerable variation exists in sporulation levels and disease severity (Bromfield, 1984; Miles et al., 2011). A third type of resistance reaction has been rarely observed, called an HR response, in which there is a light-colored lesion with no sporulation (Paul et al., 2015).

In addition to qualitative host resistance that creates a distinct resistant reaction, some soybean accessions produce TAN lesions, but show varying levels of tolerance (Bromfield, 1984; Kawuki et al., 2004). Tolerance can be defined as yield stability under disease pressure and can be determined by comparing yield for lines under high disease pressure with and without fungicide protection (Hartman et al., 2005). In some parts of Asia such as Taiwan, *Rpp* gene resistance has been overcome by *P. pachyrhizi* populations but selection for tolerance has shown yield gains of 30 to 60% (AVRDC, 1992). In these regions where consistent rust pressure can be expected, breeding for yield stability is seen as the most-effective breeding strategy, as it does not impose selection pressure on the pathogen, and can be measured by comparing protected and unprotected yield trials (Tukamuhabwa and Maphosa, 2010).

Another type of tolerance is resistance to leaf yellowing when severely infected with *P. pachyrhizi*, as was observed in two accessions by Yamanaka et al., (2011). Similarly, differences in canopy disease severity between genotypes with TAN reactions have been observed, and QTL controlling this trait have been identified (Harris et al., 2015a). These types of resistance have value but their multigenic nature precludes the use of marker-assisted selection (MAS) for backcrossing, and requires the availability of a reliable selection environment, making them difficult to employ in a breeding program in the USA (Hartman et al., 2005). Single-gene resistance to *P. pachyrhizi* has been discovered in numerous

United States Department of Agriculture (USDA) Plant Introductions (PIs) and *Rpp* genes have been mapped to at least seven different loci to date.

Rpp1 was discovered in PI 200492 following inheritance studies of bi-parental crosses screened with the single-spore purified *P. pachyrhizi* isolate Q-1, collected from infected soybean leaves at Redland Bay, Queensland, Australia (McLean and Byth, 1980). PI 200492, collected in 1952 from Shikoku, Japan, showed resistance to *P. pachyrhizi* in Taiwan in the 1960's (Cheng and Chan, 1968) and provides an IM response to specific pathotypes of *P. pachyrhizi. Rpp1* was mapped to a 0.8 cM [151.5 kilobase (kb)] interval on Chr 18 (Hyten et al., 2007). *Rpp1* has shown resistance to *P. pachyrhizi* populations in the USA in most years but is susceptible to *P. pachyrhizi* isolates collected in Brazil, Columbia, South Africa, Zimbabwe, Vietnam, and Taiwan (Akamatsu et al., 2013; Harris et al., 2015b; Walker et al., 2011, 2014a).

Rpp1-b was discovered in PI 594538A by Chakraborty et al., (2009) through molecular mapping of its resistance to *P. pachyrhizi* isolate ZM01-1, collected in Zimbabwe in 2001. PI 594538A, collected in the Fujian Province of China in 1996, has shown resistance to rust populations in Brazil, Paraguay, Thailand, Zimbabwe, and Nigeria but is susceptible to rust in the USA (Chakraborty et al., 2009; Walker et al., 2011, 2014a). *Rpp1-b* resistance maps to a 1.9 cM (445 kb) region around simple sequence repeat (SSR) marker Sat_064, which is the same locus harboring the *Rpp1* gene, but its differential reaction to *P. pachyrhizi* isolates indicates a different allelic identity (Chakraborty et al., 2009; Harris et al., 2015b).

Additional studies have identified PIs with resistance mapping to the *Rpp1*/*Rpp1-b* locus, including PI 417120, PI 423958, PI 518295, and PI 594177 (Harris et al., 2015b; Yamanaka et al., 2015a) collected from Japan or Taiwan, and PI 561356, PI 587880A, PI 587886, PI 587905, PI 594760B, PI 594767A, and PI 587855 collected in China from 1992 to 1996 (Garcia et al., 2011; Hossain et al., 2014; Kim et al., 2012; Ray et al., 2009; Yamanaka et al., 2016). Although Akamatsu et al., (2013) provides some evidence of differential alleles among some of these PIs, further characterization with allelism tests or diverse *P. pachyrhizi* isolates should be done to test if any new alleles are present.

Rpp2 was discovered in PI 230970 by its broader resistance (including resistance to *P. pachyrhizi* isolates IN73-1 and TW72-1) compared to *Rpp1* (Bromfield and Hartwig, 1980). *Rpp2* has shown an RB reaction to most *P. pachyrhizi* isolates from North and South America and Asia, but its field resistance is only moderate, as the lesions typically sporulate (Pham et al., 2009; Walker et al., 2014a). The *Rpp2* locus was first mapped by Silva et al., (2008) and was later fine-mapped by Yu et al., (2015) to a 188.1 kb region on Chr 16. In addition to PI 230970, PI 224270 and PI 417125 also have an *Rpp* gene at the *Rpp2* locus and all three accessions were collected from Japan (Calvo et al., 2008; Garcia et al., 2008; Nogueira et al., 2008). *Rpp2* has been shown to display completely dominant, incompletely dominant, and recessive gene action, depending on the *P. pachyrhizi* isolate and soybean genetic background, although it is possible that the different gene actions result from allelic variation. Yamanaka et al., (2015a) found PI 224270 to be resistant to four Brazilian isolates of *P. pachyrhizi*, while PI 230970 was susceptible to all isolates tested, providing evidence that a different allele may be involved. However, allelism tests between PI 230970 and PI 224270 showed no evidence of allelic variation (Garcia et al., 2008; Nogueira et al., 2008).

Rpp3 was discovered in PI 462312 based on its RB reaction to IN73-1 compared to the IM reaction provided by *Rpp1* (Hartwig and Bromfield, 1983). *Rpp3* has shown resistance to a similar, though somewhat more extensive, set of *P. pachyrhizi* isolates as *Rpp1*, and along with *Rpp1*, had its resistance broken by virulent *P. pachyrhizi* populations in Brazil as early as 2002 (Yorinori et al., 2005). The *Rpp3* locus has been mapped to a 1.4 cM (688 kb) position on Chr 6 (Hyten et al., 2009).

Alleles at the *Rpp3* locus showed complete dominance when mapped in PI 628932 (Brogin, 2005), incomplete dominance when mapped in PI 416764 (Hossain et al., 2014), and recessive gene action in PI 567099A (Ray et al., 2011). *Rpp?*(Hyuuga) is a dominant gene that was also mapped to the *Rpp3* locus in Hyuuga (Monteros et al., 2007). Hyuuga (PI 506764) was derived from a cross between PI 416764, which contains a gene at the *Rpp3* locus (Hossain et al., 2014), and 'Asomusume' (Yamanaka et al., 2015a), the latter which might carry the *Rpp5* gene that is also present in Hyuuga (Kendrick et al.,

2011). Using a combination of bulked segregant analysis (BSA), haplotype analysis, and differential isolate screening, Harris et al. (2015b) identified 52 out of 75 analyzed accessions to have a putative resistance gene at the *Rpp3* locus, suggesting that this gene represents the most widespread source of resistance to *P. pachyrhizi* in soybean. More work should be done to determine any allelic variation at the *Rpp3* locus.

Rpp4 was discovered in PI 459025B based on its exceptionally broad resistance (Hartwig, 1986). It was mapped to a 14.7 cM (2 Mb) position on Chr 18 (Garcia et al., 2008; Silva et al., 2008) about 26 cM from *Rpp1*. It was later fine-mapped to a 55.3 kb region and a candidate gene, *Rpp4C4*, was identified in PI 459025B (Meyer et al., 2009). PI 459025B was collected in the Fujian Province of China and has demonstrated an RB reaction to nearly all *P. pachyrhizi* pathotypes tested, but the lesions tend to sporulate quite heavily, making the *Rpp4* gene of little use as a resistance source by itself in the USA (Walker et al., 2014a). However, *Rpp4* may be a valuable addition to a multiple-*Rpp* gene pyramid (Vuong et al., 2016).

Another allele at the *Rpp4* locus, *Rpp4-b*, was recently identified in PI 423972 which was collected from Japan in 1978 (King et al., 2017). This allele provides a resistance reaction to a narrower range of rust isolates, but has shown reduced sporulation when challenged with *P. pachyrhizi* isolates in the USA, compared to *Rpp4.* PI 423972 also shows a different SNP haplotype within the resistance locus, indicating that *Rpp4-b* has a different origin than *Rpp4*. PI 567104B and PI 605791 may also contain an *Rpp* allele at the *Rpp4* locus (Harris et al., 2015b).

Rpp5 was discovered simultaneously in four PIs based on their resistance to a bulk *P. pachyrhizi* isolate from Brazil that was virulent against *Rpp1* and *Rpp3* (Garcia et al., 2008). The *Rpp5* locus was mapped to a 4.2 cM (2.8 Mb) region on Chr 3 (Garcia et al., 2008) but an additional fine mapping study has not yet been published. In addition to PI 200456, PI 200526, and PI 200487 (which were collected from Japan) and PI 471904 (which was collected from Indonesia), a cultivar from Japan (Hyuuga) also has an *Rpp* gene at the *Rpp5* locus (Kendrick et al., 2011). The gene(s) at this locus have exhibited

complete dominance, incomplete dominance (PI 471904), and recessive gene action (PI 200456; designated *rpp5*) as has been observed with the *Rpp2* gene.

Rpp5 (from PI 200526) has shown excellent resistance to *P. pachyrhizi* populations in South America, but is completely susceptible to *P. pachyrhizi* in the USA (Akamatsu et al., 2013; Walker et al., 2011, 2014a). Although Hyuuga, PI 200487, and PI 471904 show good resistance in the USA, resistance is likely being driven by an *Rpp* gene at the *Rpp3* locus (Kendrick et al., 2011). In addition, reactions to a panel of diverse *P. pachyrhizi* isolates suggest that the *Rpp5* alleles in Hyuuga, PI 200487, and PI 471904 differ from that of PI 200526 (Kendrick et al., 2011) but further work should be done to confirm this.

Rpp6 was discovered in PI 567102B, which was collected from East Java, Indonesia in 1993 (Li et al., 2012). *Rpp6* was mapped to a 23.7 cM (4 Mb) position on Chr 18, approximately 40 cM from *Rpp4* (Li et al., 2012), and was later fine-mapped to a 45 kb region by King et al. (2015). However, this same gene was also mapped in PI 567104B to an 879 kb region, slightly downstream of the King et al., mapping position (Liu et al., 2016). A different allele or tightly linked gene, *Rpp[PI567068A]* was mapped in PI 567068A near this same region (King et al., 2015). *Rpp6* has shown excellent resistance to *P. pachyrhizi* in the USA and at least some resistance to South American rust populations (Miles et al., 2008; Walker et al., 2014a).

Rpp7 was recently discovered in PI 605823 and its resistance against the *P. pachyrhizi* isolate GA12, collected in the US state of Georgia in 2012, mapped to a 0.8 cM (154 kb) region on Chr 19 (Chapter 2: Childs et al., 2017). PI 605823 was collected near Ha Giang, Vietnam in 1998 and has shown RB resistance to *P. pachyrhizi* in the USA, Columbia, Paraguay, and Australia. It has shown excellent field resistance in the USA, similar to *Rpp1* and *Rpp6* (Walker et al., 2011, 2014a).

No reports of transgenic resistance to *P. pachyrhizi* in soybean had been published until recently. Kawashima et al., (2016) reported the successful transfer of a resistance gene, named *CcRpp1*, from pigeonpea [*Cajanus cajan* (L.) Millsp.] into soybean. When homozygous in the plant, this gene provided an immune response and when hemizygous, it produced an RB reaction. *CcRpp1* provided resistance to

77 Brazilian *P. pachyrhizi* isolates as well as two isolates from the USA and two from Asia (Kawashima et al., 2016). It is hoped that this gene may provide resistance to all known *P. pachyrhizi* isolates, but further testing is needed.

Mechanisms of resistance

Plants often employ pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) when faced with pathogen infection. Since PTI can sometimes be overcome by pathogen effectors, plants possess *R* genes to detect specific pathogen avirulence (*Avr*) genes to initiate a resistance response (Ishiga et al., 2015). A biphasic gene response to infection that likely corresponds to these two major resistance mechanisms has been observed in the soybean- *P. pachyrhizi* interaction. The first phase is similar in susceptible and resistant genotypes and occurs approximately 12 hr after inoculation (HAI). The second phase of gene upregulation occurs approximately 72 HAI in the resistant genotypes and only much later and weaker in the susceptible genotypes (Meyer et al., 2009).

Rpp genes conditioning resistance to *P. pachyrhizi* in soybean appear to follow the gene-for-gene model between *R* (host) and *Avr* (pathogen) genes. *R* genes often have the CC-NBS-LRR gene structure, containing leucine-rich repeat (LRR) motifs that are involved in signal-transduction (Michelmore and Meyers, 1998). They tend to be clustered together on the genome, partly due to unequal crossing-over, gene conversion, duplications, and transposable element activity which may help *R* genes evolve faster (Meyer et al., 2009; Michelmore and Meyers, 1998). Individual *R* genes tend to demonstrate unusual rates of mutation that allows them to detect evolving pathogen effector genes (Michelmore and Meyers, 1998).

Since resistance genes typically start a signaling cascade, the activation of multiple genes downstream is expected. This has been demonstrated by non-host resistance penetration (PEN) genes that are known to prevent penetration by *P. pachyrhizi* in non-hosts (Ishiga et al., 2015). Activation of non-host resistance to *P. pachyrhizi* in *Medicago truncatula* increased the production of the phytoalexin medicarpin and upregulated chlorophyll catabolism (Ishiga et al., 2015). Differentially expressed genes in soybean after inoculation with *P. pachyrhizi* compared to a mock control included genes encoding heat

shock proteins and salicylic acid-related proteins and other genes related to stress tolerance and defense (Panthee et al., 2007). However, since this study was performed in a susceptible genotype, these genes are likely related to the initial phase of resistance that ultimately fails without the second phase associated with *Rpp* genes.

The resistance reaction of the soybean plant with an *Rpp* gene compatible with the *P. pachyrhizi Avr* gene involves a hypersensitive response (HR) that involves localized cell death to contain the pathogen (Jordan et al., 2010; Kumudini et al., 2010; Yamanaka et al., 2010). This may only involve a few cells, as in the IM response, or may include a larger area, in the case of the RB reaction (Jordan et al., 2010). In addition, a cell wall deposition (CWD) has been observed in immune reactions of kudzu toward *P. pachyrhizi* infection that physically limits hyphal spread through the leaf (Jordan et al., 2010).

The gene action of the *Rpp* genes is usually dominant; however, cases of incomplete dominance and recessive gene action have been reported (Calvo et al., 2008; Garcia et al., 2008; Hossain et al., 2014; Ray et al., 2009). Since resistance results from a host-pathogen interaction, it is possible that some exceptionally virulent *P. pachyrhizi* strains may reduce the dominance of the gene action and create a dosage effect for the *R* gene (Calvo et al., 2008; Garcia et al., 2011; Ray et al., 2009). This difference may result from the pathogen being either homozygous or heterozygous for the *Avr* genes (Calvo et al., 2008). Genes in the host background have also been known to revert the dominance of an *R* gene to a recessive gene action, likely through a gene silencing mechanism (Garcia et al., 2011). These factors suggest that all the *Rpp* genes or alleles may actually be dominant, when present in an appropriate genetic background and interacting with less virulent *P. pachyrhizi* strains.

***P. pachyrhizi* interactions with *Rpp* genes**

No single *Rpp* gene has been observed to provide resistance to all tested isolates of *P. pachyrhizi* (Bonde et al., 2006; Paul and Hartman, 2009; Pham et al., 2009) and single gene resistance to *P. pachyrhizi* has been shown to be race-specific (Hartman et al., 2005). Specific races can be identified by testing the *Rpp* genes with different isolates and comparing the patterns of RB or TAN reactions

(Bromfield, 1984). Six pathotypes were observed in a 1966 study using nine isolates collected in Taiwan, each pathotype expressing similar reactions toward specific *Rpp* genes (Lin, 1966). Three pathotypes and six aggressiveness groups were found among 72 isolates collected from the southern USA in 2006-2009 (Twizeyimana and Hartman, 2012). Similarly, a single virulence group and 6 to 7 aggressiveness groups were reported for 24 USA isolates from 2007-2008 (Paul et al., 2015). Unfortunately, a standard set of pathogen races has not yet been established.

P. pachyrhizi populations can be characterized based on their DNA sequence variations, especially in the ITS region and the gene encoding an ADP-ribosylation factor (ARF) (Jorge et al., 2015). In addition, AFLP markers have been used to characterize the molecular diversity of *P. pachyrhizi* across its genome (Rocha et al., 2015). Isolates show relatively little variation between locations and more variation within a location (Jorge et al., 2015; Rocha et al., 2015). This may indicate that inoculum is widely dispersed between locations by wind currents, yet new variation is created over time in a particular location (Jorge et al., 2015). The *P. pachyrhizi* diversity has been seen to vary more by year than by location in a South American study, suggesting that new inoculum may be arriving in South America on east-to-west trade winds from the African continent in a yearly recurrent manner (Rocha et al., 2015). New pathogen diversity in the USA may also come from urediniospores blown north across the ocean from South America (Twizeyimana and Hartman, 2012).

Although it may appear unusual that *P. pachyrhizi* displays so much diversity without sexual reproduction, other mechanisms of gene flow have been observed. In particular, hyphal anastomosis commonly occurs and likely leads to heterokaryosis, nuclear fusion, and genetic recombination (Vittal et al., 2011).

P. pachyrhizi strains have been observed to overcome resistance genes (Hartman et al., 2005; Paul et al., 2015). This was seen in 2002 when a highly virulent strain of *P. pachyrhizi* emerged in Brazil that overcame the resistance of *Rpp1* and *Rpp3* (Yorinori et al., 2005). The *P. pachyrhizi* strains in South America have continued to remain virulent on *Rpp1* and *Rpp3* and have since at least partially broken the

resistance of *Rpp2* and *Rpp4* (Yamanaka et al., 2010). In contrast, *P. pachyrhizi* in the USA must survive the winter on kudzu in the Gulf Coast region and a genetic bottleneck likely occurs yearly that may influence the virulence of the pathogen in the subsequent season (Paul et al., 2015; Twizeyimana and Hartman, 2012). This was demonstrated by *P. pachyrhizi* populations that overcame resistance from *Rpp1* and *Rpp6* in certain locations in 2009, 2011, and 2012, but could not overcome the resistance in subsequent years (Paul et al., 2013; Walker et al., 2014a). Field populations of *P. pachyrhizi* may also vary in their ability to infect depending on the level of parasitism by the mycoparasites *Trichothecium roseum* and *Verticillium psalliotae*, which can reduce penetration ability of urediniospores (Paul et al., 2015).

Screening for resistance to *P. pachyrhizi*

Screening germplasm for *P. pachyrhizi* resistance can be performed in the field or greenhouse. Field screening is limited to locations with suitable weather conditions for disease infection and in areas with reliable local *P. pachyrhizi* inoculum present. In the USA, this limits field screening in most years to the Southeastern USA Gulf Coast regions of Florida, Georgia, Alabama, Mississippi, or Louisiana. Even in these regions, *P. pachyrhizi* inoculum is not always present in sufficient abundance or sufficiently early to provide reliable disease ratings before plants are killed by frost. Artificial inoculation using locally collected inoculum and the use of susceptible spreader rows can help increase infection within the disease nursery. Furthermore, applications of the antibiotic streptomycin are recommended in this area to control bacterial pustule (*Xanthomonas campestris* pv. *glycines*) which produces disease symptoms similar to SBR (Walker et al., 2014a). Late planting (mid-July to mid-Aug) is also recommended to decrease the incidence of bacterial pustule.

Greenhouse inoculation requires the collection and maintenance of suitable *P. pachyrhizi* isolates for inoculation. An example is the bulk GA12 isolate collected in 2012 from soybean plants in Attapulgus, GA and subsequently maintained on ‘Cobb’ soybeans in a greenhouse in Griffin, GA (Walker et al., 2014b). This isolate has not been single-spore purified but provides reliable reactions on resistant

and susceptible soybeans, shows no evidence of a mixed pathotype, and has been used to screen germplasm and map multiple *Rpp* alleles (Childs et al., 2017; Harris et al., 2015a, 2015b; King et al., 2015, 2017; Walker et al., 2014b).

Walker et al. (2014b) and Harris et al. (2015b) describe the maintenance of a *P. pachyrhizi* isolate, greenhouse inoculation, and growth of soybeans in a greenhouse. In brief, soybeans are best planted two plants per 10 x 10 cm pot, inoculated twice on successive days 2 wk after planting, incubated in a humidity chamber for 48 hr after the initial inoculation, and allowed to grow for an additional 2 wk before rating their reactions to *P. pachyrhizi* infection. Using this method, disease escapes are rare and the respective lesion reactions to *P. pachyrhizi* can be easily discerned. Furthermore, many plants can be screened in a short time period using a relatively small area of greenhouse space.

Following successful infection with *P. pachyrhizi*, effective selection indices to screen for SBR resistance are needed. Lesion color (LC), as mentioned above, is the most common parameter for recording SBR resistance, likely because it can be observed macroscopically. However, LC is not always a reliable indicator since it is influenced by environment (Yamanaka et al., 2010) and includes varying levels of sporulation (Bromfield, 1984; Miles et al., 2011). The size of individual RB lesions, which directly influences the loss of green leaf area, also varies widely between soybean genotypes, from slight "flecking" to large lesions 1.5 mm or more in diameter (Bromfield, 1984; McLean and Byth, 1980).

Another resistance parameter is disease severity, which is often rated visually on a scale of 1 to 5 (Chakraborty et al., 2009; Kim et al., 2012; Miles et al., 2006; Paul et al., 2015). A more objective modification to measure disease severity was described by Kumudini (2010) and involves taking images of leaves and calculating the proportion of lesion area to total leaf area sampled. Severity, when rated on several different dates, can be used to calculate the area under the disease progress curve (AUDPC) (Miles et al., 2011). While severity ratings are useful, they have not always been strongly correlated with lesion type, which is the typical indicator of resistance (Miles et al., 2011). In addition, rust severity measured at R6 explained only about 34% of the variability in yield reduction across various genotypes in

one study in Uganda (Kawuki et al., 2004). Since severity is based somewhat on environmental conditions, including the inoculum density, an "infection index" was developed by Yamanaka (2011) to take into account the number of viable urediospores inoculated per cm^2 of leaf. Severity ratings provide a much more useful indication of resistance when combined with LC or sporulation level (SL) (Paul et al., 2015). However, severity ratings have been used successfully to map resistance genes on their own (Liu et al., 2016). A combined parameter used by Walker et al., (2014a) is a rust index (RI), formed by taking the square root of severity added to the square root of sporulation level (SL), to create a very useful measure of resistance in the field. The International Working Group on Soybean Rust (IWGSR) developed a three-digit rating scale (Shanmugasundaram, 1977) for field evaluation that incorporates the progression of the disease in the plant canopy, the lesion density, and the reaction type into one value that has been used successfully for screening (Krisnawati, 2016).

Sporulation level (SL), rated on either a 1 to 3 or 1 to 5 scale relative to a susceptible check, is a direct measure of the reproductive capacity of the pathogen in a particular host genotype and is one of the most reliable parameters for assessing resistance (Chakraborty et al., 2009; Hossain et al., 2014; Miles et al., 2011; Walker et al., 2014; Yamanaka et al., 2010). SL consistently produced the highest QTL peaks for mapping resistance genes compared to other parameters in a study by Lemos et al., (2011).

Frequency of open uredinia has been used to rate soybean genotypes for *P. pachyrhizi* resistance (Miles et al., 2011; Yamanaka et al., 2010), but is not very reliable on its own, as it sometimes causes resistant genotypes to be classified as susceptible (Yamanaka et al., 2011). It also produced QTL with effects even lower than those for LC when used to map resistance genes (Lemos et al., 2011). This parameter may best be combined with quantification of the number of uredinia per lesion for a more informative index (Hossain et al., 2014; Miles et al., 2011; Yamanaka et al., 2011).

Another less common resistance parameter is the frequency of lesions having uredinia (Lemos et al., 2011; Yamanaka et al., 2010, 2011). Selection of the appropriate disease phenotype for resistance

screening should be based on practicality and reliability across the screening environments, and using a combination of methods is likely the best strategy.

Breeding for resistance to *P. pachyrhizi*

Rpp genes from unadapted germplasm can be introduced into elite cultivars through backcrossing. Backcrossing, originally proposed by Harlan and Pope (1922) replaces unfavorable alleles from the exotic donor line by repeatedly crossing back to an elite recurrent parent. This is important since most of the PI sources harboring *Rpp* genes have poor agronomic performance, such as small seed size, extreme lodging tendency, very late maturity, and low yield (https://npgsweb.ars-grin.gov). Backcrossing should be continued for 4 -5 generations, selecting for the *Rpp* loci at each generation through phenotyping or the use of genetic markers (marker-assisted backcrossing, MABC) (Young and Tanksley, 1989).

Acceleration of the backcrossing process requires the development of genetic markers tightly linked to *Rpp* loci that utilize a cost- and time-effective genotyping platform (Diers et al., 2013). One such genotyping platform is Kompetitive Allele Specific PCR (KASP) that offers lower cost high-throughput genotyping for single nucleotide polymorphism (SNP) markers (Semagn et al., 2014). In soybean, primers can be developed using the Williams 82 reference genome (www.soybase.org) and fingerprinting SNP data from the SoySNP50K array (Song et al., 2013). Primers can also be designed around genomic deletions (Shi et al., 2015a).

It is important to recognize that while backcrossing can eliminate nearly all unlinked donor genome content, the introgressed fragment size remains relatively large, which could potentially cause yield drag associated with the introgressed *Rpp* locus (Hospital, 2001). Large fragment sizes can also limit stacking of genes, such as *Rpp1* and *Rpp4,* that are only 4.6 Mb apart. The use of molecular markers on the outskirts of the resistance locus to identify recombinant individuals with reduced fragment size should be performed more widely (Hospital, 2001; Young and Tanksley, 1989).

Backcrossing results in near-isogenic lines (NILs) which can then be used in a forward breeding strategy to achieve high yield and additional disease-resistances (Diers et al., 2013). Molecular markers linked to *Rpp* loci can be used for MAS during this part of the breeding program, as has been done with several other traits, such as soybean cyst nematode (SCN) resistance (Shi et al., 2015b; Young, 1999). Disease screening to verify the presence of introgressed *Rpp* loci would be required at later stages of the breeding cycle.

Verification of multiple *Rpp* gene pyramids in soybean cultivars is possible with the use of MAS, and pyramiding has the potential to provide much broader resistance and help protect against resistance break-down (Hartman et al., 2005; Pedersen and Leath, 1988). Furthermore, *Rpp* gene pyramids can provide a higher level of resistance than that provided by single genes, even when one of the pyramided *Rpp* genes has been overcome by *P. pachyrhizi* populations (Bhor et al., 2015; Lemos et al., 2011; Maphosa et al., 2012; Yamanaka et al., 2015b). More studies need to be performed to find the best *Rpp* gene combinations to provide resistance in the USA and to characterize the effect of pyramiding on resistance and yield performance. One limitation of gene pyramiding is the large population sizes needed to recover individuals containing all the desired genes.

NILs released from the University of Illinois involved backcrossing *Rpp1*, *Rpp1-b*, *Rpp?*(Hyuuga), and *Rpp5* into MG II and MG IV elite backgrounds (Diers et al., 2013). Additional NILs released by the University of Georgia with *Rpp1, Rpp2, Rpp3,* and *Rpp4* backcrossed into a MG VII elite background have also been developed (King et al., 2016). In addition, a breeding line was released in 2007 by the Georgia Agricultural Experiment Stations that placed the *Rpp?(Hyuuga)* gene into the background of ‘Dillon’ (MG VI) (Boerma et al., 2011).

In the USA, elite soybean cultivars with resistance to *P. pachyrhizi* have yet to be deployed, as of 2016. The only known exception is AG 5232, released by the Monsanto Co in 2012 with *Rpp1* resistance. However, it did not become popular and was discontinued (J. Gilsinger, personal communication). The difficulty of recovering high-yielding breeding lines with native *Rpp* resistance has driven Dupont

Pioneer to seek transgenic alternatives, such as that provided by *CcRpp1*, from pigeonpea [*Cajanus cajan* (L.) Millsp.] (Kawashima et al., 2016; Jillian Foerster, personal communication).

In Brazil where SBR pressure is highest, EMBRAPA released the cultivars BRS MG771F and BRS MG780FRR in 2012 with *Rpp5* resistance to SBR (M.H. Todeschini, personal communication). In addition, the breeding company TMG has successfully released soybean cultivars such as TMG 7062 IPRO, with *Rpp5* resistance, patented as Inox Technology® (www.tmg.agr.br). Approximately 1.5 million ha of Inox soybean are currently planted in Brazil, especially in the south of the country (Alexandre Garcia, personal communication). These cultivars slow the disease severity, but still require at least one fungicide application. Additional work to add another *Rpp* gene (Generation II) as well as a gene for tolerance (Generation III) is underway, but the identity of these genes is not readily available (Revistarural.com, 2016).

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CHAPTER 2

DISCOVERY OF A SEVENTH *RPP* SOYBEAN RUST RESISTANCE LOCUS IN SOYBEAN ACCESSION PI 605823

Introduction

Soybean [*Glycine max* (L.) Merr.] is one of the world's most important agronomic crops, with over 320 million metric tons produced globally (ASA, 2016). Soybean rust (SBR) is a disease of soybean caused by the obligate biotrophic fungal pathogen *Phakopsora pachyrhizi* Syd. & P. Syd. This disease threatens soybean production in tropical and subtropical regions of the world, as it can spread rapidly through windborne urediniospores, and if untreated, causes yield losses of up to 80% (Bromfield, 1984).

Phakopsora pachyrhizi was first described on *Glycine soja* Siebold & Zucc. in 1902 in Japan, but received its current scientific name after being found on jícama [*Pachyrhizi erosus* (L.) Urb.] in 1913 in Taiwan (Bromfield, 1984). *P. pachyrhizi* can now be found in many soybean growing regions around the world, and reached the continental USA in 2004 (Bromfield, 1984; Schneider et al., 2005). *P. pachyrhizi* is unusual among rust pathogens because it penetrates directly through the leaf epidermis and can infect more than 80 species in the legume subfamily Papilionideae (McLean, 1979; Slaminko et al., 2008). Furthermore, it has a latent period of only 1 to 2 wk (Bromfield, 1984). However, its spread is limited by its requirement of several hours of surface moisture for urediniospore germination and successful infection, its sensitivity to solar irradiation and temperatures greater than 30°C, and its inability to overwinter in the absence of physiologically active host tissue (Li et al., 2010; Young et al., 2012). Symptoms of infection on susceptible soybean begin as small chlorotic spots on older leaves. These lesions darken with age and produce volcano-shaped uredinia, primarily on the abaxial side of an infected leaf, which produce an abundance of urediniospores (Bromfield, 1984). Infected leaves undergo chlorosis and early senescence, causing reduced seed size, seed weight, oil content, and total yield.

In the Americas, SBR has been managed by fungicide sprays (Sikora et al., 2009; Yorinori et al., 2005). In Brazil, annual fungicide costs for SBR control average US $1.98 billion (Godoy et al., 2016). In the southeastern USA, fungicide usage on soybeans sharply increased after the occurrence of SBR in the continental USA, and growers currently spend at least US $18.75 million annually on fungicide applications, assuming 25% of the 2 million hectares of soybean in the southeastern USA are sprayed one time at US $37 ha^{-1} (Robert Kemerait, personal communication). Effective management in the southern USA has been aided by the availability of information from a coordinated sentinel plot and scouting system to monitor the distribution and spread of the fungus each growing season (Kelly et al., 2015). Some *P. pachyrhizi* populations in Brazil have developed tolerance to major fungicides such as tebuconazole (Aguiar et al., 2016), and overuse of fungicide for SBR management has undesirable environmental impacts (Langenbach et al., 2016). A more diversified disease management program that includes cultivars with *P. pachyrhizi* resistance should prolong the usefulness of some fungicides and reduce economic losses caused by this disease (Hartman et al., 2005).

Resistance to *P. pachyrhizi* (*Rpp*) genes, which provide host plant resistance against specific *P. pachyrhizi* pathotypes, have been discovered in numerous United States Department of Agriculture (USDA) soybean Plant Introductions (PIs), and have been mapped to six different loci to date (*Rpp1, 2, 3, 4, 5*, and *6*) (Table 2.1) (Garcia et al., 2008; Hyten et al., 2007, 2009; Li et al., 2012; Silva et al., 2008). In addition, at least six additional alleles have been discovered which show differential responses to selected *P. pachyrhizi* pathotypes, or demonstrate different gene actions, but were mapped to previously reported *Rpp* loci [Chakraborty et al., 2009; Garcia et al., 2008; King et al., 2015, 2017; Ray et al., 2011].

Phakopsora pachyrhizi has a high level of pathotype diversity, and the virulence and aggressiveness of field populations can vary substantially among locations and growing seasons (Akamatsu et al., 2013; Paul et al., 2015; Pham et al., 2009; Twizeyimana and Hartman, 2012). No single *Rpp* gene provides resistance to all pathotypes of the fungus (Bonde et al., 2006; Harris et al., 2015; Paul and Hartman, 2009). Moreover, while numerous germplasm accessions have shown resistance in the

USA, the *P. pachyrhizi* resistance genes from a large portion of accessions were mapped to the *Rpp3* locus, thus limiting potential combinations of *Rpp* genes that could be stacked in cultivars (Harris et al., 2015). It would therefore be useful to find additional *Rpp* genes at other loci that can be deployed in soybean cultivars to provide more durable and broader resistance (Maphosa et al., 2012).

Host resistance to *P. pachyrhizi* results in one of two general types of reactions. An immune (IM) response is characterized by the absence of macroscopically visible lesions or by the development of faint HR lesions with no sporulation (McLean and Byth, 1980; Paul et al., 2015). A more common resistance reaction results in reddish-brown (RB) lesions instead of the typical TAN reaction associated with susceptibility (Bromfield 1984; Harris et al., 2015). The RB type of reaction is considered to indicate incomplete resistance, and there are typically reduced levels of sporulation from the uredinia in the lesions (Hartman et al., 2005; Miles et al., 2011; Rosa et al., 2015). In contrast to the resistance reactions, the TAN type of reaction is characterized by increased uredinia formation and more profuse sporulation from the uredinia.

In a greenhouse assay, the soybean germplasm accession PI 605823 developed an RB/mixed resistance reaction and moderate disease severity after being inoculated with a mixture of *P. pachyrhizi* isolates originating from Thailand (TH01-1), Brazil (BZ01-1), Paraguay (PG01-2), and Zimbabwe (ZM01-1) (Miles et al., 2006). In field assays conducted in the southern USA between 2006 and 2012, resistance of PI 605823 to local *P. pachyrhizi* populations ranged from moderate to very high, depending on the year and location (Walker et al., 2011; 2014a). PI 605823 also had an RB reaction with variable sporulation to bulk (i.e., unpurified) isolates collected in Georgia in 2007, 2008, and 2012 (Walker et al., 2014b). Although the observed resistance was incomplete (i.e., limited sporulation of the fungus occurred from the uredinia of lesions that developed on infected plant leaves), the resistance was considered consistent and effective enough to be useful for SBR management. The objectives of this study were to investigate the genetic control of resistance to *P. pachyrhizi* in PI 605823 and to map the genomic location(s) of any *Rpp* gene(s) involved.

Materials and methods

The cross of 'Williams 82' × PI 605823, was made in Urbana, IL in March 2010. F_2 seeds were harvested, of which 100 F_2 seeds were used for phenotyping and BSA, and additional F_2 seeds were grown in the field to obtain 90 $F_{2:3}$ families for further phenotyping and genotyping. PI 605823 is a MG IX landrace collected in 1998 near Ha Giang, Vietnam (https://npgsweb.ars-grin.gov/gringlobal/). It has resistance to *P. pachyrhizi*, as mentioned previously, and has an indeterminate growth habit, purple flowers, grey pubescence, and a yellow seed coat. Williams 82 is a MG III cultivar that was released in 1981 by the USDA-Agricultural Research Service (ARS) and the Illinois Agricultural Experiment Station (Bernard and Cremeens, 1988), and it is susceptible to *P. pachyrhizi*. A second population derived from the cross of '5601T' × PI 605823 was created in Urbana, IL in June 2008. The cultivar 5601T is a MG V cultivar that was released in 2001 by the University of Tennessee (Pantalone et al., 2003), and it is susceptible to *P. pachyrhizi*. The population was advanced from the F_2 generation using the pedigree method. The F_4 generation was grown in Athens, GA, in 2014, and 114 single plants were used to create $F_{4:5}$ RILs for mapping the resistance gene(s).

The 100 F_2 plants of the Williams 82 × PI 605823 population were screened in July 2014, and 90 $F_{2:3}$ families from this population were screened in October 2015. The 114 $F_{4:5}$ RILs from the 5601T × PI 605823 population were screened in April 2015.

Plants were grown in a greenhouse at the University of Georgia campus in Griffin, GA in the same manner as that described by Harris et al. (2015). A randomized complete block design (RCBD) with two replications was used for the Williams 82 × PI 605823 $F_{2:3}$ screening assay and a completely randomized design (CRD) was used for the other populations. Susceptible and resistant checks were included in each experiment in addition to the segregating populations. Black plastic trays containing 15 Kord Presto sheet pots (10cm × 10cm; Griffin Greenhouse Supplies, Inc., Tewksbury, MA) were filled with Fafard® 3B greenhouse media (Sun Gro Horticulture, Agawam, MA). The middle three pots were

not used in each tray to allow better light penetration, leaving 12 pots per tray. Two to three seeds were sown per pot and seedlings were thinned to two plants per pot.

Approximately 14 days after planting, the plants were inoculated with a urediniospore suspension of the GA12 *P. pachyrhizi* bulk isolate. This isolate was collected from naturally-infected soybean plants in southern Georgia in the summer of 2012 and was maintained on SBR-susceptible 'Cobb' soybean plants in a greenhouse (Harris et al., 2015; Hartwig and Jamison, 1975; Walker et al., 2014b). Although this isolate was not purified, it induced uniform reactions on resistant and susceptible checks, and has been used successfully in previous studies to screen germplasm and map *Rpp* genes (Harris et al., 2015; King et al., 2015, 2017). The urediniospore inoculum consisted of freshly-collected urediniospores, sterile water, and 0.04% Tween, with a minimum of 50,000 urediniospores mL^{-1}. Inoculum was sprayed on the foliage with an atomizer until runoff occurred, and the plants were immediately placed in a shaded dew chamber for 24 hr. The dew chamber consisted of a flooded tray on a greenhouse bench with a white polyethylene enclosure covered in 90% black shadecloth. The plants were inoculated a second time 24 hr after the first inoculation before being transferred back to the greenhouse after another 24 hr in the dew chamber, as previously described (Harris et al., 2015).

Approximately 14 days after inoculation (DAI), plants were rated for their reactions. Plants with a TAN reaction were considered susceptible and those with an RB reaction were considered resistant. No IM reactions were observed in either of the two populations. F_2 plants from the Williams 82 × PI 605823 population were evaluated on an individual plant basis, and the resistant and susceptible classifications were used for bulked segregant analysis (BSA; Michelmore et al., 1991). For the Williams 82 × PI 605823 $F_{2:3}$ population, families with fewer than 12 $F_{2:3}$ progeny plants were excluded from the analysis, and the remaining families were used to infer the phenotype of the corresponding F_2 plants. The phenotypes of the 5601T × PI 605823 $F_{4:5}$ RILs were determined based on the evaluation of 10 to 12 plants per line. Germination was unusually poor in this population and some lines had to be excluded from the analysis because insufficient plants were available.

Families and RILs were considered to have residual heterozygosity if they had at least two plants with susceptible reactions and two plants with resistance reactions. Families and RILs with no more than one plant with a susceptible reaction were classified as homozygous resistant, and those with no more than one plant with a resistance reaction were classified as susceptible (King et al., 2015). Misclassification due to disease escape, seed contamination, or ambiguity between a TAN reaction and a sporulating RB reaction might have resulted in these off-types.

For bulked segregant analysis, a 5 cm^2 section of young leaf tissue was collected from each of a minimum of 20 F_2 resistant plants and 20 F_2 susceptible plants, and the leaf tissue from each was combined into separate 50 mL Falcon tube (Fisher Scientific, Waltham, MA), lyophilized, and ground to a fine powder in a Genogrinder (SPEX US, Metuchen, NJ). DNA was extracted as per Keim et al., (1988). The two bulks, as well as their parents, were genotyped with the SoySNP50K iSelect SNP BeadChip (Song et al., 2013) at the Soybean Genetics Lab at Michigan State University, East Lansing, MI. The BSA data were analyzed with Microsoft Excel and the Graph Builder function of JMP® Pro 12 (SAS Institute Inc., Cary, NC). The putative resistance locus indicated by BSA was further mapped using KASP (LGC Genomics, Middlesex, UK) SNP assays. Leaf tissue from 10 to 12 plants of each $F_{2:3}$ family or $F_{4:5}$ RIL was combined into a single 50 mL Falcon tube (Fisher Scientific, Waltham, MA). Tissue from sibling plants was then lyophilized and ground to a fine powder with a Genogrinder (SPEX US, Metuchen, NJ) for DNA extraction. DNA was extracted per Keim et al. (1988). SNP markers were selected and SNP assays at the target genomic location were developed in-house based on the SoySNP50K data between 5601T and PI 605823 and the Williams 82 reference genome. These SNPs were then used to genotype the 5601T × PI 605823 $F_{4:5}$ RIL population. Additional SNP assays were developed based on the polymorphisms between Williams 82 and PI 605823 in the same resistance locus indicated by the BSA for mapping the gene in the Williams 82 × 605823 $F_{2:3}$ population. The genotyping calls were determined using either a Tecan M1000 Pro Infinite Reader (Tecan Group Ltd., Männedorf, Switzerland) with KlusterCaller software, or a Roche LightCycler 480 II with LightCycler® software

(Roche Diagnostics Corporation, Indianapolis, IN). Calls that were ambiguous were classified as missing data.

An integrated genetic map was generated from the combined Williams 82 × PI 605823 and 5601T × PI 605823 population data using JoinMap 4.1 software (Van Ooijen, 2006). Linkage between the trait and markers was analyzed using the regression function with a LOD score of 3, and recombination distances were calculated using Kosambi's mapping function. In addition, MapChart (Voorrips, 2002) was used to create a map of the physical position of the genetic markers used in this study based on the Glyma.Wm82.a2 reference genome (Soybase.org).

PI 605823 and the susceptible parents Williams 82 and 5601T were evaluated for resistance to nine different *P. pachyrhizi* isolates maintained by the USDA-ARS Foreign Disease-Weed Science Research Unit (FDWSRU) in a Biosafety Level 3 (BSL-3) plant pathogen containment facility at Ft. Detrick, MD (Table 2.2). These isolates have been used previously to differentiate *Rpp* genes due to their ability to induce pathotype-specific resistance reactions (Harris et al., 2015; Kendrick et al., 2011; King et al., 2015, 2017).

Isolate reaction tests for Williams 82 and PI 605823 were performed in May 2015 and a reaction test for 5601T was performed in September 2016 at the FDWSRU using methods similar to those described in detail by Kendrick et al. (2011). Briefly, each parent was challenged individually with all nine of the *P. pachyrhizi* isolates in a RCBD with four replicates (pots) per isolate. Isolate types were evaluated separately, and for each isolate-specific experiment, two blocks (inoculation chambers) were used, with two replicates included in each block. Each replicate consisted of two seedlings in a single pot, with pots randomized in trays. Seedlings were grown for 2 wk in a greenhouse before being transferred to a BSL-3 plant pathogen containment facility and inoculated with *P. pachyrhizi* isolates. Seedlings were rated as RB, INT (intermediate, reddish-brown but somewhat smaller, with sporulating uredinia), or TAN 2 wk after inoculation.

The SoySNP50K haplotype data are available at Soybase.org for 19,652 *Glycine max* accessions from the USDA Soybean Germplasm Collection (ARS-GRIN.gov). Using these data, a unique four-SNP haplotype was identified that spans the resistance locus of PI 605823. This haplotype was examined across the 19,652 genotyped soybean accessions (Table 2.3). Whole-genome relatedness using SOYSNP50K data of the mapping parents, 32 major ancestors of North American soybean cultivars (Gizlice et al., 1994), all sources of mapped *Rpp* resistance genes (Table 2.1), and four accessions that share an identical four-SNP haplotype with PI 605823 and show resistance to *P. pachyrhizi* were used to create a phylogenetic tree using Tassel 5.2.31 (Bradbury et al., 2007). The default software setting for creating relatedness cladograms was used i.e. genetic distances were calculated using a modified Euclidean distance, where a homozygote is 100% similar to itself and a heterozygote is only 50% similar to itself. The neighbor-joining algorithm was used to generate the cladogram, which was transformed into a circular figure using FigTree v1.4.3 (http://tree.bio.ed.ac.uk).

Results

Both the Williams 82 × PI 605823 $F_{2:3}$ and 5601T × PI 605823 RIL populations showed segregation for resistance to the GA12 *P. pachyrhizi* bulk isolate inoculation in the greenhouse. The resistant individuals had reddish-brown (RB) lesions with reduced sporulation, similar to PI 605823, and the susceptible individuals had TAN lesions with abundant sporulation similar to the susceptible parents 5601T and Williams 82 (Fig. 2.1). The respective segregation ratios indicated that the resistance of PI 605823 to the GA12 isolate was controlled by a single, dominant allele (Table 2.4).

BSA was conducted in the Williams 82 × PI 605823 F_2 population by first selecting the homozygous SNP calls that were polymorphic between the Williams 82 parent and the PI 605823 parent. SNP marker alleles from the susceptible bulk genotype which matched the SNP genotype of the Williams 82 parent were then selected, and if the SNP allele from the resistant bulk genotype simultaneously matched that of the PI 605823 parent, that marker was considered to be associated with a putative resistance locus. BSA indicated 10 SNPs within a 1.2 Mb region on chromosome (Chr) 19 (Gm19:

39,213,174 – 40,448,735 Glyma.Wm82.a2) that met the genotype-phenotype association criteria, suggesting that this genomic region contains a gene responsible for the observed resistance to *P. pachyrhizi* (Fig. 2.2). No SNPs in any other genomic regions were detected by BSA.

The putative resistance genomic locus identified on Chr 19 through BSA was more precisely mapped using 87 $F_{2:3}$ families and 84 $F_{4:5}$ RILs. The Williams 82 × PI 605823 $F_{2:3}$ population was genotyped with seven polymorphic SNP markers and the 5601T × PI 605823 $F_{4:5}$ RIL population was genotyped with 11 polymorphic SNP markers from the target region to identify recombination within the region. An integrated linkage map was generated from the combined data, and this placed the putative *Rpp* locus in a 154 kb region between the markers GSM0546 and GSM0463 (Gm19: 39,462,291 – 39,616,643 Glyma.Wm82.a2). The *Rpp* locus co-localized with the markers GSM0547 and GSM0548 (Fig. 2.3). Since the name "*Rpp7*" has been approved by the Soybean Genetics Committee, this designation is henceforth used in this article to refer to the *P. pachyrhizi* resistance gene from PI 605823 and the locus on Chr 19 at which it resides.

The marker alleles of recombinant families or RILs from each mapping population were compared, along with the phenotypic data, to further verify the mapped position of the resistance gene (Tables 2.5a, b). The mapping interval for the 5601T × PI 605823 $F_{4:5}$ RIL population (154 kb) is within the larger interval of the Williams 82 × PI 605823 $F_{2:3}$ population (655 kb). In the Williams 82 × PI 605823 $F_{2:3}$ population, *Rpp7* co-localized with markers GSM0463, GSM0466, and GSM0469 within a 655 kb interval, based on nine recombinant families (Table 2.5a). In the 5601T × PI 605823 $F_{4:5}$ RIL population, additional recombination was observed within 20 recombinant RILs, and *Rpp7* co-localized with markers GSM0547 and GSM0548 within a 154 kb interval (Table 2.5b).

The susceptible mapping parents Williams 82 and 5601T developed a TAN (i.e., susceptible) reaction to each of six international and three domestic isolates evaluated at the FDWSRU. The resistant mapping parent PI 605823 developed an RB reaction to four isolates, a TAN reaction to four isolates, and an RB/INT reaction to one isolate. This was a unique pattern of resistance reactions compared to each of

the accessions with previously discovered *Rpp* genes that have been tested in previous studies (Table 2.2). PI 605823 had an RB or RB/INT reaction to all of the U.S.-collected isolates on the panel, but had a TAN reaction to isolates collected from India, Taiwan, Vietnam and Zimbabwe (Table 2.2).

A four-SNP haplotype spanning the *Rpp7* locus in PI 605823 was examined across 19,652 genotyped accessions from the USDA Soybean Germplasm Collection (Soybase.org). Of those accessions, 251 were found to have the same rare four-SNP haplotype as PI 605823. Four of these accessions (PI 81765, PI 232988, PI 437633 and PI 612753) developed an RB reaction to the GA12 *P. pachyrhizi* isolate (Table 2.3), but all of these accessions were collected from northern China and are from much earlier maturity groups (MG 0 to II), so a common, identical-by-descent ancestry seems highly unlikely. In addition, a whole-genome dendrogram of relatedness that was created using the SoySNP50K chip data for the four accessions, PI 605823, all previously reported *Rpp* gene sources, and the 32 soybean ancestors, did not indicate that any of the four resistant accessions is closely related to PI 605823 (Fig. 2.4).

Discussion

Management of soybean rust continues to be a major challenge for soybean production in many parts of the world, so the resistance provided by *Rpp* genes needs to be incorporated into cultivars targeted to those regions to protect yield and reduce fungicide usage and the risk of tolerance to fungicides (Godoy et al., 2016; Hartman et al., 2005). Because of the pathogenic diversity in the fungus and the variation in the amount of protection that individual *Rpp* genes provide against different pathotypes, resistance gene pyramids have been proposed and evaluated as a means of obtaining broader and more durable resistance (Yamanaka et al., 2013). The search for novel *Rpp* genes and alleles continues, as resistance from known *Rpp* genes is pathotype-specific, often incomplete, and can lose efficacy (Bonde et al., 2006; Yamanaka et al., 2010; Yorinori et al., 2005). Discovery of new sources of resistance with unique genes tagged with closely linked genetic markers would give breeders more options in breeding for resistance to *P. pachyrhizi*. Although resistance genes have previously been

reported at six independent loci, the ability to develop cultivars with different *Rpp* gene pyramids is limited by several factors, including the following: (1) many of the soybean germplasm accessions with resistance to U.S. populations of *P. pachyrhizi* have a resistance gene at the same *Rpp3* locus (Harris et al., 2015); (2) the *Rpp1* and *Rpp4* loci are only about 30 cM apart, so trying to combine resistance genes from those loci in the same genetic background could limit the recombination; (3) the *Rpp5* gene from PI 200526 has not provided resistance to U.S. populations of *P. pachyrhizi*; and (4) although the sources of the *Rpp6*/*Rpp(PI567068A)* locus condition very effective resistance against most rust populations in the USA (King et al., 2015; Walker et al., 2014a), the accessions are very poor agronomically, so linkage drag might be a problem following introgression of the gene.

In this study, the resistance to *P. pachyrhizi* in PI 605823 was discovered to be associated with a novel genomic location on Chr 19 based on BSA of genotypic and phenotypic data from an F_2 population (PI 605823 × Williams 82) (Fig. 2.2). This locus was further examined in two other independent mapping populations. Eighty-seven PI 605823 × Williams 82 $F_{2:3}$ families and 84 PI 605823 × 5601T $F_{4:5}$ RILs were used for genetic mapping with KASP SNP marker assays and phenotyping with the *P. pachyrhizi* GA12 bulk isolate. Resistance segregation ratios of the $F_{2:3}$ families and $F_{4:5}$ RILs indicated that resistance to this isolate is inherited as a single gene, and ratios of F_3 and F_5 plants showed that the gene acts in a completely dominant fashion (Table 2.4). The gene conditioning the RB resistance reaction against the GA12 isolate mapped to a 154 kb region on Chr 19 between the KASP markers GSM0546 and GSM0463 (Gm19: 39,462,291 – 39,616,643 Glyma.Wm82.a2) (Fig. 2.3; Tables 2.3, 2.6). Within this region, additional SNP markers GSM0547 and GSM0548 co-segregated with variation in the trait, and the lack of observed recombination between these markers and the resistance source in 84 RILs indicates that these markers would be effective to select for inheritance of the *Rpp7* gene in segregating breeding populations (Fig. 2.3; Tables 2.3, 2.6). Additionally, Sat_150 is the closest SSR marker to *Rpp7* (40,265,193 bp), and could be used for selection, although occasional recombination between the marker and trait is possible (Table 2.5).

PI 605823 had a unique pattern of resistance and susceptibility reactions to nine *P. pachyrhizi* isolates compared to other sources of mapped *Rpp* genes (Table 2.2). The *Rpp7* gene resulted in resistance reactions to *P. pachyrhizi* isolates from Australia (AU79-1) and Columbia (CO04-2), and to U.S. isolates from Georgia (GA12-1), Louisiana (LA04-1), and Hawaii (HW98-1), indicating its potential for widespread utilization as a source of resistance to *P. pachyrhizi*. PI 605823 also had an RB reaction with very low sporulation in a field screening at Itapúa, Paraguay in 2006 (Miles et al., 2008), and showed resistance to field populations of *P. pachyrhizi* in evaluations conducted between 2007 and 2016 in the U.S. states of AL, FL, GA, and SC (Walker et al., 2011, 2014a) (Table 2.7).

The PI 605823 haplotype at four SNP loci within the mapped resistance region was unique compared to the haplotypes of the susceptible mapping parents, the 32 major ancestors of North American soybean cultivars, and all sources of previously mapped *Rpp* genes (Table 2.3). When this haplotype was examined across the entire USDA Soybean Germplasm Collection, it was found in 251 accessions, of which four had an RB reaction to the GA12 *P. pachyrhizi* isolate in greenhouse assays (data not shown). However, these four accessions were collected in northeastern China (i.e., >1000 km from Vietnam) and do not show much whole-genome similarity to PI 605823 (Fig. 2.4). The resistance of those four accessions is surprising because they are all in early maturity groups (i.e., MG 0 to II), they originated from a region with very cold winters and little or no soybean rust (Manchuria), and they are some of the only PIs from China with resistance to *P. pachyrhizi* isolates or field populations from the USA. Further genomic comparison studies would be needed to elucidate whether or not they contain the same *Rpp* allele found in PI 605823, but this seems unlikely considering the many differences between them and their geographical origins.

The 154 kb region on Chr 19 in which *Rpp7* is located contains 16 annotated genes in the Williams 82 reference genome (Soybase.org) (Table 2.8). Of these, three genes are predicted to encode nucleotide-binding site leucine rich repeat (NBS-LRR) disease resistance proteins. One of these genes has been isolated as a candidate disease-resistance gene and named EU888329.1 by researchers at the

Institute of Soybean Research in Harbin, China (annotated on Soybase.org). Re-sequencing this region in PI 605823 could identify the nucleotide variation that is conferring resistance in the PI 605823 genotype.

The long arm of Chr 19, which harbors the *Rpp7* locus, contains relatively few reported disease resistance loci compared to Chr 18, on which *Rpp1, 4, 6,* and R gene loci associated with resistance to several other soybean diseases are located (Soybase.org). However, QTL for resistance to sudden death syndrome (Abdelmajid et al., 2012) and white mold (Arahana et al., 2001) have been mapped to the same general region as the *Rpp7* locus (37,185,287 – 40,265,091 and 40,154,846 – 40,637,071, respectively) (Glyma.Wm82.a2; Soybase.org). Interestingly, in addition to the cluster of three putative R genes in the vicinity of the *Rpp7* locus, three other putative R gene clusters are annotated on the Williams 82 reference genome slightly downstream on Chr 19, between 39,660,000 and 40,120,000 bp (Soybase.org). Clustering of R genes and copy number variation are common phenomenon due to tandem and segmental duplication, and to unequal crossing over, respectively (McHale et al., 2006). A major maturity/flowering-time locus (*E3/FT3*) is also located at approximately 47,640,000 bp (Glyma.Wm82.a2; Soybase.org)(Watanabe et al., 2009), only 8 Mb downstream of *Rpp7*. As PI 605823 is a MG IX soybean, linkage between these traits would need to be broken to obtain an earlier-maturing cultivar with *Rpp7*-mediated resistance.

Conclusion

The gene designation *Rpp7* has been approved by the Soybean Genetics Committee for the soybean rust resistance gene from PI 605823, as it maps to an entirely different genomic region than any of the previously reported *Rpp1* through *Rpp6* loci, and this locus was detected in two segregating populations derived from PI 605823. For the combination of mapping populations and isolate used, there was no evidence that PI 605823 carries any additional *Rpp* genes. *Rpp7* may prove useful to soybean breeders as an additional gene that can be pyramided with other resistance genes to provide broader and more durable resistance to pathogenically diverse strains of *P. pachyrhizi*.

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Figures and tables

Fig. 2.1 The reactions of mapping population parents to the Georgia 2012 (GA12) *P. pachyrhizi* bulk isolate 2 wk after inoculation: **a** Williams 82, **b** 5601T, **c** and **d** PI 605823. The TAN infections of Williams 82 and 5601T (**a, b**) have diffuse edges, abundant uredinia, and dust-like urediniospores. The RB lesions of PI 605823 (**c, d**) have sharply delimited edges and a few volcano-shaped uredinia, but no urediniospores. *Black arrows* point to clusters of urediniospores. *Bar* 1mm.

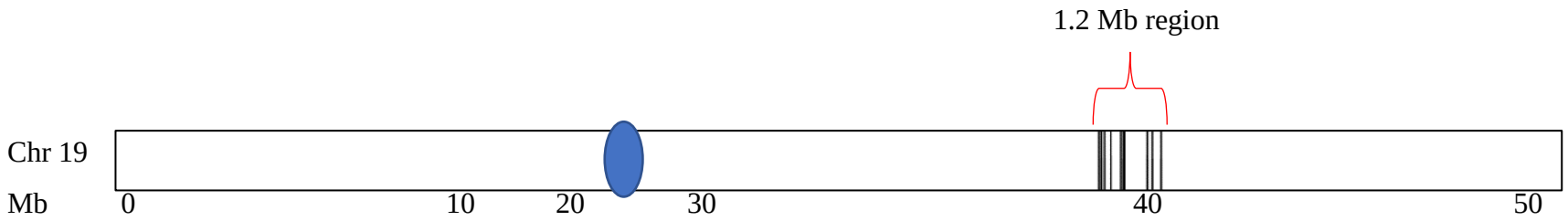

Fig. 2.2 Bulked segregant analysis (BSA) results from the Williams 82 × PI 605823 F_2 population indicating the presence of a soybean rust resistance gene on *Glycine max* chromosome 19. The 2,315 SNPs from the SOYSNP50K chip on chromosome 19 are represented across the length of the chromosome figure. A putative genomic region was determined by identifying homozygous SNP calls that were polymorphic between the Williams 82 parent and PI 605823 parent, whereby the susceptible bulk genotype matched that of the Williams 82 parent and the resistant bulk genotype simultaneously matched that of the PI 605823 parent. The 10 SNPs highlighted by BSA are represented by black lines and are located within a 1.2 megabase (Mb) region toward the lower distal end of the chromosome (Gm19: 39,213,174 – 40,448,735 Glyma.Wm82.a2). The approximate centromeric location is represented by a blue oval, and the chromosome distances are in Mb.

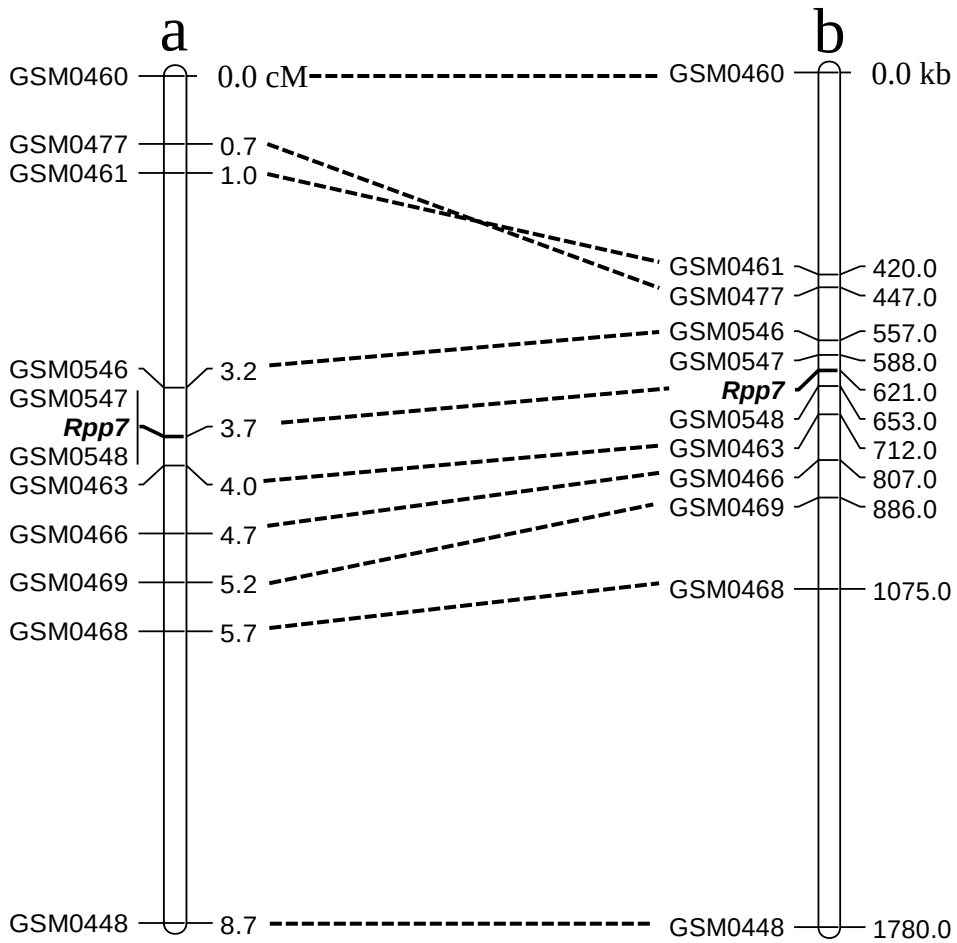

Fig. 2.3 Genetic and physical maps of the *Rpp7* locus on soybean chromosome 19: **a** Integrated genetic map of the *Rpp7* gene locus generated with data from 87 Williams 82 × PI 605823 $F_{2:3}$ families and 84 5601T × PI 605823 $F_{4:5}$ RILs. The units on the right side of the chromosome map are in centiMorgans (cM) and the names of the KASP markers are on the left side of the map. **b** The physical map of the *Rpp7* locus. The units on the right of the chromosome map are in kilobases (kb) and the physical positions of the genetic markers were taken from Soybase.org (Glyma.Wm82.a2). The approximate position of the *Rpp7* locus is based on the genetic distances between closest markers GSM0547 and GSM0548.

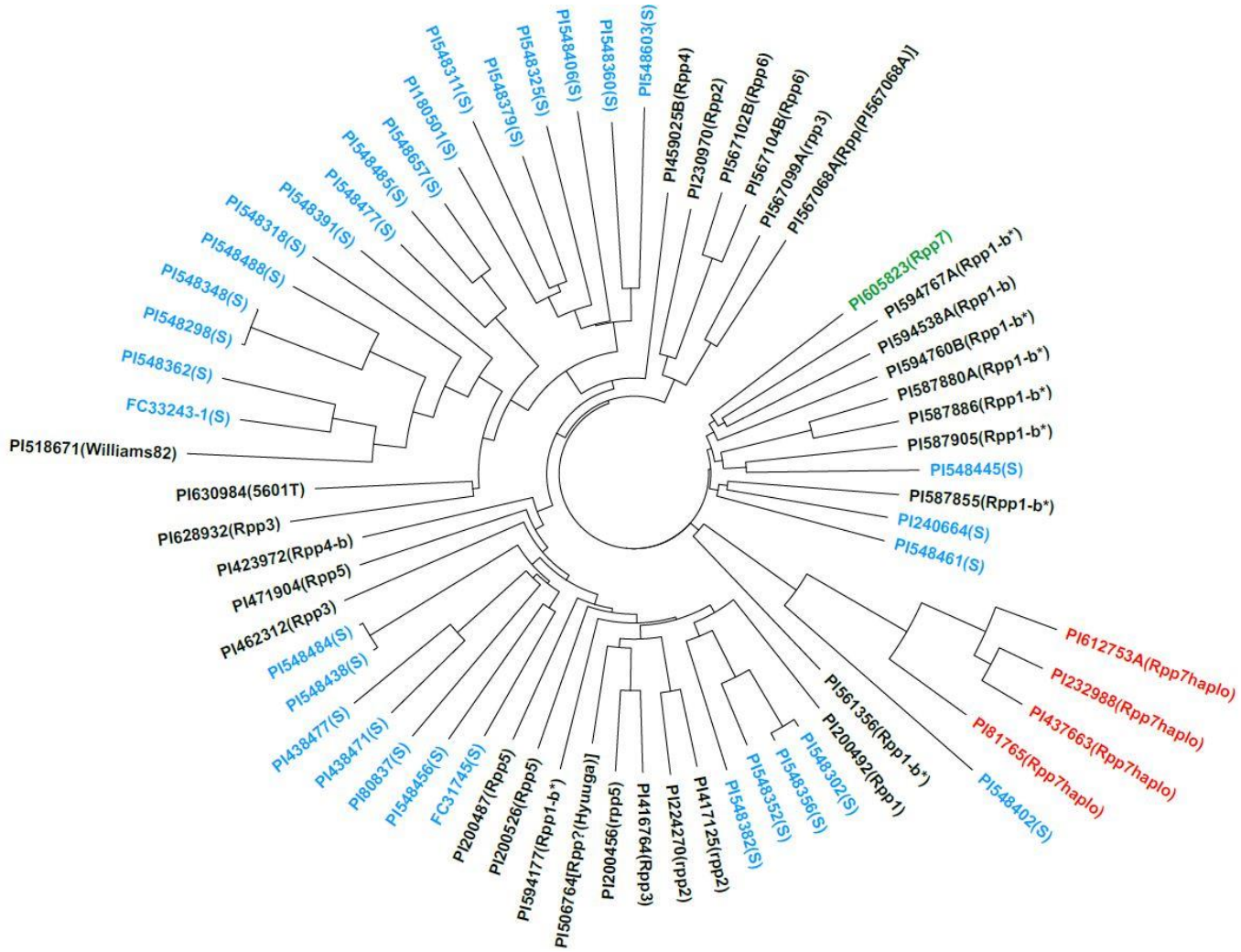

Fig. 2.4 Dendrogram of whole-genome relatedness using SOYSNP50K data: green color indicates the source of *Rpp7* resistance, red indicates the four accessions that share a four-SNP haplotype with PI 605823 and show resistance to *P. pachyrhizi* (Table 3), black indicates the sources of published *Rpp* genes, and blue indicates SBR-susceptible mapping parents and the major ancestors of North American soybean cultivars. *Note*: accessions with *Rpp1-b** have an *Rpp* gene at either the *Rpp1* or *Rpp1-b* locus.

Table 2.1 Genomic positions and germplasm accession sources of mapped *Rpp* genes.

Gene locus	Chr	Physical position Glyma.Wm82.a2[†]	Source	References
Rpp1/ *Rpp1-b*	18 (G)	56,182,523-56,797,174	PI 200492 PI 594538A PI 587886 PI 587880A PI 594760B PI 561356 PI 594767A PI 587905 PI 594177 PI 587855	Chakraborty et al., 2009; Garcia et al., 2011; Hossain et al., 2014; Hyten et al., 2007; Kim et al., 2012; McLean and Byth, 1980; Ray et al., 2009; Rocha et al., 2016; Yamanaka et al., 2015, 2016
Rpp2/*rpp2*	16 (J)	27,937,049-30,478,472	PI 230970 PI 224270 PI 417125	Bromfield and Hartwig, 1980; Calvo et al., 2008; Garcia et al., 2008; Hartwig and Bromfield, 1983; Nogueira et al., 2008; Silva et al., 2008; Yu et al., 2015
Rpp3/*Rpp?* (*Hyuuga*)	6 (C2)	44,049,891-45,995,029	PI 462312 PI 506764[‡] PI 628932 PI 567099A PI 416764	Brogin, 2005; Hartwig and Bromfield, 1983; Hossain et al., 2014; Hyten et al., 2009; Kendrick et al., 2011; Monteros et al., 2007; Ray et al., 2011
Rpp4/*Rpp4-b*	18 (G)	51,397,064-51,584,617	PI 459025B PI 423972	Garcia et al., 2008; Hartwig, 1986; King et al., 2017; Meyer et al., 2009; Silva et al., 2008
Rpp5/*rpp5*	3 (N)	29,862,641-32,670,690	PI 200526 PI 200456 PI 200487 PI 471904 PI 506764[‡]	Calvo et al., 2008; Garcia et al., 2008; Kendrick et al., 2011
Rpp6/ *Rpp [PI567068A]*	18 (G)	5,953,237 - 6,898,528	PI 567102B PI 567068A PI 567104B	King et al., 2015; Li et al., 2012; Liu et al., 2016

[†]Physical positions of the markers used for mapping were taken from Soybase.org.

[‡]PI 506764 (Hyuuga) has an *Rpp* gene at both the *Rpp3* and *Rpp5* locus (Kendrick et al., 2011).

Table 2.2 Phenotypic reactions of sources of soybean rust resistance to international rust isolates.

	AU79-1[†]	CO04-2	GA12-1	HW98-1	IN73-1	LA04-1	TW72-1	VT05-1	ZM01-1
Williams 82[‡]	TAN[§]	TAN	TAN	TAN	TAN	TAN	TAN	TAN	TAN
5601T	TAN	TAN	TAN	TAN	TAN	TAN	TAN	TAN	TAN
PI 200492 (*Rpp1*)	TAN	TAN	IM	IM	IM/RB	IM	TAN	TAN	TAN
PI 594538A (*Rpp1-b*)	RB	-	TAN	RB	RB	TAN	RB	-	RB
PI 230970 (*Rpp2*)	RB	-	RB	-	RB	-	RB	-	RB
PI 462312 (*Rpp3*)	MIX	TAN	RB	RB	RB	RB	TAN	TAN	TAN
PI 506764 [*Rpp?*(*Hyuuga*)]	-	RB	RB	RB	RB	RB	TAN	RB	RB
PI 459025B (*Rpp4*)	RB	RB	RB	RB	RB	RB	RB	-	RB
PI 423972 (*Rpp4-b*)	RB	RB	RB	RB	TAN	RB	MIX	-	RB
PI 200526 (*Rpp5*)	-	TAN	TAN	TAN	TAN	TAN	TAN	TAN	TAN
PI 200456 (*rpp5*)	-	MIX	-	MIX	TAN	TAN	TAN	MIX	MIX
PI 471904 (*Rpp5*)	-	RB	RB	RB	RB	RB	TAN	RB	RB
PI 567102B (*Rpp6*)	RB	RB	RB	RB	RB	RB	MIX	-	RB
PI 567068A [*Rpp(PI567068A)*]	RB/INT	-	RB	RB	-	RB	TAN	-	TAN
PI 605823 (*Rpp7*)	RB	RB	RB	RB/INT	TAN	RB	TAN	TAN	TAN

[†]Isolate codes: Australia, 1979 (AU79-1); Armenia, Columbia, 2004 (CO04-2); Georgia, USA, 2012 (GA12-1); Oahu, Hawaii, 1998 (HW98-1); Pantnagar, India, 1973 (IN73-1); Louisiana, USA, 2004 (LA04-1); Taipei, Taiwan, 1972 (TW72-1); Hanoi, Vietnam, 2005 (VT05-1); Narare, Zimbabwe, 2001 (ZM01-1).

[‡]Reactions for Williams 82, 5601T, and PI 605823 were tested in this study. All other reaction data were taken from Harris et al., 2015; Hyten et al., 2007 2008; Kendrick et al., 2011; Kim et al., 2012; King et al., 2015; and Ray et al., 2009.

[§]TAN reaction is light-brown colored, with profuse sporulation from uredinia; RB reaction is reddish-brown colored with sporulation absent or reduced; IM reaction has no macroscopic lesion formation; MIX is a mixture of RB and TAN reactions; RB/IM is a mix of IM and small RB reactions; RB/INT is a mixture of RB reactions and intermediate reactions (dark-colored lesions similar to the RB type that are relatively smaller and are sporulating more vigorously). Gray shading highlights susceptible lesion reactions.

Table 2.3 Reactions of accessions with *Rpp7* haplotypes, the parents of the mapping populations used in this study, 32 soybean ancestors of North American cultivars, all reported PIs with mapped genes, and resistant accessions with the PI 605823 haplotype to the GA12 isolate of *Phakopsora pachyrhizi*.

							Gm19_Glyma.Wm82.a2[#]			
							Gm19_39493123	Gm19_39500215	Gm19_39505777	Gm19_39557854
PI[†]	**Cultivar**	**Year**[‡]	**Origin (Country, Region)**	**MG**[§]	**Reaction to GA12 isolate of *Phakopsora pachyrhizi*[¶]**	**Known *Rpp* gene**	ss715634822 (GSM0547)	ss715634825	ss715634826	ss715634832 (GSM0548)
PI 605823	SAMPLE 87	1998	Vietnam, Ha Giang	IX	R	*Rpp7*	C	T	C	C
PI 81765	Moshito	1929	China, Manchuria	I	R	None	C	T	C	C
PI 232988	Harbin No. 413	1956	China, Manchuria	II	R	None	C	T	C	C
PI 437663	Gun'-tszu-lin' 691	1980	China	II	R	None	C	T	C	C
PI 612753A	ZY 645	2000	China	0	R	None	C	T	C	C
PI 200492	Komata	1952	Japan, Shikoku	VII	R	*Rpp1*	T	T	C	T
PI 594177	Himeshirazu	1996	Japan	VIII	R	*Rpp1?*	C	C	T	T
PI 594538A	Min hou bai sha wan dou	1996	China, Fujian	IX	S	*Rpp1-b*	C	C	T	C
PI 561356	Jin yun dou	1992	China, Zhejiang	V	S	*Rpp1-b?*	T	T	C	T
PI 587880A	Huang dou	1995	China, Zhejiang	VI	S	*Rpp1-b?*	T	C	T	T
PI 587855	Jia bai jia	1995	China, Zhejiang	VIII	S	*Rpp1-b?*	T	C	T	T
PI 587886	Bai dou	1995	China, Zhejiang	VI	S	*Rpp1-b?*	T	T	C	T
PI 587905	Xiao huang dou	1995	China, Zhejiang	VII	S	*Rpp1-b?*	T	C	T	T
PI 594760B	Gou jiao huang dou	1996	China, Guangxi	IX	S	*Rpp1-b?*	T	T	C	T

PI 594767A	Zhao ping hei dou	1996	China, Guangxi	IX	S	*Rpp1-b?*	C	C	T	C
PI 417125	Kyushu 31	1977	Japan, Kanagawa	VIII	R	*rpp2*	C	C	T	T
PI 224270	Howgyoku	1955	Japan, Hyogo	VII	R	*rpp2*	C	C	T	T
PI 230970	NA	1956	Japan, unknown	VII	R	*Rpp2*	C	C	T	T
PI 567099A	MARIF 2740	1993	Indonesia, East Java	IX	M	*rpp3*	C	C	T	T
PI 462312	Ankur	1981	India, Uttar Pradesh	VIII	R	*Rpp3*	T	C	T	T
PI 416764	Akasaya	1977	Japan, Kanagawa	VIII	R	*Rpp3*	T	C	T	T
PI 628932	FT-2	2002	Brazil	VII	R	*Rpp3*	T	T	C	T
PI 459025B	Bing nan	1981	China, Fujian	VIII	R	*Rpp4*	C	C	T	C
PI 423972	Takema	1978	Japan, Kumamoto	IX	R	*Rpp4-b*	C	C	T	C
PI 200456	Awashima Zairai	1952	Japan, Shikoku	VIII	S	*rpp5*	T	C	T	T
PI 200526	Shira Nuhi	1952	Japan, Shikoku	VIII	S	*Rpp5*	C	C	T	C
PI 200487	Kinoshita	1952	Japan, Shikoku	VIII	R	*Rpp5*	T	C	C	T
PI 471904	Orba	1982	Indonesia, Java	IX	R	*Rpp5*	C	C	T	T
PI 567102B	NA	1993	Indonesia, East Java	IX	R	*Rpp6*	T	T	C	T
PI 567104B	MARIF 2769	1993	Indonesia, East Java	IX	R	*Rpp6*	T	T	C	T
PI 567068A	MARIF 2666	1993	Indonesia, East Java	VII	R	*Rpp(PI567068A)*	C	C	T	T
PI 506764	Hyuuga	1986	Japan, Kyushu	VII	R	*Rpp?(Hyuuga)*	T	C/T	C/T	T
PI 518671	Williams 82	1981	USA, Illinois	III	S	None	C	C	T	C
PI 630984	5601T	2001	USA, Tennessee	V	S	None	T	T	C	T
PI 659315	R00-1194F	2010	USA, Arkansas	IV	S	None	-	-	-	-
FC 31745	NA	1948	unknown	VI	S	None	T	C	T	T
FC 33243-1	Anderson	1954	unknown	IV	S	None	T	T	C	T
PI 80837	Mejiro	1929	Japan, unknown	IV	S	None	T	T	C	T
PI 180501	Strain No.18	1949	Germany, unknown	0	S	None	C	C	T	T
PI 240664	Bilomi No. 3	1957	Philippines, unknown	X	S	None	T	T	C	T
PI 438471	Fiskeby III	1980	Sweden, Ostergotland	00	S	None	C	T	C	C/T
PI 438477	Fiskeby 840-7-3	1980	Sweden, Ostergotland	00	S	None	T	T	C	T
PI 548298	A.K. Harrow	1939	China, NE China	III	S	None	T	T	C	T

PI 548302	Bansei	1936	Japan, Hokkaido	II	S	None	T	T	T	T
PI 548311	Capital	1944	China, NE China	0	S	None	C	C	T	T
PI 548318	Dunfield	1923	China, Jilin	III	S	None	T	T	C	T
PI 548325	Flambeau	1944	Russia, unknown	00	S	None	T	T	C	T
PI 548348	Illini	1927	China, Heilongjiang	III	S	None	T	T	C	T
PI 548352	Jogun	1936	Korea, Hamgyong Puk	III	S	None	T	C	T	T
PI 548356	Kanro	1936	N. Korea, Pyongyang	II	S	None	T	-	T	T
PI 548360	Korean	1928	North Korea, unknown	II	S	None	T	T	C	T
PI 548362	Lincoln	1943	China, unknown	III	S	None	T	T	C	T
PI 548379	Mandarin (Ottawa)	1934	China, Heilongjiang	0	S	None	C	C	T	T
PI 548382	Manitoba Brown	1939	unknown	00	S	None	T	C	T	T
PI 548391	Mukden	1932	China, Liaoning	II	S	None	T	T	C	T
PI 548402	Peking	1910	China, Beijing	IV	S	None	T	T	C	T
PI 548406	Richland	1938	China, Jilin	II	S	None	T	T	C	T
PI 548438	Arksoy	1937	N. Korea, Pyongyang	VI	S	None	C	C	T	T
PI 548445	CNS	1943	China, Jiangsu	VII	S	None	C	-	T	C
PI 548456	Haberlandt	1907	N. Korea, Pyongyang	VI	S	None	T	C	T	T
PI 548461	Imp. Pelican	1950	China, unknown	VIII	S	None	C	C	T	C
PI 548477	Ogden	1940	unknown	VI	S	None	T	C	T	T
PI 548484	Ralsoy	1940	N. Korea, Pyongyang	VI	S	None	C	C	T	T
PI 548485	Roanoke	1946	China, Jiangsu	VII	S	None	T	C	T	T
PI 548488	S-100	1945	China, Heilongjiang	V	S	None	T	T	C	T
PI 548603	Perry	1952	USA, Indiana	IV	S	None	T	T	C	T
PI 548657	Jackson	1953	USA, North Carolina	VII	S	None	T	C	T	T

[†]PI, plant introduction ID from the USDA Germplasm Resources Information Network.
[‡]Year the plant introduction was deposited in the USDA Soybean Germplasm Collection.
[§]MG, maturity group.

¶These reaction data were taken from greenhouse studies performed at the University of Georgia using the GA12 bulk isolate unless specified. GA12 was collected from field-grown kudzu and soybean in 2012. R indicates an RB or IM resistance reaction types, M indicates a mixed RB and TAN response, S indicates a susceptible TAN lesion reaction, and NA indicates that the reaction has not been tested.
#The genomic locations are from chromosome 19 of the Glyma.Wm82.a2 sequence available at www.soybase.org/dlpages/index.php#snp50k (Song et al., 2013). The gray highlights the relatively rare haplotype allele representative of PI 605823.

Table 2.4 Single-gene inheritance and dominance *Chi*-square test for the mapping populations derived from PI 605823. The ratios do not differ significantly from the expected F_2 1:2:1 R:H:S ratio and the F_4 7:2:7 R:H:S ratio expected for a single-gene model. The R:S ratios of plants in heterozygous families do not differ significantly than the expected F_3 1.67 to 1 R:S ratio and the F_5 1.13 to 1 R:S ratio, indicating that the resistance locus is completely dominant.

		Single-gene *Chi*-square test							**Dominance *Chi*-square test**					
		No of families							No of plants					
Susceptible Parent	Gen	R†	H	S	Total	Expected ratio	$\chi 2$	p*	R	S	Total	Expected ratio	$\chi 2$	p*
Williams 82	$F_{2:3}$	17	51	19	87	1:2:1	2.68	0.26	680	445	1125	1.67:1 (F3)	2.13	0.14
5601T	$F_{4:5}$	38	11	35	84	7:2:7	0.15	0.93	66	57	123	1.13:1 (F5)	0.02	0.89

†R stands for resistant; H stands for heterozygous or heterogeneous; and S stands for susceptible.

Table 2.5a Families with recombination near the *Rpp7* locus in the Williams 82 × PI 605823 $F_{2:3}$ population. The vertical lines indicate intervals in which recombination events resulted in an alternate marker allele.

Marker		GSM0460[†]	GSM0461	*Rpp7*[‡]	GSM0463	GSM0466	GSM0469	GSM0468	GSM0448
Position		38,905,966	39,325,408		39,616,643	39,712,503	39,791,263	39,979,924	40,685,024
Family ID #	12	h[§]	a	A	a	a	a	a	a
	59	h	h	B	b	b	b	b	b
	79	a	a	H	h	h	h	h	h
	4	h	h	H	h	h	h	b	b
	29	a	a	A	a	a	a	h	h
	33	h	h	H	h	h	h	a	a
	67	h	h	H	h	h	h	h	b
	68	a	a	A	a	a	a	a	h
	77	h	h	H	h	h	h	h	b

[†]KASP marker positions on chromosome 19 are from the Glyma.Wm82.a2 map at Soybase.org.

[‡]Phenotypic reactions of the families to the GA12 isolate: susceptible (A), resistant (B), or heterozygous/heterogeneous (H).

[§]Indicates a KASP SNP allele similar to the susceptible parent (a), resistant parent (b), or that of a heterozygous/heterogeneous family (h).

Table 2.5b RILs with recombination near the *Rpp7* locus in the 5601T × PI 605823 $F_{4:5}$ population. The vertical lines indicate intervals in which recombination events resulted in an alternate marker allele.

Marker		GSM0460[†]	GSM0461	GSM0447	GSM0546	GSM0547	*Rpp7*[‡]	GSM0548	GSM0463	GSM0466	GSM0469	GSM0468	GSM0448
Position		38,905,966	39,325,408	39,352,524	39,462,291	39,493,123		39,557,854	39,616,643	39,712,503	39,791,263	39,979,924	40,685,024
RIL ID #	1	h[§]	a	a	a	a	A	a	a	a	a	a	a
	5	h	b	b	b	b	B	b	b	b	b	b	b
	30	b	h	h	h	h	H	h	h	h	h	h	-
	17	b	b	b	h	h	H	h	h	h	h	h	-
	27	a	a	a	b	b	B	b	b	b	b	b	b
	32	h	h	h	b	b	B	b	b	b	b	b	b
	76	a	a	a	h	h	H	h	h	h	h	h	h
	82	a	a	a	b	b	B	b	b	b	b	b	b
	20	h	h	h	h	a	A	a	a	a	a	a	a
	48	h	h	h	h	h	H	h	a	a	a	a	a
	15	a	a	a	a	a	A	a	a	h	h	h	-
	3	h	h	h	h	h	H	h	h	h	a	a	a
	69	b	b	b	b	b	B	b	b	b	b	a	a
	29	a	a	a	a	a	A	a	a	a	a	a	h
	40	a	a	a	a	a	A	a	a	a	a	a	b
	53	b	b	b	b	b	B	b	b	b	b	b	a
	71	b	b	b	b	b	B	b	b	b	b	b	a
	73	h	h	h	h	h	H	h	h	h	h	h	a
	77	a	a	a	a	a	A	a	a	a	a	a	h

103	h	h	h	h	h	H	h	h	h	h	h	b

[†]KASP marker positions on chromosome 19 were taken from Soybase.org (Glyma.Wm82.a2).
[‡]*Rpp7* represents the phenotypic reaction of the RIL to the GA12 isolate; susceptible (A), resistant (B), or heterozygous/heterogeneous (H).
[§]Indicates a KASP SNP allele similar to the susceptible parent (a), resistant parent (b), or that of a heterogeneous RIL (h).

Table 2.6 Primer details for KASP markers used for genetic mapping.

Assay ID	dbSNP ID[†]	SNP location[‡]	PI SNP allele[§]	Forward primer 1 5'-3' (FAM)	Forward primer 2 5'-3' (HEX)	Reverse primer 5'-3'
GSM0460	ss715634768	Gm19_38905966_RC[¶]	C	GAAGGTGACCAAGTTCATGCTCTTT TCCCAACACCCAGAACAC	GAAGGTCGGAGTCAACGGATTCTTT TCCCAACACCCAGAACAT	TTGTTACCATGCCCGCAACT
GSM0461	ss715634802	Gm19_39325408	A	GAAGGTGACCAAGTTCATGCTGTTA CAATGCCACCTAGATGAAAAAA	GAAGGTCGGAGTCAACGGATTGTTA CAATGCCACCTAGATGAAAAAG	AAGGAAGGGTTAGTAAAAGT AACATTGAC
GSM0447	ss715634808	Gm19_39352524	T	GAAGGTGACCAAGTTCATGCTTCAA CCTGGTTGGGATTCCTTAC	GAAGGTCGGAGTCAACGGATTTCAA CCTGGTTGGGATTCCTTAT	TCCCAAACATTTGATTCCTCA AT
GSM0546[#]	ss715634817	Gm19_39462291	T	GAAGGTGACCAAGTTCATGCTAAC AAAGGAGAGGCCCCTGAC	GAAGGTCGGAGTCAACGGATTAACA AAGGAGAGGCCCCTGAT	TTGTTGCCATTGCTGTGACCT
GSM0547	ss715634822	Gm19_39493123	T	GAAGGTGACCAAGTTCATGCTCTCA GCTATTTGGATATGAAAATGAAAC	GAAGGTCGGAGTCAACGGATTCTCA GCTATTTGGATATGAAAATGAAAT	CTCCTAGCTACGGGTGTGCA A
GSM0548	ss715634832	Gm19_39557854	T	GAAGGTGACCAAGTTCATGCTAAC AGACAATAGATTAAGGAAAAGGGT C	GAAGGTCGGAGTCAACGGATTAACA GACAATAGATTAAGGAAAAGGGTT	CTTCTAGGATTCCTGCCACAT TTT
GSM0463[#]	ss715634841	Gm19_39616643	A	GAAGGTGACCAAGTTCATGCTGCCC CCTCTCTCAATGTGATAA	GAAGGTCGGAGTCAACGGATTGCCC CCTCTCTCAATGTGATAC	CATGACCCTTGTTTCCCACCT
GSM0466	ss715634866	Gm19_39712503	G	GAAGGTGACCAAGTTCATGCTTCTA GCTATCACCACCTAATTAAGACCA	GAAGGTCGGAGTCAACGGATTTCTA GCTATCACCACCTAATTAAGACCG	AGATTGGTGGGGAGGGTCAT
GSM0469	ss715634880	Gm19_39791263	T	GAAGGTGACCAAGTTCATGCTCTAA TCAAGTGGTTCATTCTCTTATAAGG	GAAGGTCGGAGTCAACGGATTCTAA TCAAGTGGTTCATTCTCTTATAAGT	AAAATTATGTGGACTAACAT AAAATAAAGACAC
GSM0468	ss715634920	Gm19_39979924	G	GAAGGTGACCAAGTTCATGCTCATC TACCAAGGGTTGTGTGCA	GAAGGTCGGAGTCAACGGATTCATC TACCAAGGGTTGTGTGCG	TGAAGGGAAAAGGAGAAAA ATGG
GSM0448	ss715635026	Gm19_40685024	C	GAAGGTGACCAAGTTCATGCTCTCA TGACCATCTAATTTGGTGCAC	GAAGGTCGGAGTCAACGGATTCTCA TGACCATCTAATTTGGTGCAT	GCTAATGCCATTGTCGCCTTC

[†]dbSNP ID found at www.Soybase.org/dlpages/index.php#snp50k (Song et al., 2013).
[‡]Physical genomic locations are based on the Glyma.Wm82.a2 sequence of the SNP available at www.Soybase.org/snps/index.php.
[§]SNP allele found in the PI 605823 parent.
[¶]RC indicates the reverse compliment orientation of the sequence was used to design the KASP markers.
[#]SNP allele flanking the *Rpp7* gene.

Table 2.7 Soybean rust index (RI) ratings in nine year-location environments in the southeastern USA for PI 605823 (the source of the *Rpp7* gene, accessions with known *Rpp* genes, and a susceptible check)*.

		Location	Quincy, FL	Fairhope, AL	Quincy, FL	Fairhope, AL	Bossier City, LA	Quincy, FL	Quincy, FL	Attapulgus, GA	Attapulgus, GA
Rpp gene	Source	Year	2008	2008	2009	2009	2009	2011	2012	2012	2016
Susceptible Check	Williams 82		5.0[†‡]	-	5.0	5.0	4.9	2.9[§]	4.5	4.5	4.6
Rpp1	PI 200492		2.3	1.0	1.3	-	3.7	-	4.3	-	1.4
Rpp1-b	PI 594538A		-	-	-	-	-	-	4.4	4.2	4.2
Rpp2	PI 230970		3.8	3.5	3.3	-	-	3.6	3.2	2.2	2.1
Rpp3	PI 462312		3.5	3.9	2.4	3.6	4.9	3.7	4.5	2.4	1.0
Rpp4	PI 459025B		4.5	4.5	3.5	3.5	4.9	3.2	3.7	3.7	1.2
Rpp5	PI 200526		-	-	4.5	5.0	5.0	4.1	4.9	5.0	4.2
Rpp6	PI 567102B		1.4	1.4	1.4	1.0	4.2	1.9	2.8	1.0	1.3
Rpp7	PI 605823		2.4	1.4	1.4[‡]	1.2	4.4	2.9	3.3	2.2	1.0

*Table adapted from Walker et al., 2011 and 2014a, and supplemented with unpublished data.

[†]RI ratings were calculated as the square root of the factor obtained by multiplying the disease severity and sporulation ratings. Each of these was rated on a scale of 1 to 5, where 1 indicated no disease symptoms.

[‡]Gray shading indicates a RI value that was higher than that of PI 605823.

[§]Calculated from an incomplete data set.

Table 2.8 The 16 annotated genes of the Williams 82 soybean reference genome in the 154 kb *Rpp7* region on chromosome 19.

Gene name[†]	Glyma.Wm82.a2.v1 annotation	Additional names	Paralogs	Position[‡]	Size[§]	Notes
Glyma.19g133500	NA	NA	Glyma.03g131600	39,464,716 – 39,465,776	1060	NA
Glyma.19g133600	Gibberellin regulated protein	AT1G74670.1 PF02704	Glyma.03g131700	39,470,881 – 39,473,502	2621	NA
Glyma.19g133700	N-acetylglucosaminyl transferase component (Gpi1)	AT3G57170.1 PF05024	Glyma.03g131800	39,477,915 – 39,481,558	3643	NA
Glyma.19g133800	dehydration-induced protein (ERD15)	AT2G41430.1	Glyma.03g131900	39,488,115 – 39,490,016	1901	NA
Glyma.19g133900	Protein Y48E1B.2, isoform A	AT3G57180.1 PF01926	Glyma.03g132000	39,490,822 – 39,494,317	3495	50S ribosome-binding GTPase
Glyma.19g134000	PAX transcription activation domain interacting protein	AT2G41450.1 PF00583	Glyma.03g132200	39,497,308 – 39,503,963	6655	N-acetyltransferase
Glyma.19g134100 [¶]	Leucine rich repeat-containing protein	AT3G14460.1 PF00931	None	39,510,186 – 39,512,804	2618	LRR and NB-ARC domains-containing disease resistance protein
EU888329.1	NBS-LRR disease resistance protein mRNA	NA	NA	39,510,765 - 39,512,452	2085	"Isolation of a candidate disease-resistance gene from soybean" (2008) Li, W., Y. Han, and W. Chang. College of Agronomy, NEAU, Institute of Soybean Research, Gongbin Road, Harbin, Heilongjiang 150030, China; UNPUBLISHED
Glyma.19g134200	Leucine rich repeat-containing protein	AT3G14460.1 PF00931	None	39,523,361 - 39,525,814	2453	LRR and NB-ARC domains-containing disease resistance protein
Glyma.19g134300	Leucine rich repeat-containing protein	AT3G14470.1 PF00931	None	39,537,942 - 39,540,667	2725	LRR and NB-ARC domains-containing disease resistance protein
Glyma.19g134400	NA	NA	None	39,551,222 – 39,551,455	233	NA

Glyma.19g134500	Extended synaptotagmin-related	AT3G08550.1	Glyma.03g132300	39,551,479 – 39,556,860	5381	Elongation defective 1 protein / ELD1 protein
Glyma.19g134600	NA	AT5G03990.1	None	39,566,372 – 39,569,820	3448	NA
Glyma.19g134700	Glycosyl hydrolases family 17	AT3G57270.1 PF00332	Glyma.03g132700	39,576,172 – 39,579,436	3264	Carbohydrate metabolic process
Glyma.19g134800	Glycosyl hydrolases family 17	AT3G57270.1 PF00332	Glyma.03g132900	39,581,336 – 39,584,321	2985	Carbohydrate metabolic process
Glyma.19g134900	Mitochondrial DNA repair protein RECA homolog	AT3G10140.1 PF00154	Glyma.03g133000	39,588,459 – 39,594,665	6206	recA bacterial DNA recombination protein
Glyma.19g135000	Transmembrane proteins 14, 15	AT3G57280.1 PF03647	Glyma.03g133200	39,595,797 – 39,601,116	5319	Predicted membrane protein

[†]Taken from http://www.Soybase.org/gb2/gbrowse/gmax2.0.
[‡]Position on chromosome 19.
[§]Size in base pairs (bp).
[¶]Annotated genes in bold print are candidate genes for the *Rpp7* resistance locus.

CHAPTER 3

CHARACTERIZATION OF *RPP* GENES AND IMPLEMENTATION OF MOLECULAR BREEDING FOR SOYBEAN RUST RESISTANCE

Introduction

Soybean [*Glycine max* (L.) Merrell] yield is affected by numerous diseases and pests, among which soybean rust (SBR), caused by *Phakopsora pachyrhizi* Syd. & P. Syd., is one of the severe foliar diseases (Bromfield, 1984). SBR, a problem in Asia since the 1960's, has spread around the world, reaching the Western Hemisphere in the early 2000's (Morel et al., 2001; Schneider et al., 2005; Yorinori et al., 2005) and hampers soybean production in sub-tropical regions by reducing yield and seed quality (Bromfield, 1984). The rapid dissemination of *P. pachyrhizi* via wind-blown urediniospores and the ability of the fungus to overwinter on alternative hosts allows SBR to quickly reach epidemic proportions when humid, moderate conditions prevail (Bromfield, 1984).

Fungicide protection programs are widely deployed and historically highly effective, but come at a considerable economic and environmental cost (Godoy et al., 2016; Langenbach et al., 2016; Robert Kemerait, personal communication). In addition, *P. pachyrhizi* insensitivity to some fungicide chemistries has hampered control efforts in Brazil (Aguiar et al., 2016). Cultural control methods, such as early planting and control of overwintering inoculum, have been implemented in several regions of Brazil, with variable results (Godoy et al., 2016).

Use of host plant resistance genes in modern cultivars is a promising approach to management of SBR (Hartman et al., 2005). This approach involves the discovery of resistance genes in landrace soybeans, such as the plant introductions (PIs) within the USDA Germplasm Collection, and the transfer of resistance genes into high-yielding modern cultivars. Multiple studies have been conducted to discover sources of resistance and identify the genomic locations of such resistance genes, known as *Rpp*

(resistance to *Phakopsora pachyrhizi*) genes. Currently, *Rpp* genes have been mapped to seven genetic loci and multiple resistance alleles or tightly-linked genes have been identified at these loci (Chakraborty et al., 2009; Childs et al., 2017; Garcia et al., 2008; Hossain et al., 2014; Hyten et al., 2007, 2009; Kim et al., 2012; King et al., 2015, 2017; Li et al., 2012; Nogueira et al., 2008; Ray et al., 2009, 2011; Silva et al., 2008; Yamanaka et al., 2015, 2016).

Evaluation of putatively resistant soybean PIs with local *P. pachyrhizi* isolates or populations is important to the advancement of knowledge about *P. pachyrhizi* resistance (Walker et al., 2014a). Since *Rpp* genes are pathotype-specific and only some alleles provide a high level of resistance to rust populations in any one location, identification of the best alleles, or combination of alleles, is important for regional breeding efforts (Hartman et al., 2005). Evaluating resistance sources with *P. pachyrhizi* isolates in the greenhouse can also provide evidence for allelic diversity among *Rpp* genes mapped to the same genomic location (King et al., 2015). Field evaluations are also vital to monitoring any changes in the virulence of local *P. pachyrhizi* populations (Walker et al., 2014a). Since race or pathotype development within populations of *P. pachyrhizi* could potentially overcome *Rpp* gene resistance (Paul et al., 2013), screening germplasm to identify potential new sources of resistance will help future rust resistance breeding efforts.

An especially useful method to identify new *Rpp* genes or alleles is characterization of sources of resistance with geographically diverse *P. pachyrhizi* isolates, such as those maintained at the USDA-ARS Foreign Disease-Weed Science Research Unit (FDWSRU) in Ft. Detrick, MD (Kendrick et al., 2011). Many of the known *Rpp* genes have been characterized for their resistance reaction phenotype using these isolates which were collected as early as 1972 from multiple countries around the world. Their reaction phenotypes provide useful information to help understand the plant host resistance provided by each *Rpp* gene, to confirm the presence of pyramided genes in breeding lines, and to discover the effect of pyramided genes on resistance to different *P. pachyrhizi* pathotypes. Another methodology that greatly facilitates discovery and genetic mapping of novel *Rpp* loci by saving time and genotyping costs is bulked

segregant analysis (BSA; Michelmore et al., 1991). This procedure involves combining DNA from multiple resistant and susceptible plants into respective bulks which can be assayed with high-density genetic markers to identify genomic regions associated with resistance. This technique has limitations, however, as it can only detect major resistance loci that are effective against the *P. pachyrhizi* isolate used for screening (Kendrick et al., 2011).

Many germplasm accessions with resistance to *P. pachyrhizi* populations in the USA have an *Rpp* gene at the *Rpp3* locus (Harris et al., 2015). The *Rpp3* resistance region contains an abundance of repetitive DNA content (Okii et al., 2014). Within this region, a 90-bp deletion has been identified in the original source of *Rpp3* (PI 462312) (Hyten et al., 2009) and other *Rpp3* sources such as PI 506764 (Hyuuga), PI 200487, and PI 471904 (Kendrick et al., 2011) but was not found in susceptible lines and other *Rpp* sources (K.S. Pedley, unpublished data). This deletion can be used as a marker to screen germplasm and breeding lines for the *Rpp3* allele from PI 462312. A molecular marker could identify potential allelic diversity among the sources of resistance mapping to the *Rpp3* locus and could also be used to screen germplasm with unknown resistance to separate putative *Rpp3* resistance from novel *Rpp* loci. This marker for *Rpp3* and other molecular markers linked to *Rpp* loci can be used for marker-assisted selection (MAS) to introgress these resistance loci from exotic germplasm into high-yielding cultivars, as has been done with several other traits, such as soybean cyst nematode (SCN) resistance (Shi et al., 2015b; Young, 1999).

The breeding technique known as backcrossing is widely deployed to introgress the target alleles from the exotic donor by repeatedly crossing back to an elite recurrent parent. Selection for the trait of interest at each generation is performed through phenotyping or the use of genetic markers (Young and Tanksley, 1989). Acceleration of the backcrossing process requires the development of genetic markers tightly linked to *Rpp* loci that utilize a cost- and time-effective genotyping platform (Diers et al., 2013). One such genotyping platform is Kompetitive Allele Specific PCR (KASP™) that offers affordable high-throughput genotyping for single nucleotide polymorphism (SNP) markers (Semagn et al., 2014). In

soybean, SNP marker assays can be developed using the Williams 82 reference genome (www.soybase.org) and fingerprinting SNP data from the SoySNP50K array (Song et al., 2013) or can be designed around genomic deletions (Shi et al., 2015a).

A major breeding challenge when backcrossing resistance loci into elite cultivars is the large number of unfavorable alleles present in unimproved landraces that tend to reduce quality and yield (Boerma et al., 2011). Most of the PI sources harboring *Rpp* genes have poor agronomic performance, such as small seed size, extreme lodging susceptibility, very late maturity, and low yield (https://npgsweb.ars-grin.gov). It is important to recognize that while backcrossing can eliminate nearly all unlinked donor genome content, the introgressed fragment size remains relatively large, which could potentially cause yield drag associated with the introgressed *Rpp* locus (Hospital, 2001). Large fragment sizes can also limit stacking of pyramided genes, such as *Rpp1* and *Rpp4*, that are only 4.6 Mb apart. Previous studies have measured the size of exotic introgressions in chickpea (Varshney et al., 2014), rice (Jia et al., 2012), tomato (Lin et al., 2014), wheat (Salina et al., 2003), and soybean (Ortega et al., 2017). Pyramiding multiple *Rpp* genes into soybean cultivars is possible with the use of MAS, and has the potential to provide much broader resistance and help protect against resistance break-down (Hartman et al., 2005). Furthermore, *Rpp* gene pyramids can provide a higher level of resistance than that provided by single genes, even when one of the pyramided *Rpp* genes has been overcome by *P. pachyrhizi* populations (Bhor et al., 2015; Lemos et al., 2011; Maphosa et al., 2012; Yamanaka et al., 2015b). A limitation of gene pyramiding is the large population sizes needed to identify individuals possessing all the desired genes.

The objectives of this study were to: 1) perform greenhouse and field disease screening of diverse soybean PIs and breeding lines with putative resistance to *P. pachyrhizi*; 2) determine the reaction of selected breeding lines and PIs to nine geographically diverse *P. pachyrhizi* isolates; 3) perform BSA on five mapping populations to determine the genomic location(s) of resistance; 4) develop a robust

molecular marker to select for *Rpp3* resistance; 5) perform MAS to pyramid *Rpp1* and *Rpp3* loci in breeding lines; and 6) characterize the size of *Rpp* gene introgressions in breeding lines.

Materials and methods

Field and greenhouse screening

Field and greenhouse screening for SBR was performed on 328 PIs and 132 breeding lines (Tables 3.2 and 3.3). PIs were selected for screening based on previous research indicating they may have resistance to *P. pachyrhizi*. Some accessions harbor resistance alleles that have been previously mapped to *Rpp* loci. Other accessions have shown resistance in field or greenhouse assays in the USA or other countries but the resistance loci have not been mapped. Still other accessions had not been previously screened with *P. pachyrhizi* pathotypes from the Southeastern USA, but originated in countries or within maturity groups from which other resistant germplasm were found. In particular, accessions from Japan, Indonesia, Vietnam, and China in the maturity groups V to IX were of special interest. Furthermore, some PIs that shared the same SNP haplotype allele with the source of a mapped *Rpp* gene were included. Breeding lines developed from backcrossing *Rpp* alleles into elite germplasm were also included for screening.

SoySNP50K iSelect BeadChip data (Song et al., 2013) from 324 PIs included in field and greenhouse screening were obtained from SoyBase (www.soybase.org) (data were unavailable for four PIs). These data were used to create a relatedness cladogram using TASSEL 4.3.15 (Bradbury et al., 2007). Genetic distances were calculated using a modified Euclidean distance, and the neighbor-joining algorithm was used to generate the cladogram. The cladogram was transformed into a circular figure using FigTree v1.4.3 (http://tree.bio.ed.ac.uk), in which accessions were color-coded by country of origin.

Greenhouse screening for resistance to *P. pachyrhizi* was conducted at the Griffin campus of the University of Georgia. Plantings were made in Dec. 2015, Jan., Feb., Mar., and Apr. 2016, and Jan. and Mar. 2017. The protocol for planting, inoculation, and rating was similar to that described in detail by Walker et al., (2014b) with a few modifications. In this study, a randomized complete block design

(RCBD) with two replications of six plants was used for each of the plantings, with the two blocks located adjacent to each other in the same greenhouse room. Inoculation with a urediniospore suspension of the GA12 *P. pachyrhizi* isolate was performed approximately 14 d after planting, at the V2 stage when the second trifoliolate leaf was unfurling. Due to uneven plant emergence and difference in vigor among the diverse germplasm, occasionally some plants were inoculated at a more or less advanced stage. Trays with plants were inoculated using an atomizer and then immediately placed into a dew chamber consisting of a PVC frame 3.0 m x 1.0 m x 0.8 m, completely enclosed in white polyethylene plastic, placed on a greenhouse bench, and further enclosed on the top and sides with 90% black shadecloth. Two to three similar chambers were utilized per planting. After trays were placed inside, the floor of the chamber was flooded with water to create a high humidity environment and the plastic covering was securely closed. Inoculation was performed in the morning and plants were left in the inoculation chamber for 24 h before a second fresh urediniospore suspension was again sprayed over the tops of the plants within the inoculation chamber and the humid environment was maintained for an additional 24 h. After 48hr in the chamber, plants were removed and placed in the greenhouse for continued growth.

Approximately 14 d after the first inoculation, plants were rated for their reactions to the GA12 *P. pachyrhizi* isolate. At least one leaflet was examined per plant using an illuminated magnification lens or a dissecting microscope. The first trifoliolate leaf was the most informative leaf for rating but unifolate leaves were occasionally used if no lesions were present on the trifoliolate leaves. The reaction type was recorded as TAN (fully susceptible and highly sporulating); RB (reddish-brown with limited or no sporulation); HR (hypersensitive reaction with faint discoloration and no sporulation); and IM (immune with no lesions present). Sometimes the distinction between IM and RB was not clear, as small "flecking" was observed that was smaller than the typical RB reaction, and in this case "IM/RB" was recorded. Also, if some plants produced RB lesions and other plants of the same genotype produced an IM reaction, "IM/RB" was recorded for the genotype. If some plants produced TAN lesions and other plants of the same genotype produced resistant lesions, the reaction of the entry was recorded as "MIX". No genotype

produced both TAN and RB lesions on the same plant in this study, which would have indicated a mixed *P. pachyrhizi* pathotype.

In addition to reaction type, a severity rating was recorded for all genotypes and a sporulation rating was recorded for some of the genotypes. The severity rating was based on a visual assessment of the relative number of lesions and amount of yellowing present on a representative leaf of each plant and corresponded to a 1 to 5 scale spanning from completely immune to severely infected and yellowed. The 1 to 5 sporulation rating was made by examining a representative leaf under a dissecting microscope where "1" was immune (similar to PI 200492), "2" had only a few RB sporulating lesions (similar to PI 605823), "3" had clearly reduced sporulation from RB lesions but nearly all lesions were sporulating (similar to PI 459025B), "4" had a moderate sporulation level from TAN reactions (similar to Williams 82), and "5" had exceptionally heavy sporulation from TAN lesion (similar to PI 200526). The severity and sporulation ratings were averaged across plants within the genotype to provide an average rating for the entry. If an entry was included in more than one planting, as was the case with some resistant check accessions, data were averaged across the replications.

Field screening for resistance to *P. pachyrhizi* was conducted at the University of Georgia Attapulgus Research and Extension Center as described by Walker et al., (2014a). Entries were planted in single 3 m rows with approximately 40 plants per row and two replications were planted per soybean genotype. A susceptible cultivar, 5601T, was planted in a double row on the perimeter of the field and through the center of the field to help spread the disease. Seed was planted on 14 July 2016, an earlier date than previous year's plantings for rust infection, in an attempt to achieve greater infection. Although natural rust infection was reported in Decatur Co., Georgia as early as 14 June 2016, rust infection was not seen within the screening nursery as late as 30 September. Consequently, infected leaves from Grady Co., which is approximately 32 km east of Attapulgus, were collected from a soybean farm and used to inoculate the disease nursery on 28 Sept. 2016. To create a spore suspension, infected leaves were immersed in a tank of water and agitated to dislodge spores. The resultant solution was strained through

cheesecloth and applied to the field via a power sprayer attached to an ATV equipped with a handheld spray nozzle and having an approximate spray range of 9 m. Inoculation was performed in the evening and achieved by driving the ATV on one side of the field and spraying across the field, repeating the procedure on the opposite side of the field.

Plants were rated for disease symptoms on 18-19 Oct. 2016, approximately 3 wk after inoculation with the bulk *P. pachyrhizi* population obtained from the local area. Due to time constraints, leaf samples from most plots were only taken from one of the two reps, although both reps were sampled for some resistant checks. Three infected leaves were collected at random from the canopy of each plant row and placed into a sealed plastic bag. Samples were kept on ice or refrigerated for 2 to 3 d, until ratings were performed. Leaves were examined under a dissecting microscope and lesion type (IM, RB, or TAN) was recorded. Disease severity (1 to 5 scale) was determined using a visual assessment of each leaf, in a similar manner to that used for greenhouse screening. Sporulation level (1 to 5) was also determined with the aid of a dissecting microscope, using the same scale previously described for greenhouse screening. Data were averaged over the three leaves collected for each genotype.

Characterization of selected breeding lines and accessions with a panel of* P. pachyrhizi *isolates

Selected breeding lines and PIs to be challenged with the nine *P. pachyrhizi* isolates were planted at the USDA-ARS, FDWSRU, BSL-3 Containment Facility at Ft. Detrick, MD. Breeding lines were selected if they had a putative multiple-*Rpp*-gene stack, based on molecular marker analysis. Some breeding lines were also included if molecular markers indicated that they had single *Rpp* gene introgressions. PIs were selected because they were putative novel sources of resistance and parents of mapping populations.

Separate plantings were made in Jul. and Aug. 2016, and Jan., Feb., and Mar. 2017. Planting and inoculation protocols were conducted per Kendrick et al., (2011). Isolates used for inoculation were collected in diverse locations and at different times (Table 3.1). The isolate LA04-1 was single-spore purified but all other isolates were collected from field populations of *P. pachyrhizi* without purification

(Pham et al., 2009). Urediniospores for each isolate were propagated on susceptible soybean 'Williams 82', collected with a cyclone collector (Cherry and Peet, 1966), and stored under liquid nitrogen (–196°C) at the USDA-ARS, FDWSRU, BSL-3 Containment Facility (Pham et al., 2009).

Bulked segregant analysis

To identify potential novel genes for rust resistance, selected plant introductions that showed consistent resistance reactions in field and greenhouse studies were crossed to susceptible parents 'Boggs', G00-3213, G00-3880, or 'Prichard'. F_2 seeds were grown to obtain $F_{2:3}$ families for all populations except PI 567061 x Prichard that was advanced to the F_5 generation via single-seed descent, and subsequently selfed to obtain $F_{5:7}$ RILs. Populations were planted in the University of Georgia Griffin Campus pathology greenhouse for inoculation with the GA12 *P. pachyrhizi* isolate. For each population, 120 pots were planted, with one family or RIL per pot. Four seeds were planted per pot and seedlings in each pot were later thinned to two plants per pot. Due to uneven germination, some pots only had one plant available for rating.

An approximate 5 cm^2 section of a leaf from each of at least 20 susceptible plants were collected and bulked into a single Falcon tube (Fisher Scientific, Waltham, MA). Leaf tissue was similarly collected and bulked from families with resistance reactions. Since only two plants were available to infer the phenotype of the respective family or RIL, determination of homozygous or heterozygous resistant plants was only possible at 44% probability ($n = \frac{\log(1-P1)}{\log(1-P2)}$ where n= number of plants, P_1 = probability of distinguishing homozygous and heterozygous individuals, and P_2 = fraction of homozygous resistant plants) (Garcia et al., 2008). Therefore, some plants included in the resistant bulk may have heterozygous alleles at the resistance loci (assuming a dominance inheritance model).

DNA extraction was performed per Keim et al., (1988) and fingerprinting of the bulks with the SoySNP50K iSelect BeadChips (Song et al., 2013) was performed at the USDA-ARS Soybean Genomics and Improvement Laboratory, Beltsville, MD. SNP alleles were auto-called using the software GenomeStudio V2011.1 (Illumina, San Diego, USA). Fingerprinting data of the parents were obtained

from SoyBase (www.soybase.org) or the UGA soybean breeding marker database. SNPs that were monomorphic between the two parents were filtered out for each population. A SNP position where the susceptible bulk matched that of the susceptible parent, and the resistant bulk simultaneously matched that of the resistant parent was considered a "strong hit" and coded with "1". SNPs where the resistant bulk was heterozygous while the susceptible bulk matched the susceptible parent was considered a "weak hit" and was coded with "0.5". All other polymorphic SNPs were coded with "0". The SNP position and the scores of 0, 0.5, or 1 were loaded into JMP® Pro 12 (SAS Institute Inc., Cary, NC) and a figure was generated using the Graph Builder function to visualize the BSA hits across the genome or on individual chromosomes. A genomic region was thought to be a putative resistance locus if nearly all the BSA hits fell on a single chromosome.

Development of a robust marker for the* Rpp3 *allele from PI 462312

A deletion within the candidate *Rpp3* gene Glyma06g40740 has been found to co-segregate with *Rpp3* resistance (Dr. K.S. Pedley, unpublished data). Forward primer 5'-CAAAGATCTCAACATAGTTTCGTTG-3' and reverse primer 5' GAAAGCTTAGTAAGTGTTCCTG-3' produce a PCR product at approximately Gm06: 44,468,577 – 44,469,056 (Glyma.Wm82.a2) that has a fragment size of 474 bp in a susceptible line (Williams 82) and 384 bp in *Rpp3* sources PI 462312, Hyuuga, PI 200487, and PI 471904 (Fig. 3.1). Conversion of this gel-based marker to a KASP marker was accomplished through examination of the sequences of Williams 82 and PI 462312 immediately surrounding the InDel. Sequencher™ 5.1 (Gene Codes, Anna Arbor, MI) was used to align the sequences and select primers. Melting temperature (Tm) was calculated using Primer Express 3.0 (Applied Biosystems, Foster City, CA), internal structures were analyzed with IDT OligoAnalyzer 3.1 (http://www.idtdna.com/calc/analyzer), reverse primers were selected with Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi), and specificity was determined with NCBI Primer Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/).

The KASP marker assay to detect the *Rpp3* deletion was named GSM0551 and used to genotype a panel of resistant and susceptible soybean accessions. These accessions included the 32 major soybean ancestors (Gizlice et al., 1994), 51 PIs with resistance to *P. pachyrhizi* that were mapped to the *Rpp3* locus (Harris et al., 2015), 18 PIs from which mapping populations were derived, and 16 sources of mapped *Rpp* loci. DNA was extracted from young leaf tissue collected from 8 to 15 plants per line (Keim et al., 1988) and diluted to a final concentration of 5 – 25 ng μL^{-1}. The KASP reactions were carried out in the same manner as Pham et al., (2013) and endpoint reading was determined using a Tecan M1000 Pro Infinite Reader (Tecan Group Ltd., Männedorf, Switzerland) with KlusterCaller software (LGC Group, Teddington, UK). In addition, the gel-based marker developed by K.S. Pedley was used to genotype a subset of these lines to verify the KASP marker performance.

SoySNP50K data for the 66 accessions with an *Rpp* gene mapped to the *Rpp3* locus were used to create a relatedness cladogram, as previously described. Accessions with the *Rpp3* deletion found in PI 462312 were color-coded green, and accessions that did not have the deletion, based on the results of GSM0551, were color-coded red. DNA from an additional collection of 345 PIs available in our lab was used to further test the usefulness of GSM0551 as a marker which could be used to identify additional germplasm with the *Rpp3*(PI 462312) allele.

Marker-assisted selection to pyramid* Rpp *loci

Breeding lines with *Rpp* genes in their pedigrees were analyzed with KASP markers to select lines with one or more *Rpp* loci. The *Rpp1* allele originally came from PI 200492 and the *Rpp3* allele came from PI 506764 (Hyuuga). KASP markers flanking *Rpp1* and *Rpp3* were developed in a similar manner as described for GSM0551. However, in this case, polymorphic SNPs were identified by comparing SoySNP50K data (www.soybase.org) for resistant and susceptible parents (if available), and flanking sequences were used to design primers. The SNP markers GSM0422 and GSM0549 were used for MAS of *Rpp1*, GSM0551 (described previously) and the SNP marker GSM0412 was used for MAS of *Rpp3*, and GSM0004 was used for *Rpp5* selection.

Determination of introgression fragment size

Breeding lines with *Rpp* gene introgressions along with their parental lines were fingerprinted with the SoySNP50K iSelect BeadChips. SNPs that were polymorphic between the original PI sources of the *Rpp* locus and all the susceptible parents were extracted from the dataset and used for further analysis. L85-2378, a Williams 82 NIL with an *Rpp1* introgression, was included in the analysis to compare the size of its *Rpp1* introgression to other derived breeding lines with additional generations of introgression.

Homozygous SNP alleles that were derived from the resistant allele donor were coded as "1", heterozygous alleles were coded as "0.5", and alleles derived from the elite parents were coded as "0". Data were then loaded into JMP® Pro 12 (SAS Institute Inc., Cary, NC) and a figure was generated using the Graph Builder function to visualize the introgression region. Since it is expected that a small percentage of SNP alleles could be miscalled on the SoySNP50K iSelect BeadChips, single SNP alleles that appeared to be introgressed were ignored.

The theoretical amount of retained donor genome is expected to equal $\left(\frac{1}{2}\right)^{t+1}$ where *t* is the number of backcross generations (Stam and Zeven, 1981). The equation $\left(\frac{2}{L}\right)\left[\left(\frac{1}{t+1}\right)\left(1-e^{-\frac{(t+1)L}{2}}\right)\right]$ where *t* is the number of backcross generations and *L* is the length of the carrier chromosome in Morgans provides a theoretical estimate of the length of introgressed fragments (Hanson 1959; Muehlbauer et al., 1988; Naveira and Barbadilla, 1992; Stam and Zeven, 1981). These equations were used to determine the theoretical introgression sizes in breeding lines for comparison with actual introgressions measured by fingerprinting SNP data.

Results and discussion

Field and greenhouse screening for SBR, including all Rpp *sources*

The genetic diversity of soybean PIs screened for their resistance to *P. pachyrhizi* can be observed in a dendrogram of relatedness (Fig. 3.2). The four major countries of origin for PIs were Japan, China, Vietnam, and Indonesia. In general, PIs from the same country of origin were grouped together with a few exceptions. Vietnamese accessions tended to be the most scattered. Two major divergent groups were observed: one representing modern cultivars from the USA (on the right side of the dendrogram), and the other (on the left) representing a group of accessions that share the *Rpp7* SNP haplotype found in PI 605823.

Results from diverse germplasm screened in both the greenhouse and field can be seen in Table 3.2. The SBR lesion color reactions of accessions and breeding lines were 92% similar between the field and greenhouse, with the differences likely a result of seed contamination and/or disease escape in the field. Susceptible checks were severely infected with *P. pachyrhizi*, with TAN reactions and high severity and sporulation ratings. The resistant sources mapped to the *Rpp1* locus are separated into two groups – one with an immune reaction and the other with a TAN reaction (Table 3.2). PI 200492 (the original source of *Rpp1*) (Hyten et al., 2007), the G00-3213 *Rpp1* NIL (King et al., 2016), and the Williams 82 *Rpp1* NIL (L85-2378) all carry the same allele and had similar IM reactions. Additional lines had a locus mapped to the *Rpp1* locus, PI 417120, PI 423958, PI 518295 (Harris et al., 2015), and PI 594177 (Yamanaka et al., 2015a), and also had an IM reaction. Since they share the same haplotype with PI 200492 in a 10-SNP window at the *Rpp1* locus, they likely contain the original *Rpp1* allele. Furthermore, using whole-genome SNP marker data, PI 518295 grouped tightly with PI 200492 (the original source of *Rpp1*) and PI 417120, PI 423958, and PI 594177 grouped together separately within the same Japanese clade. The accessions PI 561356, PI 587855, PI 587880A, PI 587886, PI 587905, PI 594760B, and PI 594767A (Garcia et al., 2011; Hossain et al., 2014; Kim et al., 2012; Ray et al., 2009; Yamanaka et al., 2015a, 2016) all had a TAN susceptible reaction similar to PI 594538A, the source of *Rpp1-b*

(Chakraborty et al., 2009). However, while none of these accessions shared the *Rpp1* haplotype of PI 200492, they showed significant haplotype variation at the *Rpp1* locus compared to PI 594538A, indicating that additional *Rpp1* alleles may exist. Furthermore, PI 587855, PI 587880A, PI 587886, PI 587905, PI 594760B, and PI 594767A all grouped together while PI 561356 and PI 594538A (the source of *Rpp1-b*) each come from different phylogenetic clades.

The sources of resistance mapped to the *Rpp2* locus all had an RB reaction with slight sporulation (Table 3.2). The sources of both dominant and recessive alleles at this locus [PI 230970, PI 230971 (the original sources of *Rpp2*), the G00-3213 *Rpp2* NIL (King et al., 2016), PI 224270 (*rpp2*), PI 417125, and PI 417126] all had similar severity and sporulation when inoculated with the GA12 *P. pachyrhizi* isolate. Furthermore, using whole-genome SNP markers, PI 230970 and PI 230971 grouped tightly together, PI 224270 and PI 417126 grouped together, and PI 417125 was separated but still within the Japanese clade.

Most of the accessions possessing a resistance allele at the *Rpp3* locus had excellent resistance, with small RB lesions and very little to no sporulation, but did not cluster tightly together when analyzed with whole-genome SNP markers (Table 3.2; Fig. 3.2). However, PI 416873B, PI 416886, and PI 628932 (Brogin, 2005; Harris et al., 2015) had some plants with a TAN reaction. This may result from seed contamination in the PI source, phenotyping error, or the presence of another allele. Furthermore, PI 567099A, the source of *rpp3* (Ray et al., 2011), had a TAN reaction in both field and greenhouse which provides evidence that this is indeed a different *Rpp* allele than the original source of *Rpp3* (PI 462312).

The sources of *Rpp4* and *Rpp4-b* (PI 459025B and PI 423972) showed similar reactions to *P. pachyrhizi* in Georgia, although PI 423972 (*Rpp4-b*) had slightly reduced sporulation and severity compared to PI 459025B (*Rpp4*) (Table 3.2). The sources of *Rpp5* that also likely contain an *Rpp3* allele (PI 200487, PI 471904, and PI 506764) (Kendrick et al., 2011) had similar reactions as the PI 462312 source of *Rpp3*. *Rpp5* from PI 200526 and *rpp5* from PI 200456 (Garcia et al., 2008) both had a TAN reaction, although PI 200456 had somewhat reduced sporulation compared to PI 200526. PI 200526

consistently had the highest severity and sporulation of any PI screened in the greenhouse, even exceeding the susceptible checks. The three sources (PI 567068A, PI 567102B, and PI 567104B) of resistance mapped to the *Rpp6* locus (King et al., 2015; Li et al., 2012; Liu et al., 2016) had equally good resistance and no sporulation was observed. However, PI 567068A had a nearly immune reaction and grouped separately when analyzed with whole-genome SNP markers, while PI 567102B had a light-colored hypersensitive lesion reaction, and PI 567104B had an RB response and both grouped together using whole-genome SNP markers.

Of the 82 PIs from China screened for SBR reaction, only nine had a resistant reaction. Of these, four were identified by their identical haplotype to PI 605823 at the *Rpp7* locus (Childs et al., 2017). The additional five resistant PIs are of special interest since only PI 459025B (*Rpp4*) and the sources of *Rpp1-b* resistance have originated from China, and none of these PIs grouped together or with PI 459025B using whole-genome SNP markers, suggesting that they may be unique sources of resistance. Of the 8 PIs from Indonesia screened, seven were found to be resistant and all grouped together using whole-genome SNP markers. These may not be novel *Rpp* alleles as many PIs from Indonesia have been found to have resistance at the *Rpp3* and *Rpp6* loci. Of the 70 PIs from Japan screened, 40 were resistant. Many *Rpp* alleles (*Rpp1, Rpp2, rpp2, Rpp3, Rpp4-b, Rpp5, rpp5*), have originated from Japan and at least 28 Japanese PIs have resistance at the *Rpp3* locus, but resistant accessions did not group tightly together when analyzed with whole-genome SNP markers (Fig. 3.2) (Harris et al., 2015). Of the 47 PIs from Vietnam screened, 27 were resistant but these also did not group tightly together with whole-genome SNP marker analysis. At least 12 accessions from Vietnam have resistance mapped to the *Rpp3* locus, and PI 605823, the source of the recently mapped *Rpp7* locus, also originated in Vietnam.

Representative lesions illustrating the reactions of the major sources of *Rpp* genes to greenhouse assays with the GA12 *P. pachyrhizi* isolate are pictured in Fig. 3.3. The reactions were similar to what was observed in previous reports (Walker et al., 2014b). Both *Rpp1* and *Rpp6* provide a nearly immune or

hypersensitive response. *Rpp3* and *Rpp?(Hyuuga)* produce an RB lesion, and *Rpp7* provides an exceptionally dark-brown RB lesion. *Rpp2* and *Rpp4* show intermediate RB lesions with sporulation, and *Rpp5* has a TAN reaction.

Screening was completed for 132 breeding lines developed at the University of Georgia with *Rpp1* or *Rpp3* sources in their pedigrees (Table 3.3). Two pedigrees had either *Rpp1* or *Rpp3* resistant sources and two pedigrees contained both *Rpp1* and *Rpp3* resistance sources in their pedigrees. Screening results had approximately 92% agreement with molecular marker results (data from two markers for both *Rpp1* and *Rpp3*). The main discrepancy came from disease escapes in the field or from breeding lines for which the *Rpp* alleles were not fixed in the population and resulted in mixed TAN/RB results when the marker results indicated homozygosity. Breeding lines with *Rpp* introgressions had similar results compared to the original sources of resistance.

Some crosses produced more resistant lines than others. This is not surprising since selection for *Rpp* alleles was performed using SSR markers that were not tightly-linked to the resistance loci (data not shown) and some genotyping errors are expected. In particular, no *Rpp3* resistant lines were identified in the [P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)] pedigree and no *Rpp1* resistant lines were identified in the {G00-3213(3)RR2Y x [G00-3213(2) x [G00-3209 x G01-PR68(*Rpp3)*]]} x {G00-3213(3) x [P97M50(3) x L85-2378(*Rpp1*)]} pedigree. As a result, no stacked lines were identified in these populations. This is similar to the results of Diers et al., (2013), where *Rpp3* was lost when attempting to pyramid *Rpp1* and *Rpp3*.

The Japanese soybean cultivar Hyuuga (PI 506764) has an *Rpp* allele at both *Rpp3* and *Rpp5* loci (Kendrick et al., 2011). Hyuuga was used as the source of *Rpp3* in most of the breeding lines analyzed in this study. A surprising result of our attempts to pyramid *Rpp1* and *Rpp3* loci was the introgression of the *Rpp5* allele from Hyuuga into lines with *Rpp1* resistance. This likely occurred through phenotypic selection of resistant breeding lines, and may indicate that *Rpp5* contributed to the resistance phenotype.

The presence of the *Rpp5* introgression was verified through the SNP marker GSM0004, which is located within the *Rpp5* locus (Fig. 3.4 and Table 3.4), and was further confirmed through phenotyping with the nine *P. pachyrhizi* isolates (Table 3.5) and fingerprinting with the Soy50kSNP iSelect BeadChips (Fig. 3.5e). Although the inclusion of both *Rpp1* and *Rpp5*(Hyuuga) in a breeding line did not affect the SBR reaction, the pyramided line may be useful to protect soybean production from potential changes in *P. pachyrhizi* populations.

Reaction of breeding lines and PIs to a panel of diverse* P. pachyrhizi *isolates

Challenging breeding lines with the nine *P. pachyrhizi* isolates confirmed the marker and screening data (Table 3.5). The nine isolates (Table 3.1) used in this study represent some of the world's diversity of soybean rust populations. The *Rpp1* introgressions provided resistance against IN73-1, LA04-1, HW94-1, and SA01-1. Breeding lines having *Rpp1* stacked with the *Rpp5* allele from Hyuuga provided additional resistance against AU79-1, CO04-2, VT05-1, and ZM01-1 (Table 3.5). This is similar to the *Rpp3* + *Rpp5* resistance found in Hyuuga but the *Rpp1* + *Rpp5*(Hyuuga) lines have a nearly immune reaction against IN73-1, LA04-1, and HW94-1 in contrast to the weaker RB resistance found in Hyuuga. The *Rpp3* gene introgressed from Hyuuga [when separated from *Rpp5*(Hyuuga)] provided the same isolate pattern as PI 462312 (the original source of *Rpp3*), which provides evidence that *Rpp3* and *Rpp?(Hyuuga)* are likely the same allele. Interestingly, the breeding lines with only *Rpp5*(Hyuuga) have a completely different isolate pattern than (*Rpp5*)PI 200526. They are also different from (*rpp5*)PI 200456, as PI 200456 has a mixed reaction to CO04-2, VT05-1, and ZM01-1 in contrast to *Rpp5*(Hyuuga)'s RB response. To our knowledge, this is the first time that *Rpp5*(Hyuuga) has been separated from *Rpp3* and characterized.

Challenging resistant mapping population parents with the nine *P. pachyrhizi* isolates provided insights into their putative *Rpp* alleles (Table 3.6). PI 224270 and PI 417126 showed similar reactions to isolates CO04-2 and VT05-1 that differed from PI 230970 and PI 417125, even though these PIs have an *Rpp2* allele. An accession (PI 423960B) with Hyuuga-like resistance was identified. Two accessions (PI

417208 and PI 567189) had similar reactions as *Rpp3.* In addition, three accessions mapping to the *Rpp6* locus had reactions same as *Rpp(PI567068A).* Five additional accessions had unique reactions that resemble the *Rpp3* reactions but did not fully match and these should be investigated further.

Bulked segregant analysis results

The putative genomic regions providing resistance in 19 mapping populations are listed in Table 3.7. In addition, results are included from 1) characterization with the nine *P. pachyrhizi* isolates (Table 3.6), 2) haplotype analysis by comparing the SoySNP50K SNP alleles within each mapped *Rpp* locus to the original sources of *Rpp* resistance, and 3) analysis with an *Rpp3* marker to detect the resistance allele found in PI 462312 (Table 3.8). The five populations that were analyzed through BSA in 2016-2017 are highlighted in bold print and also pictured in Fig. 3.6.

Three accessions (PI 566956, PI 566984, and PI 567073A) appear to have an allele at the *Rpp6* locus which is likely same as the *Rpp(PI567068A)* allele, as mentioned above. PI 566984 also had BSA hits near the *Rpp4* locus and appeared to have a 2-gene segregation ratio but did not show a different isolate pattern than other accessions. Three accessions (PI 200466, PI 416935, and PI 567191) have resistance clearly mapping to the *Rpp3* locus. However, each had unique patterns of resistance to the nine *P. pachyrhizi* isolates compared to PI 462312, the original source of *Rpp3.* Also, PI 567191 had a different haplotype and marker result than PI 462312 so these could be different alleles. Another accession, PI 567189A, had the same isolate pattern and marker results as *Rpp3*(PI 462312) but had ambiguous BSA results and a different haplotype. Several PIs (PI 203398, PI 379621, PI 417208, PI 423960B, PI 423963, and PI 567061) had ambiguous BSA results but their haplotype and marker genotypes indicate they likely have the *Rpp3* allele found in PI 462312. PI 567061 may have an additional *Rpp* locus and PI 423960B likely had the same *Rpp3* + *Rpp5* stack found in Hyuuga. Two accessions mapping to the *Rpp2* locus (PI 417125 and PI 417126) appear to have the *rpp2* allele found in PI 224270, although PI 417125 has a slightly different isolate pattern but the same *rpp2* haplotype.

Development of a robust* Rpp3 *marker from PI 462312

The development of KASP marker GSM0551 resulted from the discovery of a deletion in *Rpp3* candidate gene, Glyma06g40740 (Fig. 3.1). Primer sequences of GSM0551 are found in Table 3.4 and marker performance across a panel of resistant and susceptible soybean lines is illustrated in Fig. 3.7. The cluster with the wild-type allele contained samples from susceptible soybean ancestral lines and sources of *Rpp* alleles found at the loci other than *Rpp3*. It also contained some resistant accessions which do not have this deletion and could have potentially novel alleles at the *Rpp3* locus. The cluster with the mutant allele (deletion) consisted of the samples from PI 462312, Hyuuga, and 54 other *Rpp3* lines that appear to have the same allele as PI 462312, the original source of *Rpp3*.

A third intermediate cluster with low amplification was found between the two clusters with WT and MUT alleles and contained only susceptible lines or *Rpp* sources from non-*Rpp3* loci (with the exception of PI 567099A, the source of *rpp3*). This unexpected observation may result from sequence variants in these lines that allowed limited amplification of both mutant and wild-type primers in this multiple-sequence repeat region. Additional evidence for widespread sequence changes within these lines can be found by observing the performance of the original gel-based marker (Fig. 3.1). All the lines tested that produced an intermediate cluster with GSM0551 failed to produce any PCR product band at either the wild-type or mutant cluster position. This may have resulted from a large deletion of the entire region, or is more likely the result of sequence changes that do not allow the gel-marker primers (designed for Williams 82 and PI 462312) to bind and form a product.

Of 66 *Rpp3* sources genotyped with GSM0551, it is interesting to observe that 12 sources appear not to contain the same *Rpp3* allele as found in PI 462312 (Table 3.8). There is no apparent difference in resistance to the GA12 *P. pachyrhizi* isolate between the sources of the putatively different *Rpp3* alleles. However, all the accessions with a putative novel *Rpp3* allele originated from Vietnam. These individuals from Vietnam also grouped together using whole-genome SNP marker analysis (Fig. 3.8), indicating that this putative novel *Rpp3* allele likely arose independently of the *Rpp3* allele found in PI 462312. Further

characterization of the resistance of these two groups of lines against additional isolates of *P. pachyrhizi* could provide additional evidence of allelic diversity. Sequencing the *Rpp3* region of these lines could provide insight into the differences between these two groups.

GSM0551 was also used to genotype a diverse collection of 345 PIs, with largely unknown resistance, to detect the *Rpp3* allele found in PI 462312. The intermediate marker cluster was found in 306 lines and 29 lines grouped in the wild-type cluster (data not shown). Only 13 PIs grouped in the mutant allele cluster, in addition to PI 462312 and four other *Rpp3* resistant controls (Table 3.9). Nine of these accessions originated from Japan, two from Africa, one from India, and one from China. PI 416873A and PI 417389A from Japan showed some resistance in the SBR germplasm screen by Miles et al., (2006). These 13 PIs could be further screened with a *P. pachyrhizi* isolate to verify that they provide an *Rpp3*-resistance reaction as expected.

Fig. 3.9 shows the performance of GSM0551 to select breeding lines with *Rpp3*. When used for selection in this population (Table 3.10), the heterozygous samples did not separate clearly from the mutant allele but were grouped together. Samples with heterozygous alleles appear to amplify similarly as the homozygous mutant alleles in this population and *Chi*-square analysis indicated that the marker alleles fit a 3:1 ratio (p=0.46). Although this KASP marker is useful to select *Rpp3* resistance in breeding lines, the use of an additional flanking marker is recommended (Fig. 3.9).

Marker-assisted selection to pyramid* Rpp1 *and* Rpp3 *resistance alleles

Efforts to pyramid *Rpp1* and *Rpp3* alleles into breeding lines failed in the pedigrees listed in Table 3.3. However, an $F_{2:3}$ population was available from a cross between breeding lines G12-6295 and G12-6518, which were homozygous for *Rpp1* and *Rpp3* introgressions, respectively. A total of four KASP markers linked to *Rpp1* and *Rpp3* were used to genotype 200 breeding lines from this population (Fig. 3.9, 3.10; Table 3.10). *Rpp1* was homozygous in 32 breeding lines, *Rpp3* was homozygous in 41 breeding lines, and an *Rpp1* + *Rpp3* stack was homozygous in 11 lines. This population was grown in

2015, when excessive rain before harvest caused poor seed quality. Consequently, less than six plants were sampled for genotyping in some breeding lines. These marker results will be confirmed and greenhouse screening will be performed after seed increase for these lines.

This *Rpp1* and *Rpp3* pyramid will be useful in the USA, as *Rpp1* and *Rpp3* are some of the most effective resistance genes for soybean rust in this region (King et al., 2016; Walker et al., 2015). When deployed on a field scale, the presence of multiple resistance genes should reduce the incidence of the pathogen overcoming resistance. Since *Rpp1* already provides an immune reaction, it is not possible to detect any advantage of the gene combination when using the GA12 *P. pachyrhizi* isolate for inoculation. Challenging these pyramided lines with a diverse collection of 24 purified isolates (Paul et al., 2015) collected in the USA may be more informative.

Characterization of fragment sizes

To estimate the effectiveness of backcrossing to eliminate exotic genomic content when introgressing *Rpp* loci, nine breeding lines and four NILs were fingerprinted with the SoySNP50K iSelect BeadChips (Table 3.11). Multiple generations of introgression were performed, for example by backcrossing *Rpp1* into Williams 82 (L85-2378) and then backcrossing *Rpp1* from this source into the later-maturity cultivar G00-3213 (G00-3213 *Rpp1* NIL). The pedigrees also contain multiple elite parents used in forward breeding. Since the *Rpp* locus was selected with molecular markers in each generation, all crosses to elite lines could be viewed as generations of introgression and were added together for analysis. Using this approach, breeding lines with *Rpp* loci in this study underwent 4 to 14 generations of introgression (Table 3.12).

Based on analysis of SNP data across the genome, the amount of exotic genome that was not linked to the *Rpp* introgressions but was retained in the breeding lines is listed in Table 3.12. A pictorial illustration of these introgressions is also provided in Fig. 3.11. Table 3.12 lists the theoretical sizes for comparison to the actual introgressions estimated in this study. On average, the actual and theoretical values are similar, although some lines such as G12-6536 have less retained introgressions then expected,

likely due to selection for desirable traits during the breeding process. The only exception is G13-2083R2, for which marker data were unavailable for the elite parent G09PR-54457R2, and the size of its exotic introgressions is overestimated. These data indicate the usefulness of fingerprinting breeding lines at each backcross to provide background selection to identify the most elite lines at each generation.

The sizes of the introgressed genomic fragments linked to the *Rpp* loci and the theoretical introgressions of breeding lines are listed in Table 3.12 for comparison with the actual fragment sizes estimated from fingerprinting data. Pictorial illustration of fragment sizes is also provided in Fig. 3.5. Fragment sizes for *Rpp1, Rpp4,* and *Rpp5*(Hyuuga) introgressions were similar for theoretical and actual values and ranged from 1.2 to 6.4 Mb. However, actual fragment sizes for *Rpp2* and *Rpp3* lines were much larger than expected (average of 26.34 Mb actual compared to 6.87 Mb theoretical). Since the equation calculation takes into account the number of backcrosses, this factor is not driving the difference between *Rpp1, Rpp4,* or *Rpp5*(Hyuuga) and *Rpp2* and *Rpp3* fragment size. A more plausible explanation is that *Rpp2* and *Rpp3* are relatively closer to the centromere than *Rpp1* and *Rpp4*, which would limit the amount of recombination in the region. However, the *Rpp5*(Hyuuga) location is also closer to the centromere than *Rpp1* and *Rpp4* and its fragment size is only 3.7 Mb (±2.9 Mb) (Fig. 3.5). Further study could be done to explain this anomaly.

Yield data from 2015 was available for nine breeding lines (Table 3.11). Yield was based on three locations in Georgia with three replications per location and data were listed as a percentage of the highest yielding check cultivar. The average yield of the breeding lines with *Rpp* introgressions was 96.4% of the highest check (from 88.6% to 109.2%). The average yield of lines with *Rpp1* introgressions showed a 2.3% yield reduction compared to a 4.7% reduction in the lines with *Rpp3*. However, this difference was not significant (t-test, p=0.31) and may not be correlated with the smaller average fragment size of *Rpp1* compared to *Rpp3* (Fig. 3.5a, c), although a smaller fragment size of genomic content from unadapted germplasm is expected to result in higher yield.

It is interesting to observe that the *Rpp1* fragment size for L85-2378 (included in Fig. 3.5a for comparison) was not substantially different from that of the G00-3213 *Rpp1* NIL which has 14 generations of breeding to elite parents compared to only five generations for L85-2378. This highlights the limitations of backcrossing in reducing the introgressed fragment size, when only phenotypic or marker selection for the trait is performed. Clearly, additional recombination within the fragment will occasionally occur, as represented by G14-1148RR (Fig. 3.5a), where the fragment size was reduced from approximately 2.6 Mb to 1.2 Mb. The use of molecular markers at the flanking regions of the resistance locus to identify recombinant individuals could drastically reduce the fragment size and should be performed more widely (Hospital, 2001; Young and Tanksley, 1989).

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Figures and tables

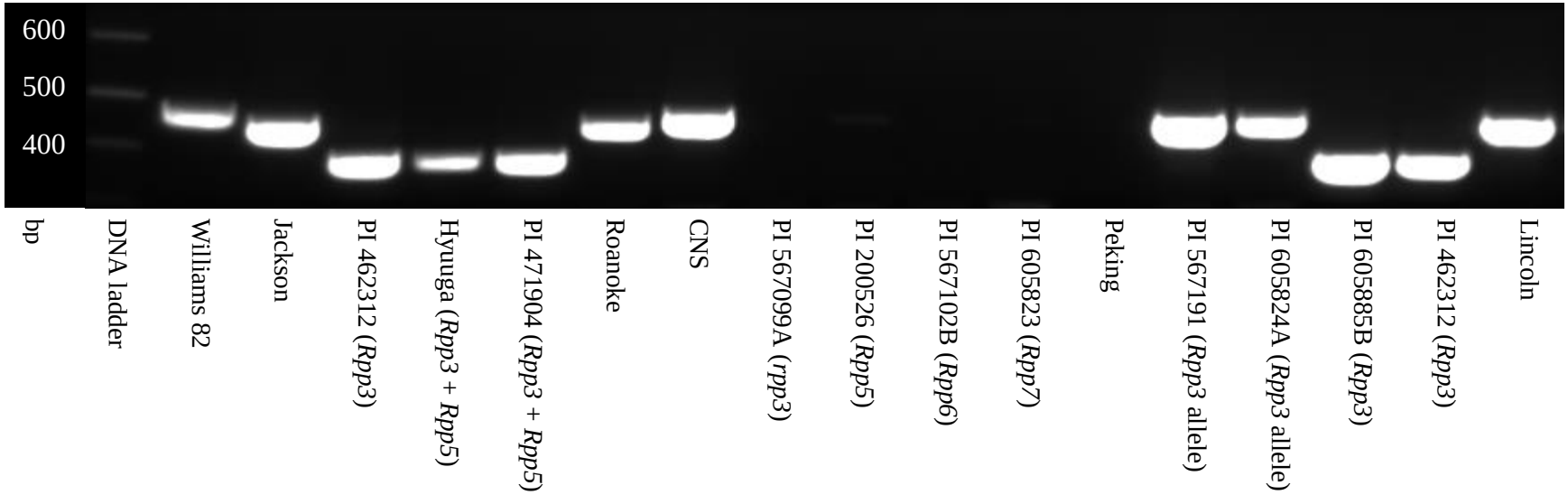


Fig. 3.1 Gel image indicating the size of PCR products amplified for the InDel region within the *Rpp3* candidate gene Glyma06g40740 among 17 selected lines. The upper fragment (wild-type allele) is 474 bp and the lower fragment (mutant allele) is 384 bp. Some samples produced no PCR product.

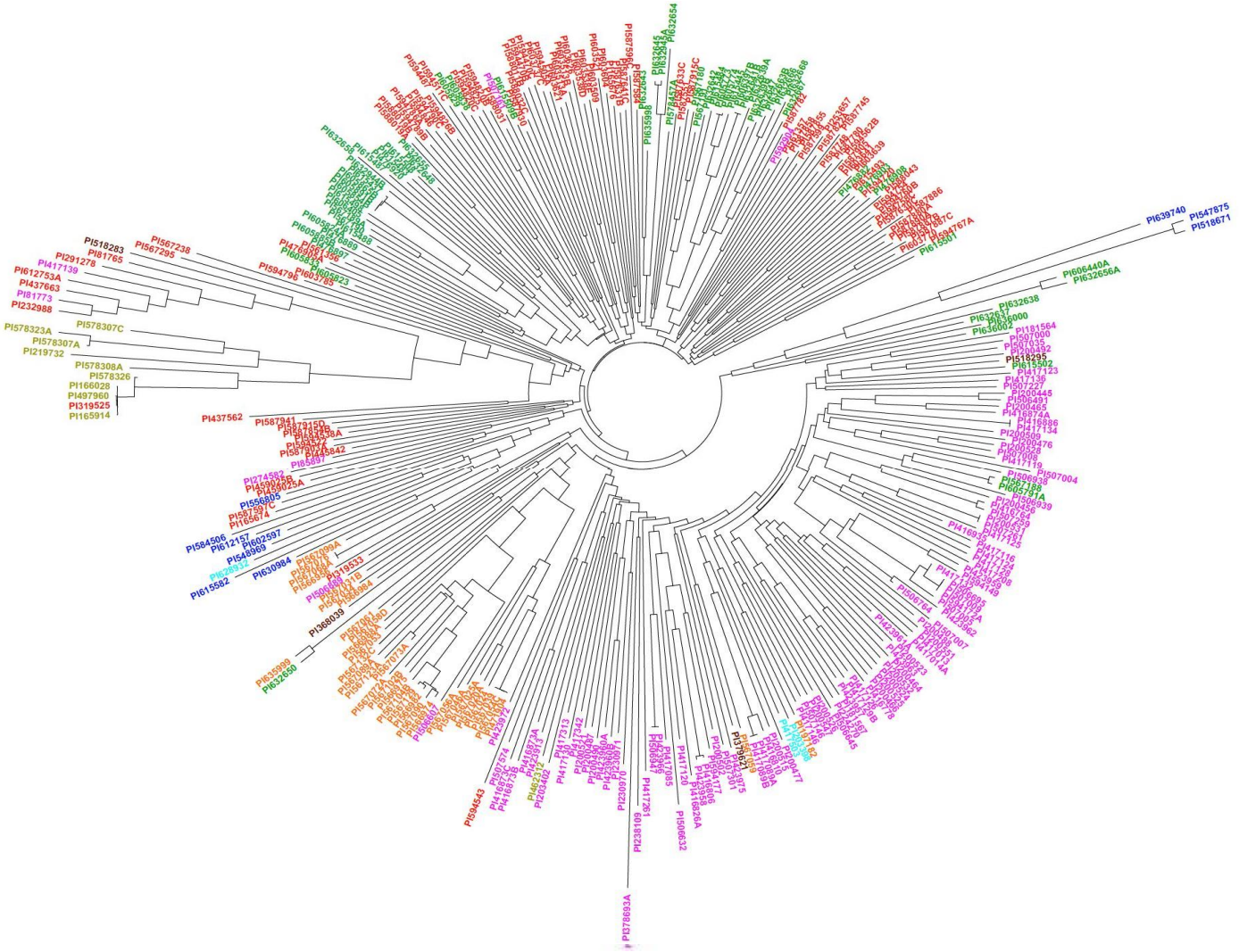


Fig. 3.2 D**endrogram of 325 soybean accessions challenged with *P. pachyrhizi* in the field and greenhouse in this study based on whole-genome analysis**. The figure was created using SoySNP50K data. Accessions were color-coded by country of origin: Vietnam (green); China (red); Japan (pink); Indonesia (orange); Nepal, Pakistan, or India (greenish-yellow); Taiwan (brown); USA (dark blue); and Brazil (light blue).

Fig. 3.3 SBR lesions of the major resistance sources inoculated with the GA12 *P. pachyrhizi* isolate. **a** Williams 82 (TAN reaction of susceptible check), **b** 5601T (TAN reaction of susceptible check), **c** PI 200492 (IM reaction), **d** PI 230970 (RBSP reaction), **e** PI 462312 (RB reaction), **f** Hyuuga (RB reaction), **g** PI 459025B (RBSP/TAN reaction), **h** PI 200526 (TAN reaction), **i** PI 567102B (HR reaction), and **j** PI 605823 (RB reaction). *Bar* = 1mm

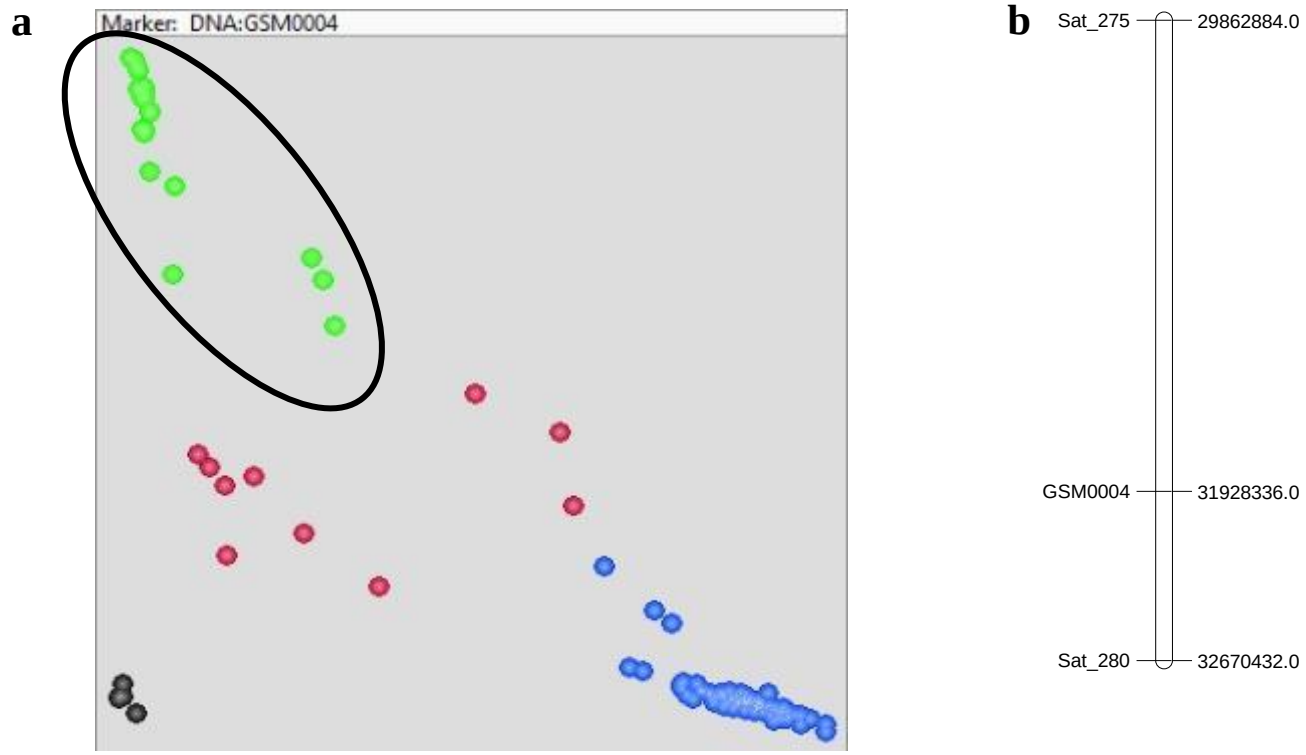
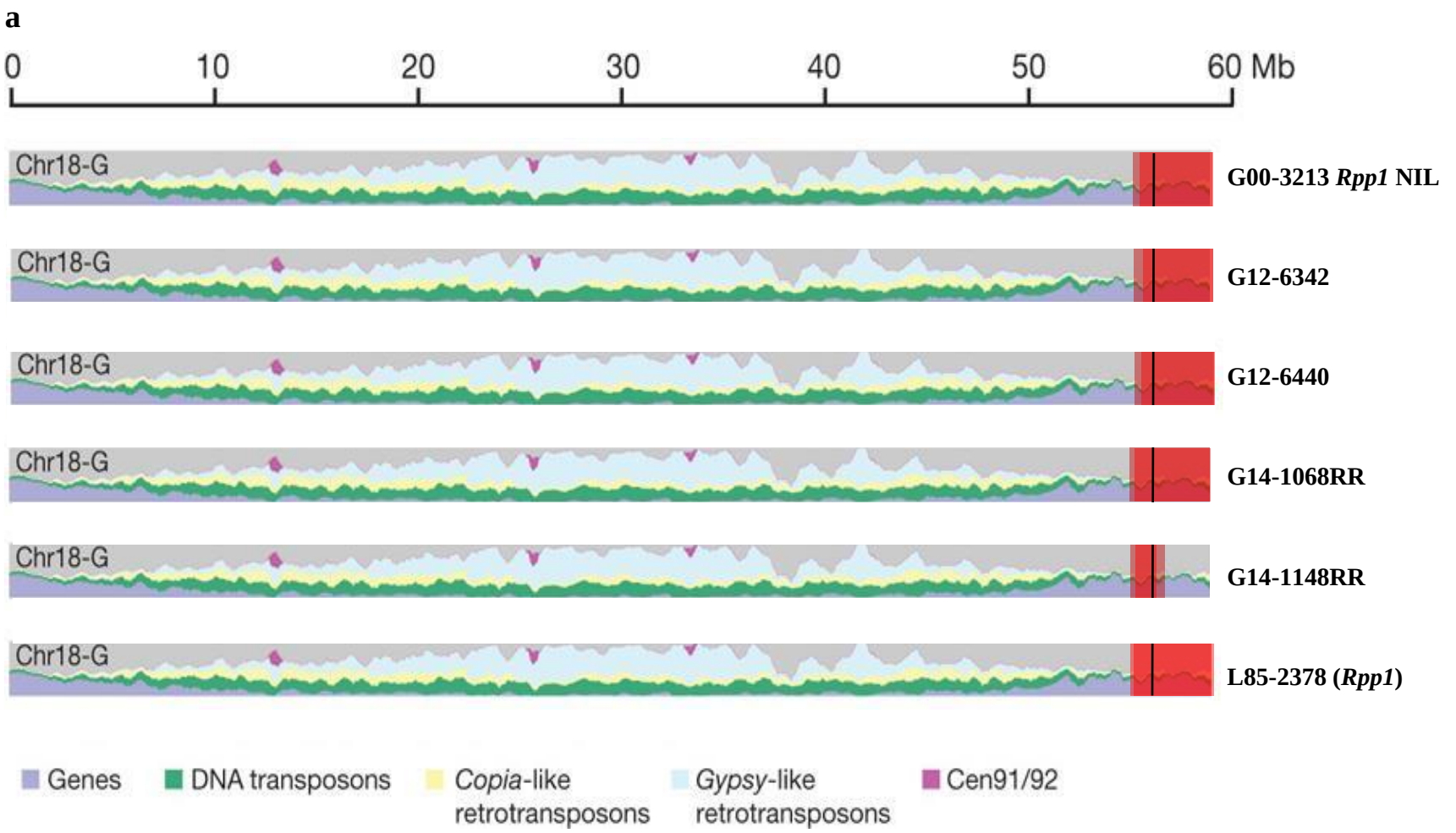
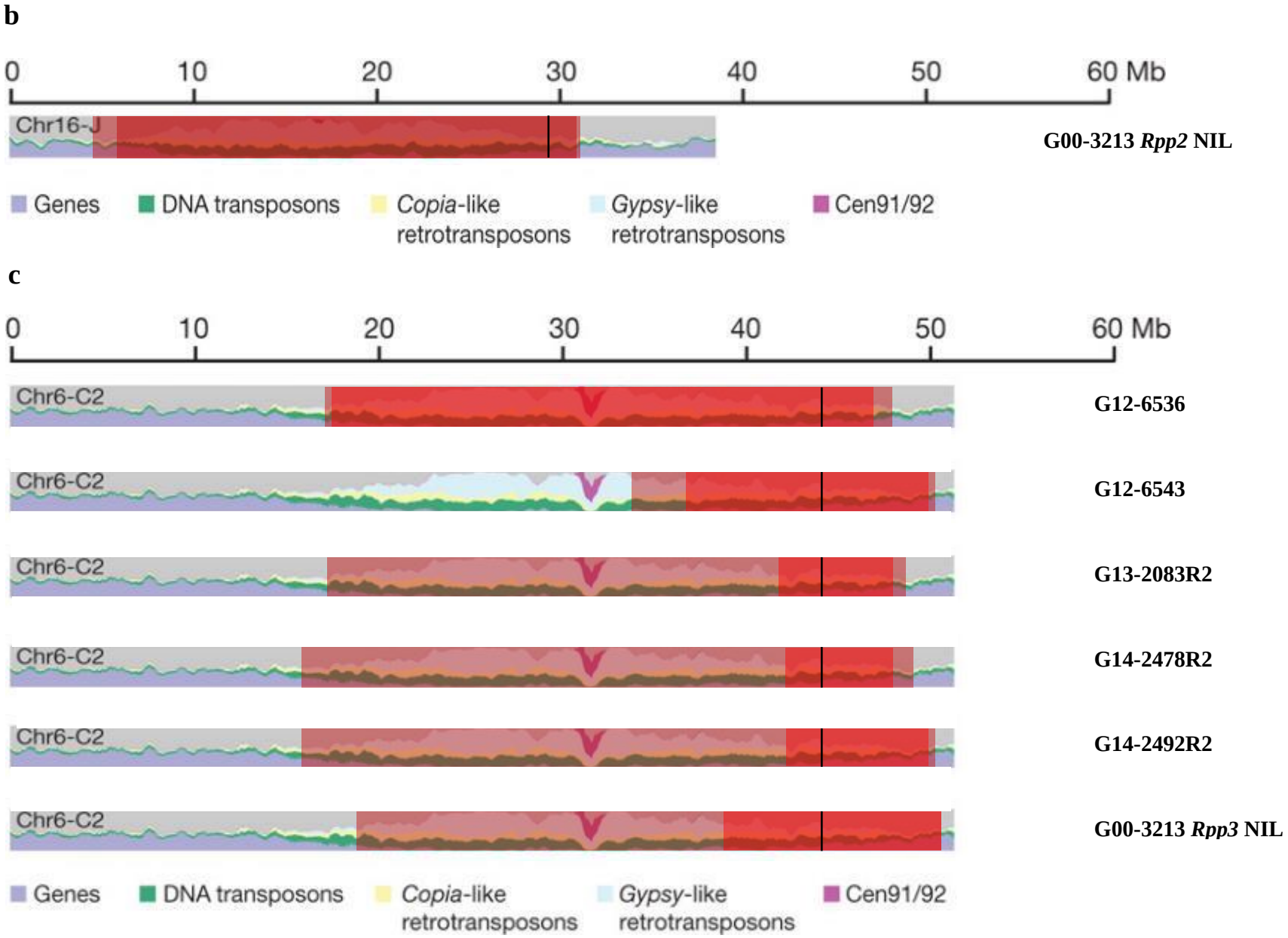


Fig. 3.4 SNP graph of GSM0004 at the *Rpp5* locus. **a** The circled samples contain the SNP allele from Hyuuga. **b** The *Rpp5* locus defined by the SSR markers used for mapping the *Rpp* gene in PI 200526 (Garcia et al., 2008). The chromosome figure was created with MapChart (Voorrips, 2002) with marker locations from SoyBase (www.soybase.org).


a
0
10
20
30
40
50
60 Mb
Chr18-G
G00-3213 Rpp1 NIL
Chr18-G
G12-6342
Chr18-G
G12-6440
Chr18-G
G14-1068RR
Chr18-G
G14-1148RR
Chr18-G
L85-2378 (Rpp1)
Genes
DNA transposons
Copia-like retrotransposons
Gypsy-like retrotransposons
Cen91/92


b
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Chr16-J
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Genes
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Gypsy-like retrotransposons
Cen91/92
c
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30
40
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60 Mb
Chr6-C2
G12-6536
Chr6-C2
G12-6543
Chr6-C2
G13-2083R2
Chr6-C2
G14-2478R2
Chr6-C2
G14-2492R2
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Cen91/92

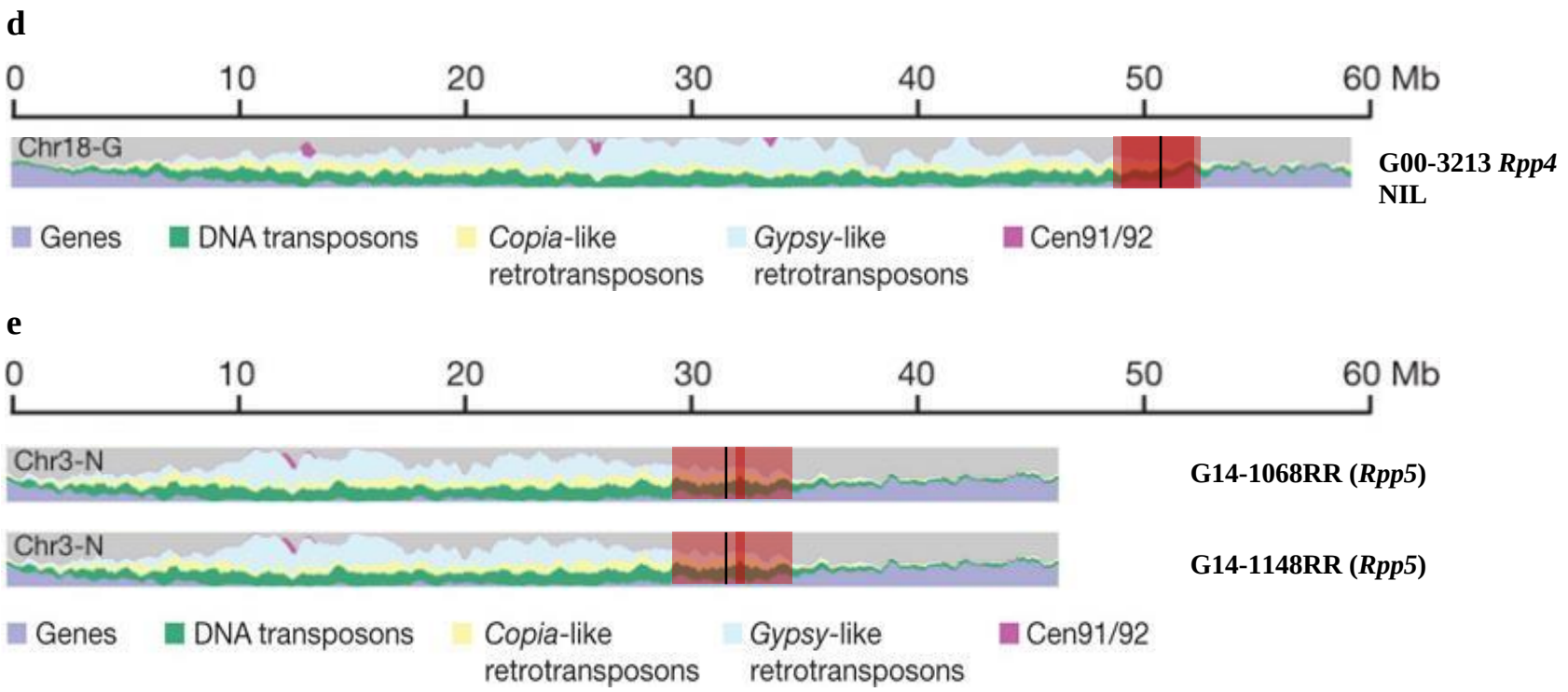

Fig. 3.5 *Rpp* gene fragment introgressions. **a** *Rpp1*, **b** *Rpp2*, **c** *Rpp3*, **d** *Rpp4*, and **e** *Rpp5*. The gray bar represents the entire chromosome and the red coloration represents a region where SNP alleles originated from an *Rpp* donor. The lighter red indicates regions where the introgression may extend but cannot be determined due to a lack of polymorphic markers. The vertical black bar indicates where the *Rpp* gene is located. Chromosome template is from Schmutz et al., 2010.

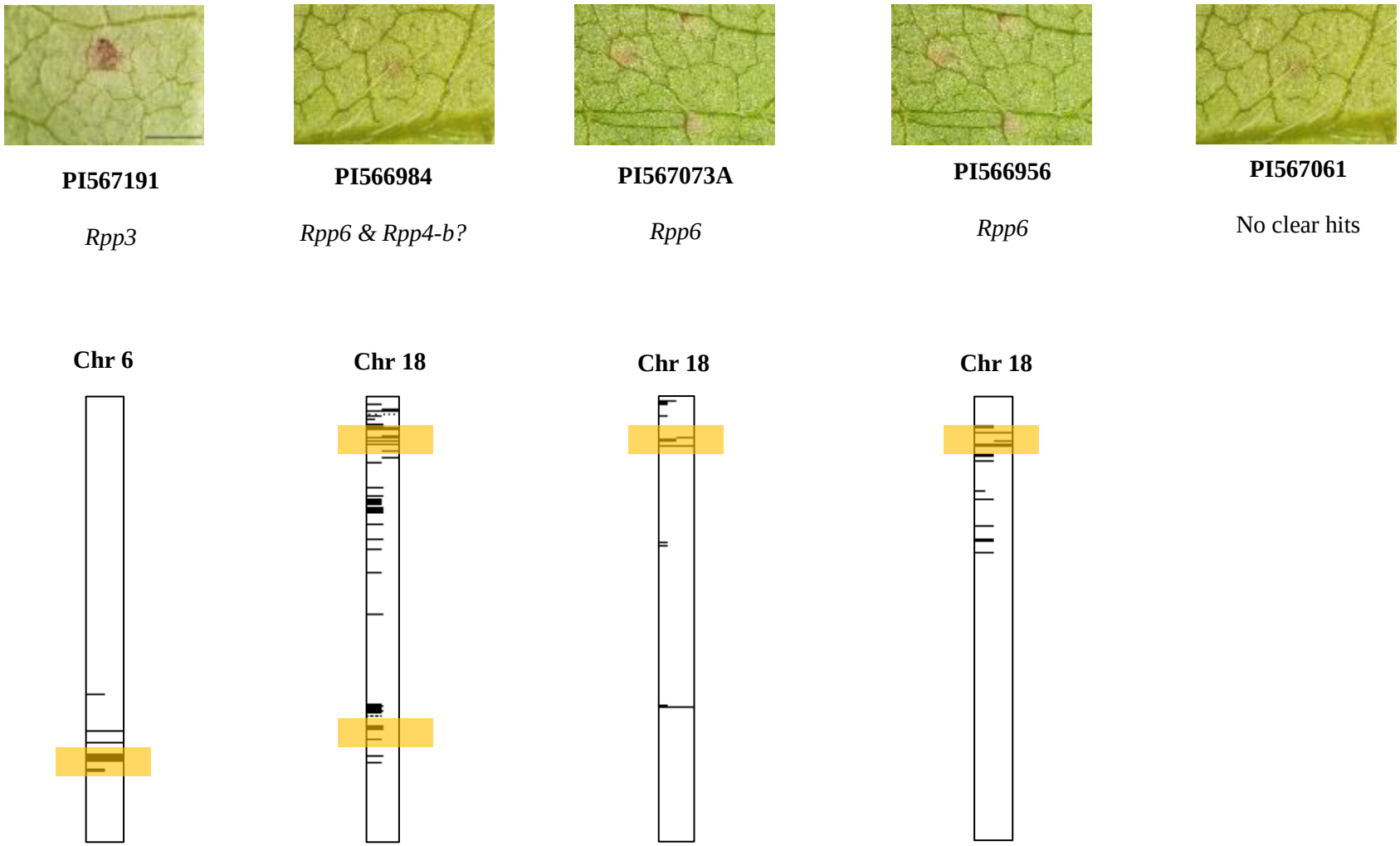

Fig. 3.6 Bulked segregant analysis (BSA) performed in 2016-2017 for 5 mapping populations. The *Rpp* loci listed are the likely *Rpp* alleles found in the resistant parents. The chromosome images show long black horizontal bars where "strong" BSA hits were found and short black bars where "weak" BSA hits were found. The yellow boxes highlight the genomic locations of previously mapped *Rpp* loci annotated for reference. The lesion images represent the reaction phenotype of the resistant parents to greenhouse inoculation with the GA12 *P. pachyrhizi* isolate.

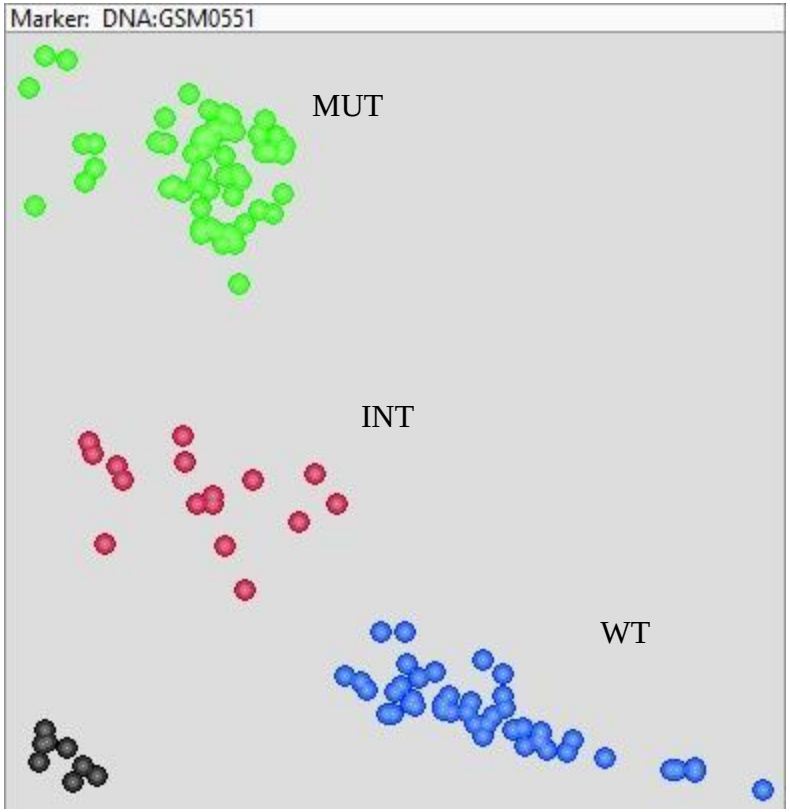

Fig. 3.7 KASP marker GSM0551 developed around an indel within the *Rpp3* candidate gene Glyma06g40740. The WT and INT clusters have a different *Rpp3* allele than that found in PI 462312. Samples in the MUT position have the same *Rpp3* allele as PI 462312.

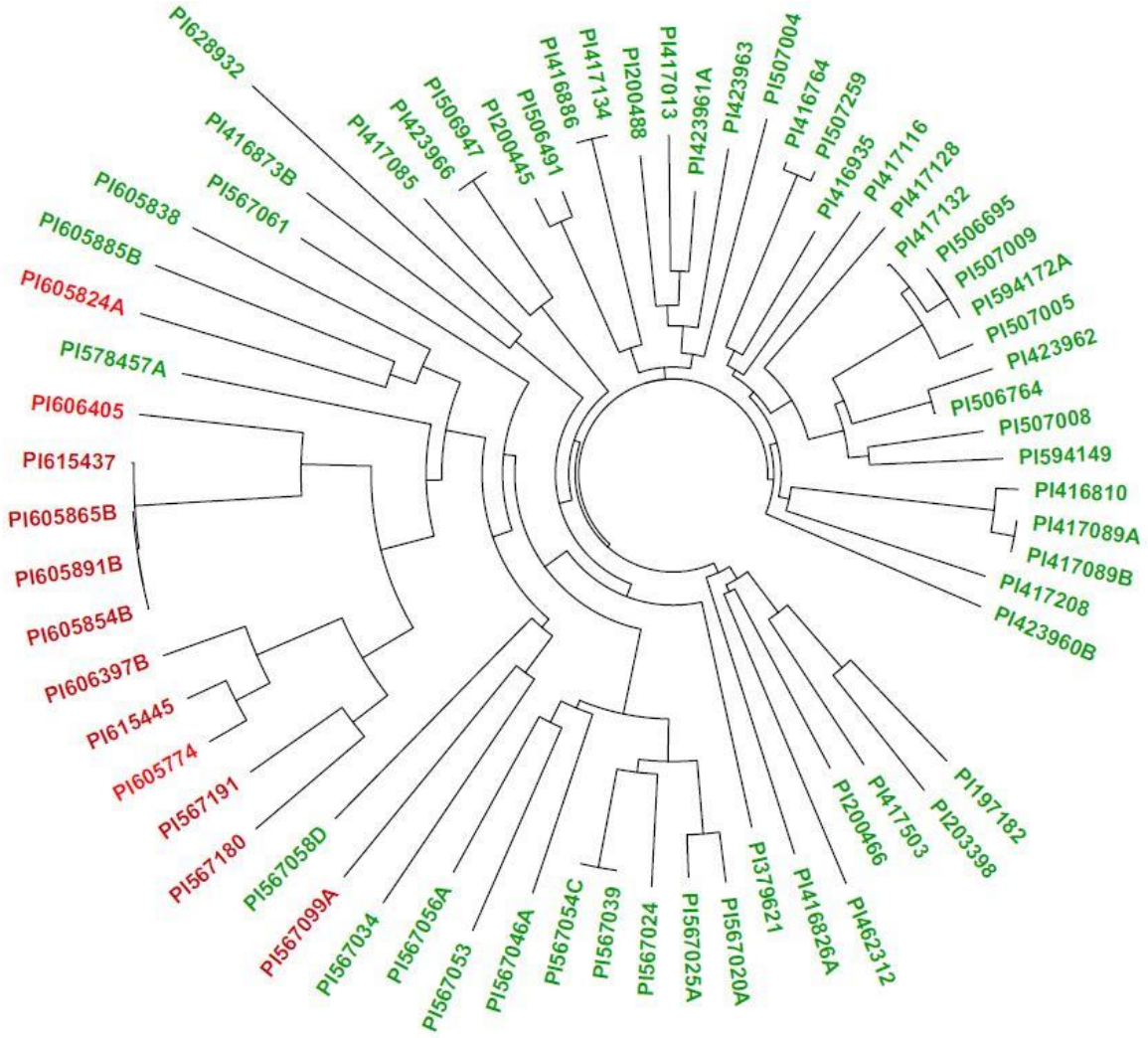

Fig. 3.8 Dendrogram of 66 soybean accessions with *Rpp* genes mapped to the *Rpp3* locus using SoySNP50K markers. Accessions in green have the same GSM0551 (*Rpp3*) marker allele as PI 462312. Red indicates accessions that do not share the same GSM0551 marker allele as PI 462312. All the red-coded accessions were also collected from Vietnam (except for PI 567099A, the source of *rpp3*, collected in Indonesia).

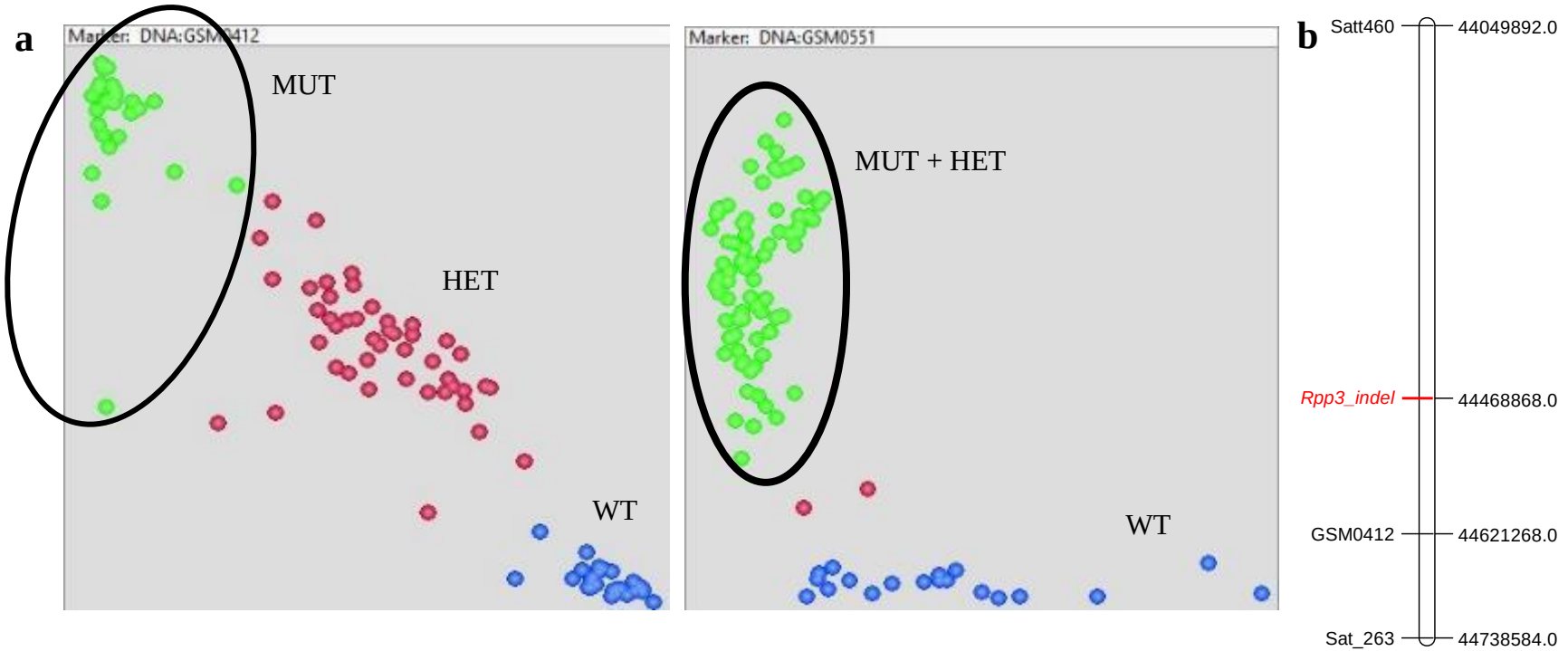

Fig. 3.9 SNP graphs of GSM0412 (left) and GSM0551 (right) at the *Rpp3* locus. **a** The circled samples contain the marker allele from Hyuuga, although the MUT and heterozygous alleles are clustered together for GSM0551. **b** The *Rpp3* locus defined by the SSR markers used for mapping the *Rpp* gene in PI 462312 (Hyten et al., 2009). The chromosome figure was created with MapChart (Voorrips, 2002) with marker locations from SoyBase (www.soybase.org).

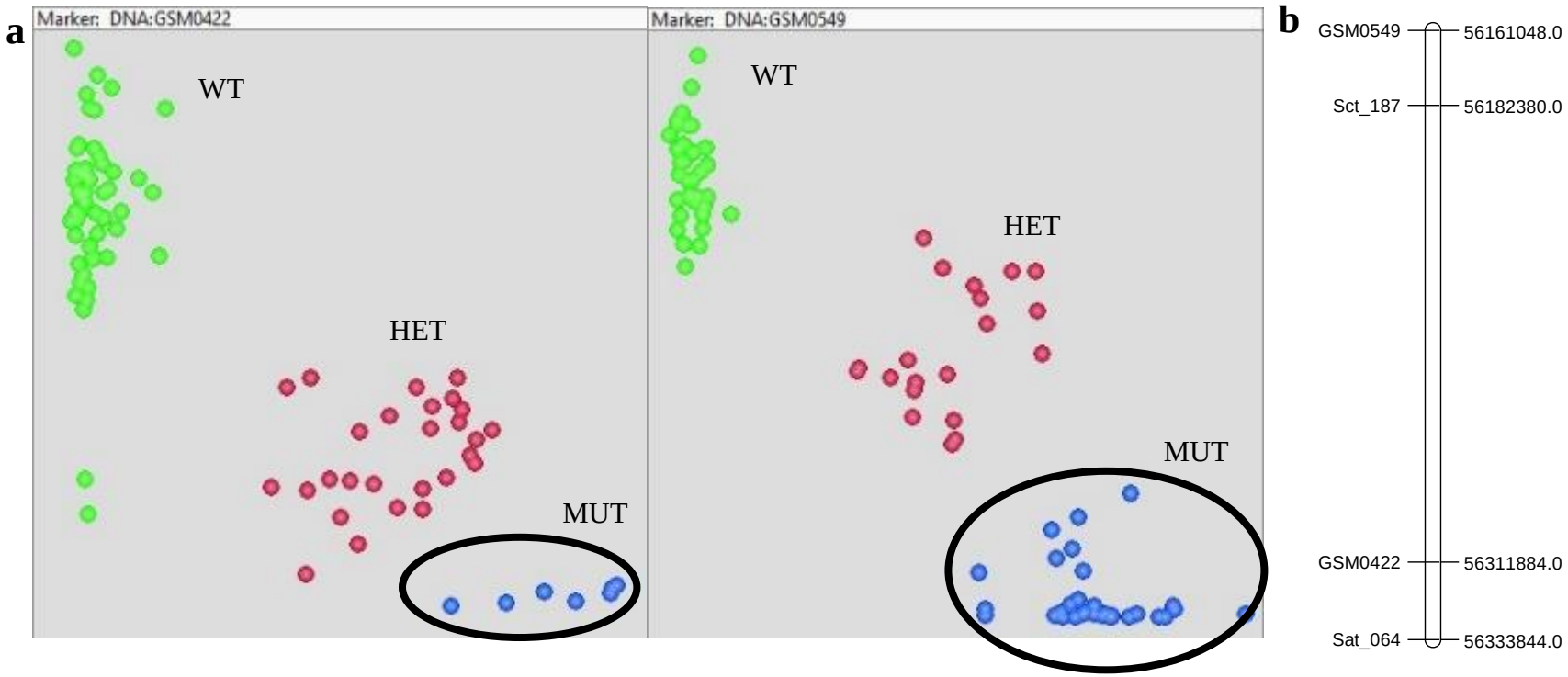

Fig. 3.10 SNP graphs of GSM0422 and GSM0549 at the *Rpp1* locus. **a** The circled samples contain the marker allele from PI 200492. **b** The *Rpp1* locus defined by the SSR markers used for mapping the *Rpp* gene in PI 200492 (Hyten et al., 2007). The chromosome figure was created with MapChart (Voorrips, 2002) with marker locations from SoyBase (www.soybase.org).

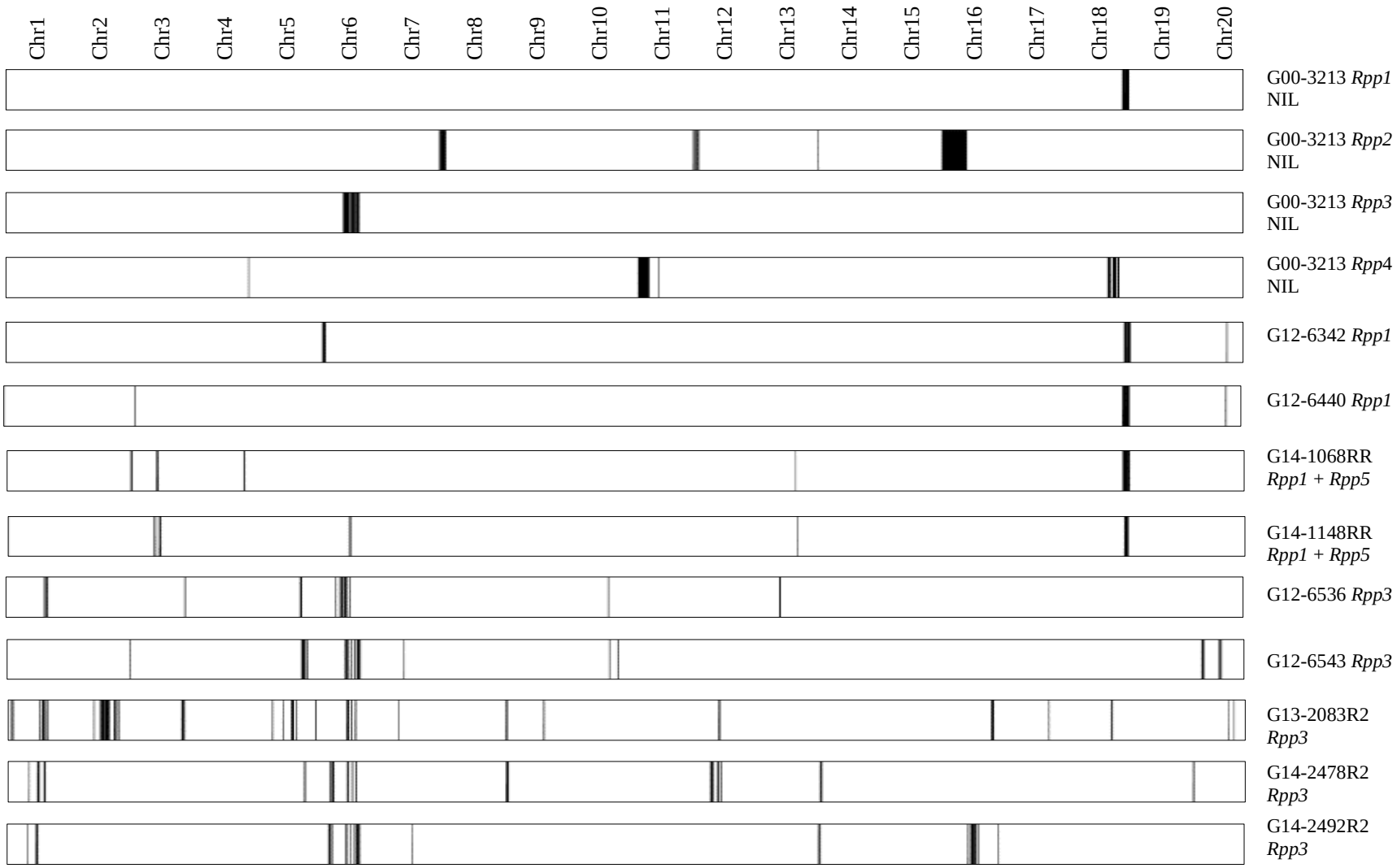

Fig. 3.11 Exotic genomic introgressions into elite breeding lines. The black bars represent regions of the genome where SNP alleles originated from PI sources of *Rpp* resistance. The approximate positions of the 20 chromosomes are indicated along the top of the figure and the identity of the breeding line is indicated on the right.

Table 3.1 The nine *P. pachyrhizi* isolates used for screening soybean germplasm.

Isolate	Country[†]	Location	Year collected	Collector
AU79-1	Australia	Redland Bay, Queensland	1979	D.E. Byth
CO04-2	Columbia	Armenia, Quindio	2004	R. Tisnes
HW94-1	USA	Oahu, Hawaii	1994	E. Kilgore
IN73-1	India	Pantnagar	1973	D.N. Thapliyal
LA04-1	USA	Ben Hur, Louisiana	2004	R. Schneider
SA01-1	South Africa	Natal Province	2001	Z.A. Pretorius
TW72-1	Taiwan	Taipei	1972	L.-C. Wu
VT05-1	Vietnam	Hanoi	2005	B. Nguyen
ZM01-1	Zimbabwe	Narare	2001	C. Levy

[†]Data taken from Bromfield et al. (1980); Hyten et al. (2009); and Pham et al. (2009); only LA04-1 has been single-spore purified – all others are bulk field isolates.

Table 3.2 Rust phenotypes of 328 soybean cultivars and accessions challenged with *P. pachyrhizi* in the field and greenhouse (2015-2017).

Cultivar/ Accession	MG[†]	Origin	Greenhouse severity[‡]	Field severity[§]	Greenhouse sporulation[¶]	Field sporulation[#]	Greenhouse reaction[††]	Field reaction[‡‡]	Comments
5601T	V	USA	4.0	-	4.7[§§]	-	TAN	-	Susceptible check
Alamo	IX	USA	3.7	-	4.6	-	TAN	-	Susceptible check
Boggs	VI	USA	4.2	4.1	4.6	5.0	TAN	TAN	Susceptible check
Carver	VII	USA	3.4	4.0	-	4.0	TAN	TAN	Susceptible check
Caviness	V	USA	4.2	-	4.8	-	TAN	-	Susceptible check
G00-3213	VII	USA	4.0	3.0	4.3	4.0	TAN	TAN	Susceptible check
Hartz 9190	IX	USA	3.4	-	4.6	-	TAN	-	Susceptible check
LD00-3309	IV	USA	4.0	-	3.9	-	TAN	-	Susceptible check
Prichard	VIII	USA	3.7	5.0	-	5.0	TAN	TAN	Susceptible check
Williams 82	III	USA	3.7	4.3	4.0	5.0	TAN	TAN	Susceptible check
PI 200492	VII	Japan	1.0	2.0	1.0	1.0	IM	IM/RB	*Rpp1* (Hyten et al., 2007)
G00-3213 *Rpp1*	VII	USA	1.3	1.0	1.0	1.0	IM/RB	IM	*Rpp1* (King et al., 2016)
L85-2378(*Rpp1*)	III	USA	1.0	1.5	-	1.0	IM	IM/RB	Williams 82 (5) x PI 200492
PI 417120	VIII	Japan	1.0	2.0	1.0	1.0	IM	IM/RB	*Rpp1* allele (Harris et al., 2015)
PI 423958	VIII	Japan	1.3	2.5	1.0	1.0	IM	RB	*Rpp1* allele (Harris et al., 2015)
PI 518295	VII	Taiwan	1.1	1.0	1.2	1.0	IM	IM	*Rpp1* allele (Harris et al., 2015)
PI 594177	VIII	Japan	1.1	-	1.0	-	IM	-	*Rpp1* allele (Yamanaka et al., 2015a)

PI 594538A	IX	China	4.3	5.0	4.1	3.5	TAN	TAN	*Rpp1-b* (Chakraborty et al., 2009)
PI 594760B	IX	China	4.0	4.0	4.3	5.0	TAN	TAN	*Rpp1-b* allele (Garcia et al., 2011)
PI 587905	VII	China	3.7	2.5	4.3	4.0	TAN	TAN	*Rpp1-b* allele (Hossain et al., 2014)
PI 594767A	IX	China	3.2	-	3.7	-	TAN	-	*Rpp1-b* allele (Hossain et al., 2014)
PI 561356	V	China	4.3	3.0	4.6	4.0	TAN	TAN	*Rpp1-b* allele (Kim et al., 2012)
PI 587880A	VI	China	3.0	3.0	4.0	4.0	TAN	TAN	*Rpp1-b* allele (Ray et al., 2009)
PI 587886	VI	China	3.8	3.5	4.5	2.0	TAN	MIX	*Rpp1-b* allele (Ray et al., 2009)
PI 587855	VIII	China	2.8	3.0	-	5.0	TAN	TAN	*Rpp1-b* allele (Yamanaka et al., 2016)
PI 230970	VII	Japan	3.9	1.5	1.3	3.0	RB	RB	*Rpp2* (Silva et al., 2008)
PI 230971	VIII	Japan	4.3	-	2.0	-	RB	-	*Rpp2* (Bromfield and Hartwig, 1980)
G00-3213 *Rpp2*	VII	USA	3.0	3.0	1.3	1.0	RB	RB	*Rpp2* (King et al., 2016)
PI 224270	VII	Japan	4.1	1.0	1.5	1.0	RB	IM	*rpp2* (Garcia et al., 2008)
PI 417125	VIII	Japan	3.1	1.5	1.1	2.0	RB	RB	*Rpp2* (Nogueira et al., 2008)
PI 417126	VIII	Japan	3.3	2.0	1.2	2.5	RB	RB	BSA parent (*Rpp2*?)

PI 197182	VIII	Malaysia	1.8	1.5	1.3	1.0	IM/RB	RB	*Rpp2* allele (Laperuta et al., 2008); *Rpp3* allele (Harris et al., 2015)
PI 462312	VIII	India	3.3	1.0	1.0	1.0	RB	IM	*Rpp3* (Hyten et al., 2009)
G00-3213 *Rpp3*	VII	USA	2.5	2.0	1.0	1.0	IM/RB	RB	*Rpp3* (King et al., 2016)
PI 628932	VII	Brazil	2.1	3.0	1.3	5.0	IM/RB	TAN	*Rpp3* (Brogin et al., 2005)
PI 416764	VIII	Japan	2.3	3.0	1.0	1.0	IM/RB	RB	*Rpp3* (Hossain et al., 2014)
PI 506764	VII	Japan	3.4	2.3	1.0	1.0	RB	RB	*Rpp?*(Hyuuga) (Monteros et al., 2007)
PI 567099A	IX	Indonesia	4.0	2.0	3.1	4.0	TAN	TAN	*rpp3* (Ray et al., 2011)
PI 200445	VIII	Japan	1.9	2.0	1.0	1.0	RB	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 200488	VIII	Japan	1.6	3.0	1.0	1.0	IM/RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 416810	IX	Japan	1.3	2.0	1.0	1.0	IM/RB	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 416826A	VIII	Japan	1.0	1.0	1.0	1.0	IM	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 416873B	VIII	Japan	1.8	2.0	1.0	2.5	MIX	TAN	*Rpp3* allele (Harris et al., 2015)
PI 416886	VIII	Japan	3.2	3.0	3.7	1.0	MIX	RB	*Rpp3* allele (Harris et al., 2015)
PI 417013	VIII	Japan	2.1	1.5	1.0	1.0	IM/RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 417085	IX	Japan	1.6	1.0	1.0	1.0	IM/RB	IM	*Rpp3* allele (Harris et al., 2015)

PI 417089A	IX	Japan	1.3	3.5	1.0	1.0	IM	RB	*Rpp3* allele (Harris et al., 2015)
PI 417089B	IX	Japan	1.0	4.0	1.0	1.0	IM	RB	*Rpp3* allele (Harris et al., 2015)
PI 417116	VII	Japan	3.4	2.0	1.0	1.0	RB	IM	*Rpp3* allele (Harris et al., 2015)
PI 417128	VII	Japan	2.4	2.0	1.0	1.0	IM/RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 417132	VII	Japan	2.1	3.0	1.0	1.0	IM	RB	*Rpp3* allele (Harris et al., 2015)
PI 417134	VIII	Japan	2.9	2.0	1.0	1.0	RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 417503	VI	Brazil	2.6	1.5	1.0	1.0	IM/RB	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 423961A	IX	Japan	1.7	2.5	1.0	1.5	IM/RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 423962	VIII	Japan	2.4	3.0	1.0	1.0	IM/RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 423966	VIII	Japan	1.0	3.0	1.0	1.0	IM	RB	*Rpp3* allele (Harris et al., 2015)
PI 506491	VIII	Japan	3.0	2.0	1.0	1.0	RB	HR	*Rpp3* allele (Harris et al., 2015)
PI 506695	VI	Japan	2.4	2.0	1.0	1.0	RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 506947	VIII	Japan	1.6	2.0	1.3	1.0	IM/RB	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 507004	VIII	Japan	1.5	2.5	1.0	1.0	IM	RB	*Rpp3* allele (Harris et al., 2015)
PI 507005	VII	Japan	2.1	3.0	1.0	1.0	IM/RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 507008	VII	Japan	1.1	2.5	1.0	1.0	IM/RB	RB	*Rpp3* allele (Harris et al., 2015)

PI 507009	VI	Japan	2.3	4.0	1.0	1.0	IM/RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 507259	VII	Japan	1.5	2.5	1.0	1.0	RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 567020A	VIII	Indonesia	1.3	2.0	1.0	1.0	IM	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 567024	VIII	Indonesia	2.4	1.5	1.0	1.0	IM/RB	IM	*Rpp3* allele (Harris et al., 2015)
PI 567025A	VIII	Indonesia	2.4	1.5	1.0	1.0	HR	IM	*Rpp3* allele (Harris et al., 2015)
PI 567034	VIII	Indonesia	1.5	1.0	1.0	1.0	IM	IM	*Rpp3* allele (Harris et al., 2015)
PI 567039	VII	Indonesia	2.3	3.0	1.0	1.0	IM	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 567046A	VIII	Indonesia	1.8	4.0	1.0	1.0	IM	RB	*Rpp3* allele (Harris et al., 2015)
PI 567053	IX	Indonesia	2.8	1.5	1.0	1.0	IM/RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 567054C	IX	Indonesia	1.8	2.0	1.0	1.0	IM/RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 567056A	VIII	Indonesia	2.3	2.0	1.0	1.0	IM/RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 567058D	IX	Indonesia	1.4	1.0	1.0	1.0	IM/RB	IM	*Rpp3* allele (Harris et al., 2015)
PI 567059	V	Indonesia	-	1.5	-	1.0	-	RB	*Rpp3* allele (Harris et al., 2015)
PI 567090	IX	Indonesia	-	2.0	-	1.0	-	RB	*Rpp3* allele (Harris et al., 2015)
PI 567180	V	Vietnam	1.8	1.5	1.0	1.0	IM/RB	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 567190	VI	Vietnam	-	2.0	-	1.0	-	RB	*Rpp3* allele (Harris et al., 2015)

PI 578457A	VIII	Vietnam	1.2	1.5	1.0	1.0	IM	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 594149	VIII	Japan	2.2	3.0	1.0	1.0	RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 594172A	VII	Japan	1.8	2.0	1.0	1.0	IM/RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 605774	V	Vietnam	1.6	1.0	1.0	1.0	IM/RB	IM	*Rpp3* allele (Harris et al., 2015)
PI 605824A	V	Vietnam	1.7	1.5	1.0	1.0	IM/RB	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 605838	V	Vietnam	2.0	1.0	1.0	1.0	IM/RB	IM	*Rpp3* allele (Harris et al., 2015)
PI 605854B	V	Vietnam	2.5	3.0	1.2	1.0	IM/RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 605865B	V	Vietnam	2.2	2.0	1.0	1.0	IM/RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 605885B	V	Vietnam	2.6	1.5	1.0	1.0	IM/RB	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 605891B	VI	Vietnam	1.3	3.0	1.0	2.5	IM	RB	*Rpp3* allele (Harris et al., 2015)
PI 606397B	V	Vietnam	1.2	1.5	1.0	1.0	IM	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 606405	V	Vietnam	1.2	2.5	1.0	1.0	IM	RB	*Rpp3* allele (Harris et al., 2015)
PI 615437	VI	Vietnam	1.4	3.0	1.0	3.5	IM/RB	RB	*Rpp3* allele (Harris et al., 2015)
PI 615445	V	Vietnam	2.0	2.0	1.0	1.0	IM/RB	IM	*Rpp3* allele (Harris et al., 2015)
PI 200466	VII	Japan	3.4	1.0	1.0	1.0	RB	IM	BSA parent (*Rpp3*?)
PI 203398	VIII	Brazil	2.8	1.0	1.0	1.0	IM/RB	IM/RB	BSA parent (*Rpp3*?)

PI 379621	VI	Taiwan	3.3	1.0	-	1.0	RB	IM	BSA parent (*Rpp3*?)
PI 416935	VIII	Japan	2.4	-	1.0	-	IM/RB	-	BSA parent (*Rpp3*?)
PI 417208	VIII	Japan	2.5	2.5	1.3	1.0	IM/RB	IM/RB	BSA parent (*Rpp3*?)
PI 423960B	IX	Japan	3.5	1.0	1.0	2.0	RB	RB	BSA parent (*Rpp3*?)
PI 567191	V	Vietnam	4.0	2.0	1.0	1.0	RB	IM/RB	BSA parent (*Rpp3*?)
PI 635999	VI	Indonesia	1.2	3.0	-	1.0	IM	RB	Rpp3 + 4 (Vuong et al., 2016)
PI 459025B	VIII	China	3.7	1.5	2.1	1.0	RB	RB	*Rpp4* (Silva et al., 2008)
G00-3213 *Rpp4*	VII	USA	3.9	1.0	2.5	1.0	RB	IM	*Rpp4* (King et al., 2016)
PI 423972	IX	Japan	3.0	2.0	1.9	1.0	RB	IM/RB	*Rpp4-b* (King et al., 2017)
PI 476905A	V	China	1.3	2.0	1.0	1.0	IM/RB	IM/RB	*Rpp4*/*Rpp6* allele (Harris et al., 2015)
PI 567076	VII	Indonesia	3.1	1.5	1.2	1.0	IM/RB	IM/RB	*Rpp4*/*Rpp6* allele (Harris et al., 2015)
PI 605791A	VI	Vietnam	3.5	3.0	2.1	1.0	RB	RB	*Rpp4*/*Rpp6* allele (Harris et al., 2015)
PI 200526	VIII	Japan	4.3	3.5	4.7	5.0	TAN	TAN	*Rpp5* (Garcia et al., 2008)
PI 200456	VIII	Japan	3.8	2.5	3.9	3.0	TAN	TAN	*rpp5* (Garcia et al., 2008)
PI 200487	VIII	Japan	1.3	1.0	1.0	1.0	IM/RB	IM	*Rpp5* (Garcia et al., 2008)
PI 471904	IX	Indonesia	1.9	1.5	1.0	1.0	RB	RB	*Rpp5* (Garcia et al., 2008)

PI 567102B	IX	Indonesia	2.5	1.9	1.0	1.0	HR	HR	*Rpp6* (Li et al., 2012)
PI 567068A	VII	Indonesia	2.6	1.0	1.0	1.0	IM/RB	IM	*Rpp(PI567068A)* (King et al., 2015)
PI 567104B	IX	Indonesia	3.3	2.0	1.0	1.0	RB	RB	*Rpp6* (Liu et al., 2016)
PI 605823	IX	Vietnam	3.7	1.0	1.9	1.0	RB	IM/RB	*Rpp7* (Childs et al., 2017)
PI 566984	VI	Indonesia	1.2	3.0	1.0	1.0	IM/RB	RB	BSA parent (*Rpp4-b*/*Rpp6*?)
PI 566956	IX	Indonesia	1.1	2.0	1.0	1.0	IM/RB	IM	BSA parent (*Rpp6*?)
PI 567073A	VIII	Indonesia	1.5	2.0	1.0	1.0	IM/RB	IM/RB	BSA parent (*Rpp6*?)
PI 81765	I	China	4.3	-	2.3	-	RB	-	*Rpp7* haplotype
PI 81773	II	Japan	4.4	-	3.3	-	MIX	-	*Rpp7* haplotype
PI 165914	VII	India	3.7	-	3.6	-	MIX	-	*Rpp7* haplotype
PI 166028	VII	India	3.3	-	3.9	-	TAN	-	*Rpp7* haplotype
PI 219732	VI	Pakistan	3.9	-	3.8	-	TAN	-	*Rpp7* haplotype
PI 232988	II	China	3.3	-	1.5	-	RB	-	*Rpp7* haplotype
PI 291278	I	China	3.9	-	2.7	-	MIX	-	*Rpp7* haplotype
PI 319525	VI	China	3.7	-	3.1	-	MIX	-	*Rpp7* haplotype
PI 437663	II	China	3.7	-	1.8	-	RB	-	*Rpp7* haplotype
PI 497960	VII	India	4.0	-	3.2	-	MIX	-	*Rpp7* haplotype
PI 578323A	VII	Nepal	4.0	-	3.8	-	TAN	-	*Rpp7* haplotype
PI 578326	VI	Nepal	3.9	-	3.6	-	MIX	-	*Rpp7* haplotype
PI 612753A	0	China	3.9	-	2.2	-	RB	-	*Rpp7* haplotype
PI 423963	VIII	Japan	1.7	1.5	1.0	1.0	IM/RB	IM/RB	BSA parent
PI 567061	VIII	Indonesia	1.1	2.0	1.0	1.0	IM	IM/RB	BSA parent

PI 567072A	VIII	Indonesia	1.7	-	1.0	-	IM/RB	-	BSA parent
PI 567089A	VIII	Indonesia	1.4	2.0	1.5	5.0	MIX	TAN	BSA parent
PI 567132C	IX	Indonesia	1.5	1.0	1.0	1.0	IM/RB	HR	BSA parent
PI 567189A	IV	Vietnam	2.6	1.5	1.0	1.0	IM	IM/RB	BSA parent
PI 594754	IX	China	3.6	-	3.6	-	MIX	-	
PI 459025A	IX	China	3.7	3.0	-	3.0	MIX	RB	
PI 594796	VIII	China	3.7	1.5	-	1.0	RB	IM/RB	
PI 588011B	VIII	China	3.4	2.0	-	3.0	RB	RB	
PI 594470B	VIII	China	2.7	1.0	-	3.0	RB	RB	
PI 594470C	VIII	China	4.4	1.0	-	3.0	RB	RB	
PI 603785	VIII	China	3.2	4.0	-	4.0	MIX	TAN	
PI 587782	VIII	China	3.4	2.0	-	4.0	RB	TAN	
PI 165674	VIII	China	3.5	-	-	-	TAN	-	
PI 165676	VIII	China	4.6	-	-	-	TAN	-	
PI 253657	VIII	China	4.4	-	-	-	TAN	-	
PI 319533	VIII	China	4.1	-	-	-	TAN	-	
PI 445842	VIII	China	3.7	-	-	-	TAN	-	
PI 561357	VIII	China	4.3	-	-	-	TAN	-	
PI 567238	IX	China	3.0	-	-	-	TAN	-	
PI 567295	VIII	China	3.2	-	-	-	TAN	-	
PI 587551	VIII	China	3.5	-	-	-	TAN	-	
PI 587584	VI	China	2.8	-	-	-	TAN	-	
PI 587596C	VIII	China	3.5	-	-	-	TAN	-	
PI 587597C	VIII	China	3.5	-	-	-	TAN	-	
PI 587599	VIII	China	4.3	-	-	-	TAN	-	
PI 587630C	VIII	China	3.9	-	-	-	TAN	-	
PI 587631B	VIII	China	3.6	-	-	-	TAN	-	

PI 587633C	VIII	China	3.1	-	-	-	TAN	-
PI 587641C	VIII	China	3.5	-	-	-	TAN	-
PI 587745	VIII	China	3.6	-	-	-	TAN	-
PI 587748	VIII	China	4.3	-	-	-	TAN	-
PI 587780	VIII	China	3.5	-	-	-	TAN	-
PI 587822A	IX	China	2.5	-	3.8	-	TAN	-
PI 587854B	VIII	China	3.3	-	-	-	TAN	-
PI 587858	VIII	China	4.4	-	-	-	TAN	-
PI 587887B	VIII	China	4.4	-	-	-	TAN	-
PI 587887C	VIII	China	3.8	-	-	-	TAN	-
PI 587890A	VIII	China	3.4	-	-	-	TAN	-
PI 587903A	VIII	China	3.4	-	-	-	TAN	-
PI 587915C	VIII	China	3.8	-	-	-	TAN	-
PI 587915D	VIII	China	3.9	-	-	-	TAN	-
PI 587930	VIII	China	4.0	-	-	-	TAN	-
PI 587941	VIII	China	3.2	-	-	-	TAN	-
PI 588019A	VIII	China	3.5	-	-	-	TAN	-
PI 588019B	VIII	China	3.7	-	-	-	TAN	-
PI 588031	VIII	China	3.5	-	-	-	TAN	-
PI 588032C	VIII	China	3.5	-	-	-	TAN	-
PI 588043	VIII	China	3.3	-	-	-	TAN	-
PI 594522	VIII	China	3.4	-	-	-	TAN	-
PI 594562B	VIII	China	3.9	-	-	-	TAN	-
PI 594720	VIII	China	4.3	-	-	-	TAN	-
PI 594758C	VIII	China	4.1	-	-	-	TAN	-
PI 594786A	VIII	China	3.0	-	-	-	TAN	-
PI 594790C	VIII	China	3.3	-	-	-	TAN	-

PI 594820B	VIII	China	3.8	-	-	-	TAN	-
PI 594820C	VIII	China	4.1	-	-	-	TAN	-
PI 594825	VIII	China	3.8	-	-	-	TAN	-
PI 594826B	VIII	China	4.3	-	-	-	TAN	-
PI 603509	VIII	China	3.3	-	-	-	TAN	-
PI 603513A	VIII	China	4.3	-	-	-	TAN	-
PI 603513B	VIII	China	4.1	-	-	-	TAN	-
PI 603521	VIII	China	3.8	-	-	-	TAN	-
PI 603538D	VIII	China	3.8	-	-	-	TAN	-
PI 603538E	VIII	China	4.4	-	-	-	TAN	-
PI 603604	VIII	China	4.4	-	-	-	TAN	-
PI 603621	VIII	China	3.0	-	-	-	TAN	-
PI 603626	VIII	China	4.2	-	-	-	TAN	-
PI 603639	VIII	China	3.9	-	-	-	TAN	-
PI 603737C	VIII	China	3.0	-	-	-	TAN	-
PI 603770	VIII	China	3.0	-	-	-	TAN	-
PI 594505A	VIII	China	2.9	2.0	-	1.0	TAN	IM/RB
PI 437562	VIII	China	2.9	3.5	-	5.0	TAN	TAN
PI 594487	VIII	China	2.8	2.0	-	5.0	TAN	TAN
PI 594511C	VIII	China	2.8	2.0	-	2.0	TAN	TAN
PI 594543	VIII	China	2.9	4.0	-	5.0	TAN	TAN
PI 594789B	VIII	China	2.8	2.0	-	5.0	TAN	TAN
PI 594846	VIII	China	2.9	2.0	-	2.0	TAN	TAN
PI 603641	VIII	China	2.3	2.0	-	5.0	TAN	TAN
PI 615493	VIII	China	4.4	5.0	4.4	5.0	TAN	TAN
PI 566982	VIII	Indonesia	1.5	-	-	-	IM/RB	-
PI 566974	IX	Indonesia	-	1.5	-	1.0	-	IM/RB

PI 566975	VIII	Indonesia	-	1.5	-	1.0	-	RB
PI 567123A	VIII	Indonesia	-	1.5	-	1.0	-	RB
PI 567129	IX	Indonesia	1.0	1.5	-	3.0	HR	RB
PI 566988A	VIII	Indonesia	2.2	3.0	-	1.0	RB	RB
PI 567031B	VIII	Indonesia	-	4.0	-	5.0	-	TAN
PI 200502	VI	Japan	-	1.0		1.0	-	IM
PI 200523	VIII	Japan	-	1.0	-	1.0	-	IM
PI 200547	VIII	Japan	-	1.0	-	1.0	-	IM
PI 238109	X	Japan	-	1.0	-	1.0	-	IM
PI 417129B	IX	Japan	-	1.0	-	1.0	-	IM
PI 423959	VIII	Japan	-	1.5	-	1.0	-	IM/RB
PI 200455	VIII	Japan	-	3.5		2.0	-	RB
PI 200524	VIII	Japan	-	2.0	-	1.0	-	RB
PI 416778	VIII	Japan	-	2.5	-	3.0	-	RB
PI 417119	VIII	Japan	-	3.0	-	1.0	-	RB
PI 506939	VI	Japan	-	2.5	-	1.0	-	RB
PI 416873C	VIII	Japan	3.3	3.0	-	1.0	HR	RB
PI 416806	VIII	Japan	1.2	2.5	1.0	1.0	IM	RB
PI 592904	VIII	Japan	1.8	2.0	-	1.0	IM	RB
PI 416874A	IX	Japan	2.2	-	1.0	-	IM/RB	-
PI 200509	VIII	Japan	1.1	1.5	1.0	1.0	IM/RB	IM/RB
PI 507007	VI	Japan	1.8	2.0	-	1.0	IM/RB	IM/RB
PI 416873A	VIII	Japan	1.6	2.0	-	1.0	IM/RB	RB
PI 423957	VIII	Japan	3.7	3.0	2.0	1.0	MIX	RB
PI 200477	VII	Japan	2.0	-	1.0	-	RB	-
PI 507146	VIII	Japan	3.0	-	-	-	RB	-
PI 200525	VIII	Japan	3.3	1.0	1.4	1.0	RB	IM

PI 200464	VII	Japan	3.1	1.5	1.9	1.0	RB	IM/RB
PI 200465	VIII	Japan	3.2	1.5	1.0	1.0	RB	IM/RB
PI 200476	VII	Japan	3.4	1.5	1.3	1.0	RB	IM/RB
PI 423960A	IX	Japan	3.3	1.0	1.1	1.0	RB	IM/RB
PI 181567	VIII	Japan	2.6	1.0	-	2.5	RB	RB
PI 200521	IX	Japan	2.0	3.0	1.0	1.0	RB	RB
PI 200551	VIII	Japan	2.8	2.0	2.4	1.0	RB	RB
PI 506938	VI	Japan	3.3	2.0	1.5	1.0	RB	RB
PI 417014A	IX	Japan	1.8	2.0	1.0	3.0	IM/RB	TAN
PI 200490	VIII	Japan	1.9	3.0	1.7	4.0	MIX	TAN
PI 417261	VIII	Japan	2.3	2.0	-	4.0	RB	TAN
PI 85897	VIII	Japan	3.4	-	-	-	TAN	-
PI 181564	VIII	Japan	3.2	-	-	-	TAN	-
PI 200531	VIII	Japan	4.0	-	3.3	-	TAN	-
PI 274582	VIII	Japan	4.1	-	-	-	TAN	-
PI 417123	VIII	Japan	3.3	-	-	-	TAN	-
PI 417124	VIII	Japan	3.5	-	-	-	TAN	-
PI 417130	VIII	Japan	3.8	-	-	-	TAN	-
PI 417136	VIII	Japan	3.0	-	-	-	TAN	-
PI 417139	I	Japan	4.0	-	-	-	TAN	-
PI 417146	VIII	Japan	4.0	-	-	-	TAN	-
PI 417313	VIII	Japan	3.6	-	-	-	TAN	-
PI 417342	VIII	Japan	4.2	-	-	-	TAN	-
PI 423913	VIII	Japan	3.0	-	-	-	TAN	-
PI 506607	VIII	Japan	3.2	-	-	-	TAN	-
PI 506632	VIII	Japan	3.6	-	-	-	TAN	-
PI 506645	VIII	Japan	3.8	-	-	-	TAN	-

PI 506889	VIII	Japan	3.7	-	-	-	TAN	-
PI 507000	VIII	Japan	3.0	-	-	-	TAN	-
PI 507161	VIII	Japan	3.8	-	-	-	TAN	-
PI 507227	VIII	Japan	2.0	-	-	-	TAN	-
PI 507261	VIII	Japan	4.6	-	-	-	TAN	-
PI 507574	VIII	Japan	3.9	-	-	-	TAN	-
PI 203402	VIII	Japan	2.9	1.0	-	1.0	TAN	IM
PI 507035	VIII	Japan	2.4	2.0	-	3.0	TAN	RB
PI 200528	VIII	Japan	4.0	5.0	-	5.0	TAN	TAN
PI 378693A	VIII	Japan	2.4	2.0	-	5.0	TAN	TAN
PI 507301	VIII	Japan	1.9	2.0	-	4.0	TAN	TAN
PI 200515	VIII	Japan	-	2.0		1.0		RB
PI 578307A	VI	Nepal	3.0	-	-	-	TAN	-
PI 578307C	VII	Nepal	3.1	-	-	-	TAN	-
PI 578308A	VI	Nepal	3.5	-	-	-	TAN	-
PI 368039	VI	Taiwan	1.0	1.5	1.0	1.0	IM	RB
PI 518283	II	Taiwan	3.7	-	-	-	TAN	-
PI 615487	V	Vietnam	-	2.0	-	1.0	-	IM
PI 605773	V	Vietnam	-	3.0	-	1.0	-	RB
PI 632639B	IV	Vietnam	1.0	-	-	-	IM	-
PI 632654	V	Vietnam	1.0	-	-	-	IM	-
PI 632666	V	Vietnam	1.0	-	-	-	IM	-
PI 632668	VI	Vietnam	1.2	-	-	-	IM	-
PI 615498	VII	Vietnam	1.0	1.0	-	1.0	IM	IM
PI 615502	VI	Vietnam	1.0	1.0	-	1.0	IM	IM
PI 615488	V	Vietnam	1.0	1.5	-	1.0	IM	IM/RB
PI 632642	IV	Vietnam	1.0	1.5	-	1.0	IM	IM/RB

PI 632650	VI	Vietnam	1.0	1.5	-	1.0	IM	IM/RB
PI 476897	VI	Vietnam	1.0	3.0	-	1.0	IM	RB
PI 632641B	V	Vietnam	1.0	2.0	-	1.0	IM	RB
PI 632658	V	Vietnam	1.0	2.0	-	1.0	IM	RB
PI 632944B	VI	Vietnam	1.0	2.0	-	1.0	IM	RB
PI 632945A	V	Vietnam	1.0	2.0	-	1.0	IM	RB
PI 632663B	V	Vietnam	2.0	-	1.0	-	IM/RB	-
PI 605829	V	Vietnam	1.5	2.0	1.0	1.0	IM/RB	IM/RB
PI 632645	IV	Vietnam	1.2	1.5	1.0	1.0	IM/RB	IM/RB
PI 632667	IV	Vietnam	2.6	1.5	1.0	1.0	IM/RB	IM/RB
PI 632637	V	Vietnam	1.4	3.0	-	1.0	IM/RB	RB
PI 567188	VI	Vietnam	3.4	2.5	2.0	2.0	RB	RB
PI 606440A	VI	Vietnam	3.1	1.5	-	1.0	RB	RB
PI 476889	V	Vietnam	3.0	-	-	-	MIX	-
PI 615508	VI	Vietnam	2.9	-	-	-	MIX	-
PI 632638	VIII	Vietnam	1.8	1.5	1.3	1.0	MIX	IM/RB
PI 476908	VI	Vietnam	4.1	3.0	3.8	3.5	MIX	RB
PI 632656A	V	Vietnam	2.4	2.5	-	1.0	MIX	RB
PI 615501	VI	Vietnam	-	2.0	-	3.5	-	RB
PI 476920	V	Vietnam	-	3.0	-	3.0	-	TAN
PI 635998	VII	Vietnam	-	4.0	-	5.0	-	TAN
PI 476882	VII	Vietnam	3.0	3.0	-	4.5	MIX	TAN
PI 615484	VI	Vietnam	2.4	3.0	-	4.0	MIX	TAN
PI 476903	IX	Vietnam	3.3	-	-	-	TAN	-
PI 605833	IX	Vietnam	4.0	-	4.1	-	TAN	-
PI 615509B	VII	Vietnam	2.8	-	-	-	TAN	-
PI 632647	IV	Vietnam	3.7	-	-	-	TAN	-

PI 632655	VI	Vietnam	3.1	-	-	-	TAN	-
PI 636000	IV	Vietnam	3.1	-	-	-	TAN	-
PI 636002	V	Vietnam	3.6	-	-	-	TAN	-
PI 632639A	IV	Vietnam	4.6	2.0	4.0	1.0	TAN	IM/RB
PI 632648	VI	Vietnam	3.3	2.0	-	3.0	TAN	RB
PI 615483	VI	Vietnam	3.8	3.0	-	4.0	TAN	TAN
PI 632643	V	Vietnam	3.8	5.0	3.9	5.0	TAN	TAN

[†]Maturity group and country of origin were taken from https://npgsweb.ars-grin.gov/gringlobal/search.aspx.

[‡]Severity was calculated on a 1 to 5 scale, with 1=immune and 5=abundance of lesions with accompanying leaf yellowing; greenhouse screening was performed in Griffin, GA. (N=12 to 24 plants)

[§]Severity was calculated same as above for field screening performed in Attapulgus, GA. (N=3 to 6 plants)

[¶]Sporulation was calculated on a 1 to 5 scale, with 1=no sporulation and 5=heavy sporulation; greenhouse screening was performed in Griffin, GA with the GA12 isolate. (N=12 to 24 plants)

[#]Sporulation was calculated same as above for field screening performed in Attapulgus, GA. (N=3 to 6 plants)

[††]TAN is susceptible with abundant sporulation; RB is a reddish-brown resistance response; IM has no visible lesion development; IM/RB has either both IM or RB reactions on separate plants or an intermediate “flecking”; HR (hypersensitive) has a ghost-like, light-colored lesion with no sporulation; MIX has a mixture of TAN and RB lesions on different plants as a result of seed admixture; greenhouse screening was performed in Griffin, GA. (N=12 to 24 plants)

[‡‡]Reactions were categorized as above for field screening performed in Attapulgus, GA. (N=3 to 6 plants)

[§§]Gray highlighting indicates a susceptible reaction (TAN lesions or sporulation >3.00).

Table 3.3 Rust reaction phenotypes of 132 breeding lines challenged with *P. pachyrhizi* in the field and greenhouse (2015-2017).

Name	Greenhouse severity[†]	Field severity[‡]	Field sporulation[§]	Greenhouse reaction[¶]	Field reaction[#]	*Rpp* gene[††]	Pedigree
G12-6342	2.1	3.0	1.0	MIX	RB	*Rpp1*	G00-3213(3) x [P97M50(3) x L85-2378(*Rpp1*)]
G12-6386	2.3	1.5	3.0	MIX	MIX	*Rpp1* het	G00-3213(3) x [P97M50(3) x L85-2378(*Rpp1*)
G12-6440	1.0	3.0	1.0	IM/RB	IM	*Rpp1*	G00-3213(3) x [P97M50(3) x L85-2378(*Rpp1*)]
G12-6515	-	2.0	1.0	-	RB		G00-3213(3) x [G00-3209 X G01-PR68(*Rpp3*)]
G12-6518	2.2	2.9	2.5	MIX	MIX	*Rpp3*	G00-3213(3) x [G00-3209 X G01-PR68(*Rpp3*)]
G12-6536	2.3	2.0	1.0	IM/RB	RB	*Rpp3*	G00-3213(3) x [G00-3209 X G01-PR68(*Rpp3*)]
G12-6543	1.6	1.5	3.0	IM/RB	MIX	*Rpp3*	G00-3213(3) x [G00-3209 X G01-PR68(*Rpp3*)]
G13-1269R2	4.3	1.0	1.0	TAN[‡‡]	IM		G09PR-54329R2 x {G00-3213(4) x [P97M50(3) x L85-2378(*Rpp1*)]}
G13-1311R2	3.8	-	-	TAN	-		G09PR-54329R2 x {G00-3213(4) x [P97M50(3) x L85-2378(*Rpp1*)]}
G13-1335R2	3.8	-	-	TAN	-		G09PR-54329R2 x {G00-3213(4) x [P97M50(3) x L85-2378(*Rpp1*)]}
G13-1338R2	3.2	2.0	5.0	TAN	TAN		G09PR-54329R2 x {G00-3213(4) x [P97M50(3) x L85-2378(*Rpp1*)]}
G13-1381R2	3.6	-	-	TAN	-		G09PR-54329R2 x {G00-3213(4) x [P97M50(3) x L85-2378(*Rpp1*)]}
G13-1449R2	3.5	3.3	4.0	MIX	RB		G09PR-54329R2 x {G00-3213(4) x [P97M50(3) x L85-2378(*Rpp1*)]}
G13-1488R2	3.2	3.9	5.0	TAN	TAN		G09PR-54329R2 x {G00-3213(4) x [P97M50(3) x L85-2378(*Rpp1*)]}

G13-1524R2	3.5	-	-	TAN	-		G09PR-54457R2 x {G00-3213(4) x [P97M50(3) x L85-2378(*Rpp1*)]}
G13-1551R2	4.0	3.3	4.5	MIX	TAN		G09PR-54457R2 x {G00-3213(4) x [P97M50(3) x L85-2378(*Rpp1*)]}
G13-1579R2	3.0	4.0	5.0	TAN	TAN		G09PR-54457R2 x {G00-3213(4) x [P97M50(3) x L85-2378(*Rpp1*)]}
G13-1621R2	4.3	-	-	TAN	-		G09PR-54457R2 x {G00-3213(4) x [P97M50(3) x L85-2378(*Rpp1*)]}
G13-1699R2	3.5	3.0	4.0	TAN	TAN		G09PR-54457R2 x {G00-3213(4) x [P97M50(3) x L85-2378(*Rpp1*)]}
G13-1754R2	2.8	4.7	5.0	TAN	TAN		G09PR-54457R2 x {G00-3213(4) x [P97M50(3) x L85-2378(*Rpp1*)]}
G13-1769R2	3.3	5.0	5.0	TAN	TAN		G09PR-54457R2 x {G00-3213(4) x [P97M50(3) x L85-2378(*Rpp1*)]}
G13-1806R2	3.5	-	-	TAN	-		G09PR-54329R2 x {G00-3213(4) x [G00-3209 x G01-PR68(*Rpp3*)]}
G13-1813R2	3.3	-	-	TAN	-		G09PR-54329R2 x {G00-3213(4) x [G00-3209 x G01-PR68(*Rpp3*)]}
G13-1834R2	3.5	-	-	TAN	-		G09PR-54329R2 x {G00-3213(4) x [G00-3209 x G01-PR68(*Rpp3*)]}
G13-1882R2	3.8	2.0	4.0	MIX	TAN	*Rpp3* het	G09PR-54329R2 x {G00-3213(4) x [G00-3209 x G01-PR68(*Rpp3*)]}
G13-1902R2	3.5	-	-	TAN	-		G09PR-54329R2 x {G00-3213(4) x [G00-3209 x G01-PR68(*Rpp3*)]}
G13-1915R2	3.2	3.0	2.0	TAN	TAN		G09PR-54329R2 x {G00-3213(4) x [G00-3209 x G01-PR68(*Rpp3*)]}
G13-2021R2	3.2	2.0	5.0	TAN	TAN		G09PR-54329R2 x {G00-3213(4) x [G00-3209 x G01-PR68(*Rpp3*)]}
G13-2073R2	2.8	3.3	3.6	TAN	TAN		G09PR-54457R2 x {G00-3213(4) x [G00-3209 x G01-PR68(*Rpp3*)]}
G13-2075R2	2.8	4.0	5.0	TAN	TAN		G09PR-54457R2 x {G00-3213(4) x [G00-3209 x G01-PR68(*Rpp3*)]}

G13-2083R2	1.3	2.0	1.0	IM/RB	IM	*Rpp3*	G09PR-54457R2 x {G00-3213(4) x [G00-3209 x G01-PR68(*Rpp3*)]}
G13-2114R2	3.5	2.5	4.0	TAN	TAN		G09PR-54457R2 x {G00-3213(4) x [G00-3209 x G01-PR68(*Rpp3*)]}
G13-2166R2	3.7	-	-	TAN	-		G09PR-54457R2 x {G00-3213(4) x [G00-3209 x G01-PR68(*Rpp3*)]}
G13-2171R2	3.8	3.5	4.5	MIX	TAN		G09PR-54457R2 x {G00-3213(4) x [G00-3209 x G01-PR68(*Rpp3*)]}
G13-2300R2	3.6	3.4	5.0	MIX	TAN	*Rpp3* het	G09PR-54457R2 x {G00-3213(4) x [G00-3209 x G01-PR68(*Rpp3*)]}
G13-2302R2	4.3	-	-	TAN	-		G09PR-54457R2 x {G00-3213(4) x [G00-3209 x G01-PR68(*Rpp3*)]}
G14-1002RR	3.3	3.0	4.5	TAN	TAN		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1003RR	3.3	4.5	5.0	TAN	TAN		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1004RR	2.4	3.5	1.0	HR	RB	*Rpp1*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1007RR	1.5	2.5	1.0	IM/RB	IM/RB	*Rpp1*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1012RR	2.8	3.0	4.5	MIX	TAN	*Rpp5*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1013RR	2.1	1.5	1.0	IM/RB	IM/RB	*Rpp1* + *Rpp5*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1015RR	2.3	1.5	1.0	MIX	IM	*Rpp1* het	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1016RR	2.2	4.0	4.0	MIX	MIX	*Rpp1* het	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1018RR	1.9	3.0	1.0	HR	RB	*Rpp1*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1019RR	2.6	3.0	5.0	TAN	TAN	*Rpp5*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]

G14-1020RR	1.6	1.5	1.0	IM/RB	IM/RB	*Rpp1*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1021RR	3.1	4.0	4.5	TAN	TAN		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1030RR	2.8	3.3	3.0	TAN	MIX		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1031RR	2.5	4.5	5.0	TAN	TAN		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1032RR	1.9	5.0	5.0	TAN	TAN		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1036RR	3.3	-	-	TAN	-	*Rpp5 het*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1038RR	2.8	3.0	4.0	TAN	TAN		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1040RR	3.4	-	-	TAN	-		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1058RR	3.1	4.5	5.0	TAN	TAN		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1063RR	2.4	3.0	2.0	TAN	RB	*Rpp5*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1064RR	2.5	2.5	4.0	TAN	MIX	*Rpp5*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1066RR	1.3	3.3	1.0	HR	IM/RB	*Rpp1* + *Rpp5*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1067RR	2.8	3.0	4.0	TAN	TAN		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1068RR	1.0	3.0	2.0	IM	MIX	*Rpp1* + *Rpp5*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1069RR	1.7	1.5	1.0	HR	IM	*Rpp1* + *Rpp5* het	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1076RR	2.4	4.5	4.5	TAN	TAN		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]

G14-1082RR	1.8	2.0	1.6	IM	MIX	*Rpp1* + *Rpp5*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1088RR	1.0	2.0	1.0	IM	IM	*Rpp1*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1089RR	2.5	3.0	3.5	TAN	TAN		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1090RR	1.6	2.0	1.0	HR	IM	*Rpp1* + *Rpp5* het	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1091RR	1.8	2.5	1.0	IM	IM	*Rpp1* + *Rpp5*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1093RR	2.0	1.5	1.0	MIX	IM	*Rpp1* + *Rpp5*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1100RR	2.0	2.5	2.0	IM/RB	IM	*Rpp1* + *Rpp5* het	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1102RR	2.5	4.0	4.8	MIX	TAN		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1103RR	1.5	1.5	1.0	IM/RB	IM	*Rpp1*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1104RR	2.4	3.0	3.0	TAN	MIX		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1107RR	3.3	-	-	TAN	-	*Rpp5*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1113RR	2.4	2.5	4.0	TAN	TAN	*Rpp5* het	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1115RR	1.9	4.0	2.6	IM/RB	MIX	*Rpp1* het	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1117RR	1.5	2.0	1.0	IM	IM	*Rpp1*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1120RR	1.2	2.5	1.0	IM	RB	*Rpp1*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1121RR	2.1	2.0	3.0	MIX	MIX	*Rpp1* het + *Rpp5* het	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]

G14-1129RR	2.3	2.5	3.5	TAN	RB		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1130RR	3.4	-	-	TAN	-	*Rpp5*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1131RR	1.2	2.5	1.0	IM	IM	*Rpp1*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1132RR	3.1	3.5	4.0	TAN	TAN		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1133RR	3.1	3.5	5.0	TAN	TAN		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1134RR	3.3	-	-	TAN	-		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1137RR	2.5	-	-	TAN	-		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1148RR	1.3	2.3	1.0	IM/RB	IM/RB	*Rpp1 + Rpp5*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1149RR	2.7	4.5	5.0	MIX	TAN	*Rpp5*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1163RR	2.1	1.5	1.0	MIX	IM	*Rpp1*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1171RR	1.1	4.0	1.0	MIX	RB	*Rpp1*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1176RR	2.7	4.0	4.0	TAN	TAN	*Rpp5 het*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1177RR	2.7	4.0	5.0	TAN	TAN	*Rpp5 het*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1181RR	2.5	4.0	5.0	TAN	TAN	*Rpp5 het*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1188RR	2.0	3.5	5.0	TAN	TAN		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1191RR	3.1	3.5	4.0	TAN	TAN		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]

G14-1193RR	2.9	-	-	TAN	-	*Rpp1* het + *Rpp5* het	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1195RR	1.5	3.0	1.0	HR	RB	*Rpp1*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1196RR	2.5	-	-	TAN	-		[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-1206RR	1.8	3.0	1.0	IM	RB	*Rpp1* + *Rpp5*	[P97M50(5) x G01-PR68(*Rpp3*] x [P97M50(6) x L85-2378(*Rpp1*)]
G14-2458R2	1.9	2.0	1.0	IM/RB	IM	*Rpp3*	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2459R2	2.7	2.5	3.0	TAN	TAN		[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2460R2	2.4	1.0	1.0	RB	IM	*Rpp3*	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2461R2	3.1	-	-	TAN	-		[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2462R2	2.5	1.5	1.0	MIX	IM	*Rpp3* het	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2463R2	3.4	4.0	5.0	MIX	TAN		[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]

G14-2464R2	2.4	4.0	1.0	MIX	RB	*Rpp3*	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2465R2	3.5	-	-	TAN	-		[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2466R2	3.3	-	-	TAN	-		[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2467R2	2.2	1.8	1.0	RB	IM/RB	*Rpp3*	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2472R2	2.8	1.5	1.0	MIX	IM	*Rpp3* het	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2473R2	1.6	3.5	1.5	IM/RB	RB	*Rpp3*	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2474R2	2.8	4.5	5.0	TAN	TAN		[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2476R2	3.3	-	-	TAN	-		[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]

G14-2477R2	2.3	3.0	3.0	TAN	TAN		[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2478R2	2.2	2.5	2.5	IM/RB	MIX	*Rpp3*	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2479R2	2.7	2.0	2.3	MIX	MIX	*Rpp3* het	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2480R2	2.9	3.5	2.3	MIX	MIX	*Rpp3* het	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2482R2	2.8	4.0	4.5	TAN	TAN		[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2487R2	2.0	2.0	1.0	IM/RB	IM/RB	*Rpp3*	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2488R2	3.5	-	-	TAN	-	*Rpp3* het	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2489R2	2.6	2.0	2.0	RB	RB	*Rpp3*	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]

G14-2492R2	2.5	2.0	1.0	RB	IM	*Rpp3*	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2493R2	2.8	4.3	4.3	TAN	MIX		[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2495R2	2.0	2.0	1.5	IM/RB	RB	*Rpp3*	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2496R2	3.1	2.0	1.0	IM/RB	IM	*Rpp3*	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2498R2	2.1	1.5	1.0	RB	IM/RB	*Rpp3*	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2499R2	2.9	3.5	2.0	TAN	RB		[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2503R2	2.1	2.5	3.5	IM/RB	MIX	*Rpp3*	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2506R2	4.0	-	-	TAN	-		[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]

G14-2508R2	2.3	1.5	1.0	MIX	IM	*Rpp3* het	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2509R2	2.5	2.5	1.0	IM/RB	RB	*Rpp3*	[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G14-2512R2	2.5	3.5	4.5	TAN	TAN		[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) x L85-2378(*Rpp1*)}]
G14-2513R2	2.0	1.5	1.0	IM/RB	RB	*Rpp3*	[[[G00-3213(3)RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp3*}]] x [[G00-3213(3)] x [{P97M50(3) xL85-2378(*Rpp1*)}]
G00-3209	-	3.5	4.0	-	TAN	Parent check	Woodruff
G00-3213	4.1	3.0	4.0	TAN	TAN	Parent check	-
G00-3213RR2Y	-	-	-	-	-	Parent check	-
G01-PR68(*Rpp3*)	-	-	-	-	-	*Rpp3* + *Rpp5*	Dillon x Hyuuga
G09PR-54329R2	-	-	-	-	-	Parent check	-
G09PR-54457R2	-	-	-	-	-	Parent check	-
Hyuuga	2.2	2.5	1.0	RB	IM/RB	*Rpp3* + *Rpp5*	-
L85-2378(*Rpp1*)	1.0	1.5	1.0	IM	IM/RB	*Rpp1*	Williams 82 (5) x PI 200492

P97M50	2.4	-	-	TAN	-	Parent check	-

[†]Severity was calculated on a 1 to 5 scale, with 1=immune and 5=abundance of lesions with accompanying leaf yellowing; greenhouse screening was performed in Griffin, GA. (N=12 plants)

[‡]Severity was calculated same as above for field screening performed in Attapulgus, GA. (N=3 to 6 plants)

[§]Sporulation was calculated on a 1 to 5 scale, with 1=no sporulation and 5=heavy sporulation; field screening was performed in Attapulgus, GA. (N=3 to 6 plants)

[¶]TAN is a susceptible reaction with abundant sporulation; RB is a reddish-brown resistance response; IM has no visible lesion development; IM/RB has either both IM or RB reactions on separate plants or an intermediate "flecking"; HR (hypersensitive) has a ghost-like, light-colored lesion with no sporulation; MIX has a mixture of TAN and RB lesions on different plants as a result of seed admixture; greenhouse screening was performed in Griffin, GA with the GA12 isolate. (N=12 plants)

[#]Reactions were categorized same as above for field screening performed in Attapulgus, GA. (N=3 to 6 plants)

[††]Results of screening with KASP markers linked to *Rpp* loci. Lines with no *Rpp* loci were left blank.

[‡‡]Gray highlighting indicates a susceptible TAN reaction. – indicates no data collected.

Table 3.4 Primer sequences of the KASP markers used for genotyping.

Assay ID	SNP ID[†]	SNP location[‡]	Gene	Mutant allele[§]	Forward primer (FAM) 5'-3'	Forward primer (HEX) 5'-3'	Reverse primer 5'-3'
GSM0422	ss715632313	Gm18_56,311,890	*Rpp1*	T	GAAGGTGACCAAGTTCATGC TCATTGGAGAGACTTCATTA TGCCAC	GAAGGTCGGAGTCAACGGA TTCATTGGAGAGACTTCATT ATGCCAT	GCTCATGTACCTT GTAAGACACCG
GSM0549	ss715632299	Gm18_56,161,046	*Rpp1*	T	GAAGGTGACCAAGTTCATGC TAGCTTCGAGTTCTCCTCAT CTTCC	GAAGGTCGGAGTCAACGGA TTAGCTTCGAGTTCTCCTCA TCTTCT	TTGTTGTAGGTCT TGTTGCTGGA
GSM0412	ss715594485	Gm06_44,621,267	*Rpp3*	C	GAAGGTGACCAAGTTCATGC TTGACCGACAAGATGGCTTC AAC	GAAGGTCGGAGTCAACGGA TTTGACCGACAAGATGGCTT CAAT	GGCCTTCACACCC TCCACT
GSM0551	N/A	Gm6_44,468,867	*Rpp3*	FAM	GAAGGTGACCAAGTTCATGC TCCATAGTTCATGAAGAAGG CTTTAAC	GAAGGTCGGAGTCAACGGA TTTAGTTCATGAAGAAGACT ATGACATTGAA	TCAGAGTCTTCTT CTAAGTCATAGTC TTCTAAG
GSM0004	N/A	near Gm03_31,928,336	*Rpp5*	C	GAAGGTGACCAAGTTCATGC TAATATGCAACACAAGGAG CCCAAC	GAAGGTCGGAGTCAACGGA TTCAATATGCAACACAAGGA GCCCAAT	GGAAGTTAGACG GAAAAAGGCCTA AATTT

[†]SNP ID found at www.Soybase.org/dlpages/index.php#snp50k (Song et al., 2013).
[‡]Physical genomic locations are based on the Glyma.Wm82.a2 sequence of the SNP available at www.Soybase.org/snps/index.php.
[§]T is the nucleotide thymine found in the mutant parent. C is the nucleotide cytosine found in the mutant parent. FAM is the KASP fluorescent dye associated with the mutant genotype.

Table 3.5 Rust reaction phenotype of sources of known *Rpp* genes, a susceptible check, and 21 breeding lines challenged with nine geographically diverse *P. pachyrhizi* isolates.

Cultivar/ Accession/ Breeding line	*P. pachyrhizi* Isolate										Marker data[‡]
	AU79-1	VT05-1	CO04-2	IN73-1	LA04-1	SA01-1	TW72-1	ZM01-1	HW94-1	GA12[†]	
Williams 82	TAN[§]	TAN	TAN	TAN	TAN	TAN	TAN	TAN	TAN	TAN	Susceptible
PI200492	TAN	TAN	TAN	IM/RB	IM	TAN	TAN	TAN	IM/RB[¶]	IM	*Rpp1*
PI462312	RB/MIX	TAN	TAN	RB	RB	RB	TAN	TAN[††]	RB[¶]	IM/RB	*Rpp3*
PI200526	TAN	TAN	TAN	TAN	TAN	TAN	TAN	TAN	-	TAN	*Rpp5*(PI200526)
Hyuuga[#]	RB	RB	RB	RB	RB	RB	TAN	RB	RB	RB	*Rpp3 + 5*
G14-1004RR	TAN	TAN	TAN	IM/RB	IM/RB	-	TAN	TAN	IM/RB	IM/RB	*Rpp1*
G14-1007RR	TAN	TAN	TAN	RB	RB	-	TAN	TAN	RB	IM/RB	*Rpp1*
G14-1020RR	TAN	TAN	TAN	RB	RB	-	TAN	TAN	RB	IM/RB	*Rpp1*
G14-1103RR	TAN	TAN	TAN	RB	RB	-	TAN	TAN	RB[¶]	IM/RB	*Rpp1*
G14-1171RR	TAN	TAN	TAN	IM/RB	IM	TAN	TAN	TAN	IM/RB	IM[¶]	*Rpp1*
G12-6386	TAN	TAN	TAN	TAN	TAN/RB	-	TAN	TAN	TAN/IM	TAN/RB	*Rpp1* het
G14-1066RR	RB	RB	RB	IM/RB	IM	RB	TAN	RB	IM/RB	IM/RB	*Rpp1 + 5*
G14-1082RR	RB	RB	RB	IM/RB	IM/RB	RB	TAN	RB	IM/RB	IM	*Rpp1 + 5*
G14-1148RR	RB	RB	RB	IM/RB	IM/RB	RB	TAN	RB	IM/RB	IM/RB	*Rpp1 + 5*
G14-1069RR	TAN/RB	TAN/RB	TAN	IM/RB	IM	RB[¶]	TAN	RB/TAN	IM/RB	IM/RB	*Rpp1 + Rpp5* het
G14-1090R2	TAN	TAN[††]	TAN	RB	RB	-	TAN	TAN	RB	IM/RB	*Rpp1 + Rpp5* het
G14-1100RR	TAN	TAN	RB/TAN	IM/RB	IM/RB	-	TAN	TAN	IM/RB	IM/RB	*Rpp1 + Rpp5* het
G14-2492R2	MIX	TAN	TAN	RB	RB	-	TAN	TAN	RB	RB	*Rpp3*
G14-2473R2	MIX	TAN	TAN	RB	RB	-	TAN	TAN	RB[¶]	IM/RB	*Rpp3*

G14-2467R2	MIX	TAN	TAN	RB	RB	-	TAN	TAN	RB	RB	*Rpp3*
G14-2478R2	RB/MIX	TAN	TAN	RB	RB	-	TAN	TAN	RB	IM/RB	*Rpp3*
G12-6518	RB	TAN	TAN	RB	IM/RB	-	TAN	TAN	IM/RB[¶]	RB/TAN	*Rpp3*
G12-6543	TAN	TAN	TAN	RB/TAN	IM/RB[¶]	-	TAN	TAN	RB/IM[¶]	IM/RB	*Rpp3*
G14-1012	-	-	-	-	-	RB	-	-	-	TAN/RB	*Rpp5(Hyuuga)*
G14-1107	RB	RB	RB	TAN	TAN	RB	TAN/INT	RB	-	TAN[††]	*Rpp5(Hyuuga)*
G14-1149	RB	RB	RB	TAN	TAN	RB	TAN/INT	RB	-	TAN/INT	*Rpp5(Hyuuga)*

[†]Isolate collected in southern Georgia in 2012, used for screening in Griffin, GA.

[‡]Based on analysis of KASP markers linked to *Rpp* gene loci.

[§]TAN is a susceptible reaction with abundant sporulation; RB is a reddish-brown resistance response; IM has no visible lesion development; IM/RB has either both IM or RB reactions on separate plants or an intermediate "flecking"; HR (hypersensitive) has a ghost-like, light-colored lesion with no sporulation; MIX has a mixture of TAN and RB lesions on the same plant; INT (intermediate) is similar to TAN, but with darker, RB-like lesions present; genotypes with more than one reaction listed have some plants with either reaction – the first listed one is predominant.

[¶]one plant TAN.

[#]Taken from Harris et al. (2015); Hyten et al. (2008); Kendrick et al. (2011).

[††]One plant RB.

Table 3.6 Rust reaction phenotype of sources of known *Rpp* genes, a susceptible check, and 15 mapping population parents challenged with nine geographically diverse *P. pachyrhizi* isolates.

Cultivar/ Accession/ Breeding line	*P. pachyrhizi* Isolates										*Rpp* genes[‡]
	AU79-1	VT05-1	CO04-2	IN73-1	LA04-1	SA01-1	TW72-1	ZM01-1	HW94-1	GA12[†]	
Williams 82	TAN[§]	TAN	TAN	TAN	TAN	TAN	TAN	TAN	TAN	TAN	Susceptible
PI200492	TAN	TAN	TAN	IM/RB	IM	TAN	TAN	TAN	IM/RB[¶]	IM	*Rpp1*
PI594538A[#]	RB	-	-	RB	TAN	RB	RB	RB	-	TAN	*Rpp1-b*
PI230970	RB	RB	RB	RB	RB	RB	RB	RB	-	RB	*Rpp2*
PI462312	RB/MIX	TAN	TAN	RB	RB	RB	TAN	TAN[††]	RB[¶]	IM/RB	*Rpp3*
PI459025B	RB	RB	RB	RB	RB	RB	RB	RB	-	RBSP[‡‡]	*Rpp4*
PI423972[#]	RB	-	RB	TAN	RB	RB	MIX	RB		RB	*Rpp4-b*
PI200526	TAN	TAN	TAN	TAN	TAN	TAN	TAN	TAN	-	TAN	*Rpp5*(PI200526)
PI567102B	RB	RBSP[‡‡]	RB	RB	RB	RBSP[‡‡]	TAN	RB	-	IM/RB	*Rpp6*
PI567068A[#]	TAN	-	-	-	RB	TAN	TAN	TAN	-	RB	*Rpp(PI567068A)*
PI605823	RB	TAN	RB	TAN	RB	-	TAN	TAN	-	RB	*Rpp7*
Hyuuga[#]	RB	RB	RB	RB	RB	RB	TAN	RB	RB	RB	*Rpp3 + 5*
PI423963	TAN	TAN	TAN	RB	RB	RB/TAN	TAN/INT	TAN	-	IM/RB	*Rpp3*/*Rpp4 ?*
PI567061	RB/INT	TAN	TAN	IM/RB	RB	RB	TAN	TAN	-	IM	*Rpp3* + ?
PI416935	RB/MIX	RB[¶]	TAN/RB	RB	RB	RB	TAN	TAN[††]	-	IM/RB	*Rpp3* allele
PI567191	MIX	RB	TAN	RB	RB	RB	TAN	TAN	-	RB	*Rpp3* allele
PI200466	MIX	TAN	TAN	RB	RB	RB	TAN/RB	TAN	-	RB	*Rpp3* allele

PI224270	RB	RB	TAN/RB	RB	RB	RB	RB	RB	-	RB	*rpp2*
PI417126	RB	RB	RB/TAN	RB	RB	RB	RB	RB	-	RB	*rpp2*
PI417125	RB	RB/TAN	RB	RB	RB	RB	RB	RB	-	RB	*rpp2*
PI423960B	RB	RB	RB	RB	RB	RB	TAN/INT	RB	-	RB	*Rpp3 + Rpp5*
PI417208	RB	TAN	TAN	RB	RB	RB	TAN	TAN[††]	-	IM/RB	*Rpp3?*
PI567189A	RB	TAN	TAN	RB	RB	RB	TAN	TAN	-	IM	?
PI566956	TAN	TAN	TAN	RB	RB	TAN	TAN	TAN	-	IM/RB	*Rpp6*
PI566984	TAN	TAN	TAN	RB	RB	TAN	TAN	TAN	-	IM/RB	*Rpp4-b/Rpp6*
PI567073A	TAN	TAN	TAN	RB	RB	TAN	TAN	TAN	-	IM/RB	*Rpp6*

[†]Isolate collected in southern Georgia in 2012, used for screening in Griffin, GA.

[‡]Based on mapping, BSA, *Rpp3* marker, or haplotype data (Table 3.7).

[§]TAN is susceptible with abundant sporulation; RB is a reddish-brown resistance response; IM has no visible lesion development; IM/RB has either both IM or RB reactions on separate plants or an intermediate "flecking"; MIX has a mixture of TAN and RB lesions on the same plant; INT (intermediate) is similar to TAN, but with darker, RB-like lesions present; genotypes with more than one reaction listed have some plants with either reaction – the first listed one is predominant.

[¶]one plant TAN.

[#]Taken from Harris et al. (2015); Hyten et al. (2008); Kendrick et al. (2011).

[††]One plant RB.

[‡‡]Red-brown lesion with sporulation.

Table 3.7 Putative *Rpp* alleles found in 19 mapping populations based on bulked segregant analysis (BSA), haplotype, *Rpp3* marker analysis, and characterization with nine diverse *P. pachyrhizi* isolates.

Name	Origin	MG	Reaction[†]	Haplotype[‡]	*Rpp3* deletion[§]	BSA[¶]	Isolate pattern[#]	Putative *Rpp* allele
PI 224270	Japan	VII	RB	*rpp2*	No	*Rpp2*	*rpp2*	*rpp2*
PI 417125	Japan	VIII	RB	*rpp2*	No	*Rpp2*	Similar to *rpp2*	*rpp2*?
PI 417126	Japan	VIII	RB	*rpp2*	No	*Rpp2*	*rpp2*	*rpp2*
PI 203398	Brazil	VIII	IM/RB	*Rpp3*	Yes	*Rpp2/Rpp3?*	-	*Rpp3*
PI 379621	Taiwan	VI	RB	*Rpp3*	Yes	*Rpp2/Rpp3?*	-	*Rpp3*
PI 567189A	Vietnam	IV	IM	*Rpp4*	No	Chr 8?	*Rpp3*	*Rpp3* allele
PI 417208	Japan	VIII	IM/RB	*Rpp3/Rpp5*	Yes	*Rpp2/Rpp3/Rpp4?*	*Rpp3*	*Rpp3*
PI 423960B	Japan	IX	RB	*Rpp3/Rpp5*	Yes	Chr 11?	*Rpp3 + Rpp5*	*Rpp3 + Rpp5*
PI 200466	Japan	VII	RB	*Rpp3/Rpp5*	Yes	*Rpp3*	Unique	*Rpp3* allele
PI 416935	Japan	VIII	IM/RB	*Rpp3/Rpp5*	Yes	*Rpp3*	Unique	*Rpp3* allele
PI 567061	Indonesia	VIII	IM	*Rpp2/Rpp3*	Yes	***Rpp3/Rpp4?***	Unique	*Rpp3* + ?
PI 423963	Japan	VIII	IM/RB	*Rpp3*	Yes	*Rpp4?*	Unique	*Rpp3* allele
PI 567191	Vietnam	V	RB	*Rpp4/Rpp5*	No	***Rpp3***	Unique	*Rpp3* allele
PI 566956	Indonesia	IX	IM/RB	*Rpp6*	No	***Rpp6***	*Rpp(PI567068A)*	*Rpp(PI567068A)*
PI 566984	Indonesia	VI	IM/RB	*Rpp4-b/Rpp6*	No	***Rpp4/Rpp6***	*Rpp(PI567068A)*	*Rpp(PI567068A)*
PI 567073A	Indonesia	VIII	IM/RB	*Rpp6*	No	***Rpp6***	*Rpp(PI567068A)*	*Rpp(PI567068A)*
PI 567072A	Indonesia	VIII	IM/RB	*Rpp4/Rpp6*	No	**in progress**	-	-
PI 567089A	Indonesia	VIII	MIX	*Rpp4/Rpp6*	No	**in progress**	-	-
PI 567132C	Indonesia	IX	IM/RB	*Rpp4/Rpp6*	No	**in progress**	-	-

[†]Reaction data came from screening accessions with the GA12 *P. pachyrhizi* isolate in the greenhouse (2016-2017) (N=12 to 24 plants). TAN is susceptible with abundant sporulation; RB is a resistance response with reduced sporulation; IM has no visible lesion development; IM/RB has either both IM or RB reactions on separate plants or an intermediate "flecking"; HR (hypersensitive) has a ghost-like, light-colored lesion with no sporulation; and MIX has some plants with RB or IM reactions and other plants with TAN reactions, due to seed admixture.

‡Haplotype analysis involved comparing the SNP alleles derived from the SoySNP50K array (soybase.org) for the resistant mapping parent with the SNP alleles within the resistance locus of known *Rpp* genes. When multiple haplotypes are listed, the parent matched the haplotype of multiple *Rpp* sources.
§Results of running the GSM0551 marker on the resistant parent (see Table 3.8).
¶The genomic region or known *Rpp* locus highlighted by BSA. Multiple loci are listed if more than one locus was indicated. Question marks indicate that the results were unclear, and bold text indicates the BSA that was performed between 2016-2017.
#An *Rpp* locus is indicated if the reaction of the resistant parent to diverse *P. pachyrhizi* isolates matched that of the sources of known *Rpp* genes (see Table 3.6).

Table 3.8 GSM0551 marker genotype of the 32 major soybean ancestors, 51 accessions with *P. pachyrhizi* resistance mapped to the *Rpp3* locus (Harris et al., 2015), 18 PI parents of mapping populations under analysis, and 16 sources of mapped *Rpp* resistance.

Name	MG	Origin	GSM0551[†]	Reaction[‡]	Comments
FC 31745	VI	unknown	WT	-	Ancestors
FC 33243-1	IV	unknown	WT	-	Ancestors
PI 080837	IV	Japan	WT	-	Ancestors
PI 180501	0	Germany	WT	-	Ancestors
PI 240664	X	Philippines	WT	-	Ancestors
PI 438471	0	Sweden	WT	-	Ancestors
PI 438477	0	Sweden	WT	-	Ancestors
PI 548298	III	China	INT	-	Ancestors
PI 548302	II	Japan	WT	-	Ancestors
PI 548311	0	China	WT	-	Ancestors
PI 548318	III	China	WT	-	Ancestors
PI 548325	0	Russia	WT	-	Ancestors
PI 548348	III	China	INT	-	Ancestors
PI 548352	III	Korea	WT	-	Ancestors
PI 548356	II	Korea	WT	-	Ancestors
PI 548360	II	Korea	INT	-	Ancestors
PI 548362	III	China	WT	-	Ancestors
PI 548379	0	China	WT	-	Ancestors
PI 548382	0	unknown	WT	-	Ancestors
PI 548391	II	China	INT	-	Ancestors
PI 548402	IV	China	INT	-	Ancestors
PI 548406	II	China	WT	-	Ancestors
PI 548438	VI	Korea	WT	-	Ancestors

PI 548445	VII	China	WT	-	Ancestors
PI 548456	VI	Korea	WT	-	Ancestors
PI 548461	VIII	China	INT	-	Ancestors
PI 548477	VI	unknown	WT	-	Ancestors
PI 548484	VI	Korea	WT	-	Ancestors
PI 548485	VII	China	WT	-	Ancestors
PI 548488	V	China	INT	-	Ancestors
PI 548603	IV	USA	WT	-	Ancestors
PI 548657	VII	USA	WT	-	Ancestors
PI 462312	VIII	India	MUT	RB	*Rpp3* (Hyten et al., 2009)
PI 416764	VIII	Japan	MUT	IM/RB	*Rpp3* (Hossain et al., 2014)
PI 628932	VII	Brazil	MUT	IM/RB	*Rpp3* (Brogin et al., 2005)
PI 506764	VII	Japan	MUT	RB	*Rpp?*(Hyuuga) (Monteros et al., 2007)
PI 567099A	IX	Indonesia	INT	MIX	*Rpp3* (Ray et al., 2011)
PI 417503	VI	Brazil	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 567020A	VIII	Indonesia	MUT	IM	*Rpp3* allele (Harris et al., 2015)
PI 567024	VIII	Indonesia	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 567025A	VIII	Indonesia	MUT	HR	*Rpp3* allele (Harris et al., 2015)
PI 567034	VIII	Indonesia	MUT	IM	*Rpp3* allele (Harris et al., 2015)
PI 567039	VII	Indonesia	MUT	IM	*Rpp3* allele (Harris et al., 2015)
PI 567046A	VIII	Indonesia	MUT	IM	*Rpp3* allele (Harris et al., 2015)
PI 567053	IX	Indonesia	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 567054C	IX	Indonesia	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 567056A	VIII	Indonesia	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 567058D	IX	Indonesia	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 200445	VIII	Japan	MUT	RB	*Rpp3* allele (Harris et al., 2015)
PI 200488	VIII	Japan	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)

PI 416810	IX	Japan	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 416826A	VIII	Japan	MUT	IM	*Rpp3* allele (Harris et al., 2015)
PI 416873B	VIII	Japan	MUT	MIX	*Rpp3* allele (Harris et al., 2015)
PI 416886	VIII	Japan	MUT	MIX	*Rpp3* allele (Harris et al., 2015)
PI 417013	VIII	Japan	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 417085	IX	Japan	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 417089A	IX	Japan	MUT	IM	*Rpp3* allele (Harris et al., 2015)
PI 417089B	IX	Japan	MUT	IM	*Rpp3* allele (Harris et al., 2015)
PI 417116	VII	Japan	MUT	RB	*Rpp3* allele (Harris et al., 2015)
PI 417128	VII	Japan	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 417132	VII	Japan	MUT	IM	*Rpp3* allele (Harris et al., 2015)
PI 417134	VIII	Japan	MUT	RB	*Rpp3* allele (Harris et al., 2015)
PI 423961A	IX	Japan	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 423962	VIII	Japan	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 423966	VIII	Japan	MUT	IM	*Rpp3* allele (Harris et al., 2015)
PI 506491	VIII	Japan	MUT	RB	*Rpp3* allele (Harris et al., 2015)
PI 506695	VI	Japan	MUT	RB	*Rpp3* allele (Harris et al., 2015)
PI 506947	VIII	Japan	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 507004	VIII	Japan	MUT	IM	*Rpp3* allele (Harris et al., 2015)
PI 507005	VII	Japan	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 507008	VII	Japan	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 507009	VI	Japan	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 507259	VII	Japan	MUT	RB	*Rpp3* allele (Harris et al., 2015)
PI 594149	VIII	Japan	MUT	RB	*Rpp3* allele (Harris et al., 2015)
PI 594172A	VII	Japan	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 197182	VIII	Malaysia	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 578457A	VIII	Vietnam	MUT	IM	*Rpp3* allele (Harris et al., 2015)

PI 605838	V	Vietnam	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 605885B	V	Vietnam	MUT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 567180	V	Vietnam	WT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 605774	V	Vietnam	WT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 605824A	V	Vietnam	WT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 605854B	V	Vietnam	WT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 605865B	V	Vietnam	WT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 605891B	VI	Vietnam	WT	IM	*Rpp3* allele (Harris et al., 2015)
PI 606405	V	Vietnam	WT	IM/RB	*Rpp3* allele (Harris et al., 2015)
PI 615437	VI	Vietnam	WT	IM	*Rpp3* allele (Harris et al., 2015)
PI 615445	V	Vietnam	WT	IM	*Rpp3* allele (Harris et al., 2015)
PI 567191	V	Vietnam	WT	IM/RB	BSA parent (*Rpp3*?)
PI 606397B	V	Vietnam	WT	RB	BSA parent (*Rpp3*?)
PI 200466	VII	Japan	MUT	RB	BSA parent (*Rpp3*?)
PI 203398	VIII	Brazil	MUT	IM/RB	BSA parent (*Rpp3*?)
PI 379621	VI	Taiwan	MUT	RB	BSA parent (*Rpp3*?)
PI 416935	VIII	Japan	MUT	IM/RB	BSA parent (*Rpp3*?)
PI 417208	VIII	Japan	MUT	IM/RB	BSA parent (*Rpp3*?)
PI 423960B	IX	Japan	MUT	RB	BSA parent (*Rpp3*?)
PI 423963	VIII	Japan	MUT	IM/RB	BSA parent
PI 567061	VIII	Indonesia	MUT	IM	BSA parent
PI 567072A	VIII	Indonesia	INT	IM/RB	BSA parent
PI 567089A	VIII	Indonesia	INT	MIX	BSA parent
PI 567132C	IX	Indonesia	INT	IM/RB	BSA parent
PI 567189A	IV	Vietnam	WT	IM	BSA parent
PI 417126	VIII	Japan	WT	RB	BSA parent (*Rpp2*?)
PI 566984	VI	Indonesia	WT	IM/RB	BSA parent (*Rpp4-b*/*Rpp6*?)

PI 566956	IX	Indonesia	INT	IM/RB	BSA parent (*Rpp6*?)
PI 567073A	VIII	Indonesia	INT	IM/RB	BSA parent (*Rpp6*?)
PI 200492	VII	Japan	WT	IM	*Rpp1* (Hyten et al., 2007)
PI 594538A	IX	China	WT	TAN	*Rpp1-b* (Chakraborty et al., 2009)
PI 224270	VII	Japan	WT	RB	*rpp2* (Garcia et al., 2008)
PI 417125	VIII	Japan	WT	RB	*rpp2* (Nogueira et al., 2008)
PI 230970	VII	Japan	WT	RB	*Rpp2* (Silva et al., 2008)
PI 459025B	VIII	China	WT	RB	*Rpp4* (Silva et al., 2008)
PI 200487	VIII	Japan	MUT	IM/RB	*Rpp3* + *Rpp5* (Garcia et al., 2008)
PI 200526	VIII	Japan	INT	TAN	*Rpp5* (Garcia et al., 2008; Kendrick et al., 2011)
PI 471904	IX	Indonesia	MUT	RB	*Rpp3* + *Rpp5* (Garcia et al., 2008; Kendrick et al., 2011)
PI 567102B	IX	Indonesia	INT	HR	*Rpp6* (Li et al., 2012)
PI 605823	IX	Vietnam	INT	RB	*Rpp7* (Childs et al.)

†WT (wild-type) alleles come from the VIC (lower-right) position of the KASP marker results; INT (intermediate) alleles come from the middle cluster of the KASP marker results; and MUT (mutant) alleles come from the FAM (upper-left) position of the KASP marker results. The MUT alleles are highlighted in gray.

‡Reaction data from screening accessions with the GA12 *P. pachyrhizi* isolate in the greenhouse (2016-2017). TAN is a susceptible reaction with abundant sporulation; RB is a resistance response with reduced sporulation; IM has no visible lesion development; IM/RB has either both IM or RB reactions on separate plants or an intermediate "flecking"; HR (hypersensitive) has a ghost-like, light-colored lesion with no sporulation; and MIX has some plants with RB or IM reactions and other plants with TAN reactions, due to seed admixture.

Table 3.9 Plant introductions which may harbor the *Rpp3*(PI 462312) allele, identified by screening 345 geographically diverse PIs with the marker GSM0551.

Plant introduction	MG[†]	Origin	Year[‡]
PI 203406	VIII	South Africa	1952
PI 416873A[§]	VIII	Japan	1977
PI 416874A	IX	Japan	1977
PI 417014A	IX	Japan	1977
PI 417129A	IX	Japan	1977
PI 417184A	VIII	Japan	1974
PI 417234	VIII	Japan	1974
PI 417389A[§]	VIII	Japan	1977
PI 423961A	IX	Japan	1978
PI 423971A	IX	Japan	1978
PI 434980B	IX	Central Africa	1979
PI 486330	VIII	India	1984
PI 603534A	VII	Shaanxi, China	1998

[†]Maturity group.

[‡]Year placed into the USDA Collection.

[§]Partially resistant to inoculation with a mixed population of *P. pachyrhizi* by Miles et al., (2006).

Table 3.10 Molecular marker genotype and inferred zygosity of *Rpp1* and *Rpp3* genes for parents and 11 breeding lines selected for stacked *Rpp1* and *Rpp3* loci.

Name[†]	Pedigree	GSM0422	GSM0549	*Rpp1*[‡]	GSM0412	GSM0551[§]	*Rpp3*[¶]
2131-34	G12-6518 x G12-6295	TT[#]	TT	MUT	CC	MUT + HET	MUT
2144-47	G12-6518 x G12-6295	TT	TT	MUT	CC	MUT + HET	MUT
2188-91	G12-6518 x G12-6295	TT	TT	MUT	CC	MUT + HET	MUT
2190-93	G12-6518 x G12-6295	TT	TT	MUT	CC	MUT + HET	MUT
2206-109	G12-6518 x G12-6295	TT	TT	MUT	CC	MUT + HET	MUT
2209-112	G12-6518 x G12-6295	TT	TT	MUT	CC	MUT + HET	MUT
2216-119	G12-6518 x G12-6295	TT	TT	MUT	CC	MUT + HET	MUT
2234-137	G12-6518 x G12-6295	TT	TT	MUT	CC	MUT + HET	MUT
2248-151	G12-6518 x G12-6295	TT	TT	MUT	CC	MUT + HET	MUT
2267-170	G12-6518 x G12-6295	TT	TT	MUT	CC	MUT + HET	MUT
2281-184	G12-6518 x G12-6295	TT	TT	MUT	CC	MUT + HET	MUT
G12-6295	G00-3213(3) x [P97M50(3) x L85-2378(*Rpp1*)]	TT	TT	MUT	TT	WT	WT
G12-6518	G00-3213(3) x [G00-3209 x G01-PR68(*Rpp3*)]	CC	CC	WT	CC	MUT + HET	MUT
G00-3213	N/A	CC	CC	WT	TT	WT	WT
G00-3209	Woodruff	CC	CC	WT	TT	WT	WT
P97M50	N/A	CC	CC	WT	TT	WT	WT
L85-2378	Williams 82 (5) x PI 200492	TT	TT	MUT	TT	WT	WT
G01-PR68	Dillon x Hyuuga	CC	CC	WT	CC	MUT + HET	MUT

[†]Combined seed lab ID (2015 F2 NNN) and molecular lab ID numbers.
[‡]Line was marked as mutant for *Rpp1* if both GSM0422 and GSM0549 had homozygous alleles from PI 200492.
[§]GSM0551 mutant and heterozygous alleles clustered together
[¶]Line was marked as mutant for *Rpp3* if both GSM0412 and GSM0551 had homozygous alleles from PI 462312.
[#]Gray shading indicates the presence of a marker allele associated with resistance

Table 3.11 Pedigrees and relative yield of 13 breeding lines or near-isogenic lines (NILs) planted at three locations in Georgia in 2015.

Name	*Rpp* gene	Pedigree	Relative yield (%) (2015)[†]
G00-3213 *Rpp1*[‡]	*Rpp1*	G00-3213(6) × [P97M50(3) × L85-2378(*Rpp1*)	NA[§]
G00-3213 *Rpp2*	*Rpp2*	G00-3213(6) × PI 230970(*Rpp2*)	NA
G00-3213 *Rpp3*	*Rpp3*	G00-3213(6) × [P97M50 × PI 462312(*Rpp3*)]	NA
G00-3213 *Rpp4*	*Rpp4*	G00-3213(6) × PI 459025B(*Rpp4*)	NA
G12-6342	*Rpp1*	G00-3213(3) x [P97M50(3) x L85-2378(*Rpp1*)]	109.2
G12-6440	*Rpp1*	G00-3213(3) x [P97M50(3) x L85-2378(*Rpp1*)]	96.5
G14-1068RR	*Rpp1* + *Rpp5*	[P97M50(5) x G01-PR68(*Rpp?Hyuuga*] x [P97M50(6) x L85-2378(*Rpp1*)]	94.3
G14-1148RR	*Rpp1* + *Rpp5*	[P97M50(5) x G01-PR68(*Rpp?Hyuuga*] x [P97M50(6) x L85-2378(*Rpp1*)]	91
G12-6536	*Rpp3*	G00-3213(3) x [G00-3209 X G01-PR68(*Rpp?Hyuuga*)]	97.2
G12-6543	*Rpp3*	G00-3213(3) x [G00-3209 X G01-PR68(*Rpp?Hyuuga*)]	88.6
G13-2083R2	*Rpp3*	G09PR-54457R2 x {G00-3213(4) x [G00-3209 x G01-PR68(*Rpp?Hyuuga*)]	89.6
G14-2478R2	*Rpp3*	[G00-3213RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp?Hyuuga*)}] x [G00-3213(3)] x [{P97M50(3) x L85-2378(*Rpp1*)]	101.5
G14-2492R2	*Rpp3*	[G00-3213RR2Y x {G00-3213(2)] x [G00-3209 x G01-PR68(*Rpp?Hyuuga*)}] x [G00-3213(3)] x [{P97M50(3) x L85-2378(*Rpp1*)]	99.4

[†]Percentage of the best yielding check cultivar (AG7733 or AG7934). Yield was based on three locations with three replications per location. Each replication consisted of double 3 m rows with approximately 40 plants per row.
[‡]The four NILs of G00-3213 have been described by King et al. (2016).
[§]Yield was not tested on the four NILs.

Table 3.12 Retained exotic introgressions *Rpp* loci indicated by SoySNP50K in soybean breeding lines.

Name	*Rpp* gene	Generations of introgression	Theoretical unlinked donor genome retained (Mb)[†]	Actual unlinked donor genome retained (Mb)	Theoretical linked donor genome retained (Mb)[‡]	Actual linked donor genome retained (Mb)
G00-3213 *Rpp1*	*Rpp1*	14	**0.03**	**0**	**2.6** ± 1.9	**2.7** ± 0.2
G00-3213 *Rpp2*	*Rpp2*	5	**16.8**	**8.0** ± 1.4	**6.1** ± 3.7	**25.2** ± 0
G00-3213 *Rpp3*	*Rpp3*	6	**8.2**	**0**	**5.6** ± 3.9	**21.3** ± 10.0
G00-3213 *Rpp*4	*Rpp*4	5	**16.6**	**7.6** ± 1.3	**6.4** ± 4.1	**5.3** ± 0.3
G12-6342	*Rpp1*	11	**0.26**	**1** ± 0.2	**3.3** ± 2.3	**2.5** ± 0.4
G12-6440	*Rpp1*	11	**0.26**	**0**	**3.3** ± 2.3	**2.6** ± 0.3
G14-1068RR	*Rpp1* + *Rpp5*	11 + 5	**0.3** & **16.6**	**2.2** ± 0.9	**3.3** ± 2.3 & **6.4** ± 4.1	**2.5** ± 0.4 & **3.7** ± 2.9
G14-1148RR[§]	*Rpp1* + *Rpp5*	11 + 5	**0.3** & **16.6**	**17.3** ± 8.7	**3.3** ± 2.3 & **6.4** ± 4.1	**1.2** ± 0.2 & **3.6** ± 2.9
G12-6536	*Rpp3*	4	**32.7**	**6.1** ± 4.8	**7.7** ± 5.1	**30.1** ± 0.6
G12-6543	*Rpp3*	4	**32.7**	**22.1** ± 18.1	**7.7** ± 5.1	**14.8** ± 1.9
G13-2083R2[¶]	*Rpp3*	6	**8.2**	**87.9** ± 37.7	**5.6** ± 3.9	**19.0** ± 12.6
G14-2478R2[¶]	*Rpp3*	11 + 4	**0.26** & **32.7**	**17.1** ± 9.9	**7.7** ± 5.1 & **3.3** ± 2.3	**36.2** ± 3.2
G14-2492R2[¶]	*Rpp3*	11 + 4	**0.26** & **32.7**	**19.5** ± 10.7	**7.7** ± 5.1 & **3.3** ± 2.3	**37.8** ± 3.0

[†]Mean obtained from the following equation $\mu = \left(\frac{1}{2}\right)^t$ (Muehlbauer et al., 1988), with whole genome distances calculated at 0.4418 Mb cM $^{-1}$ where *t* is the number of backcross generations and *e* is Euler's number (Schmutz et al., 2010; Song et al., 2004). Distances are in megabases (Mb).

‡Mean obtained from the following equation $\mu = \left(\frac{2}{L}\right)\left[\left(\frac{1}{t}\right)\left(1 - e^{-tL/2}\right)\right]$ and the SE from $V = \left(\frac{2}{L^2}\right)\left\{\left[\left(\frac{1}{t^2}\right)\left(2 - (tL + 2)e^{-\frac{tL}{2}}\right)\right] - \left[\left(\frac{1}{t}\right)\left(1 - e^{-\frac{tL}{2}}\right)\right]^2\right\}$ where *t* is the number of backcross generations, *L* is the length of the carrier chromosome in Morgans, and *e* is Euler's number (Muehlbauer et al., 1988). Centimorgan distances for calculation are from Song et al., (2004) with linked regions calculated at 0.197 Mb cM $^{-1}$ (Schmutz et al., 2010). Regions where donor introgressions are heterogeneous in the NIL are ignored.

§Also includes a marker-selected fragment near the *Rpp3* locus.

¶Includes RR2Y introgression.

CHAPTER 4

SUMMARY

Soybean rust (SBR) is a foliar disease of soybean [Glycine max (L.) Merr.] that is present in subtropical regions of the world and caused by the obligate biotrophic fungus *Phakopsora pachyrhizi* Syd. & P. Syd. SBR hampers soybean production in warmer regions of the world, such as the Southeastern USA, where the pathogen can overwinter and spread to production fields via windblown urediniospores.

A major goal of this research was to perform genetic mapping of a novel source of resistance to *P. pachyrhizi* found in PI 605823. Two mapping populations derived from PI 605823 x Williams 82 and PI 605823 x 5601T were used to map the resistance locus to a 154 kb interval on chromosome 19. This *Rpp* locus, named *Rpp7,* mapped to a different chromosome than the other six reported *Rpp* loci. *Rpp7* also provided a unique pattern of resistance reaction phenotypes when inoculated with nine geographically diverse *P. pachyrhizi* isolates, when compared to the reactions of sources of other *Rpp* genes. A four-SNP haplotype within the resistance locus was examined across 19,653 accessions in the USDA Germplasm Collection and out of 251 plant introductions (PIs) with the *Rpp7* haplotype, only four were known to be resistant to SBR.

Further research was conducted to identify additional soybean PIs with novel *Rpp* alleles. Soybean accessions were screened with *P. pachyrhizi* inoculation in field or greenhouse, and 166 out of 328 selected lines (of which many were previously screened) were resistant. Bulked segregant analysis (BSA) was performed on five mapping populations and four new sources of resistance at the *Rpp3* or *Rpp6* locus were identified. Fourteen resistant parents of mapping populations were inoculated with nine geographically diverse *P. pachyrhizi* isolaes and five lines with unique reaction phenotype patterns were identified that differed from the reactions of known *Rpp* gene sources. Additionally, based on a deletion

in the candidate gene Glyma06g40740, an *Rpp3* marker was developed and used to genotype resistant accessions. Eleven accessions from Vietnam were identified that have a potentially different *Rpp3* allele than the *Rpp3* allele found in PI 462312.

Finally, a new KASP™ SNP marker linked to the *Rpp1* resistance locus was designed and used along with three other markers to select single gene and pyramided *Rpp1* and *Rpp3* genes in University of Georgia breeding lines. Breeding lines were screened under greenhouse and field inoculation with *P. pachyrhizi* from Georgia and 57 out of 132 lines were found to be resistant. The reaction of 21 breeding lines to the nine *P. pachyrhizi* isolates confirmed that each had the same reaction phenotype as the donor sources of the respective *Rpp* genes. Furthermore, the length of donor genome content introgressed along with the *Rpp* genes was estimated to range from 1.2 – 37.8 Mb for 13 breeding lines.

This work represents significant advancement in knowledge of *Rpp* resistance to *P. pachyrhizi* in soybean. The discovery of an additional *Rpp* gene is of great interest to the soybean breeding community for development of rust resistant cultivars. Enhanced understanding of *Rpp* gene interactions with *P. pachyrhizi*, demonstration of effective molecular breeding techniques, and analysis of the effect of *Rpp* introgressions will help guide future breeding efforts. The identification of putatively novel sources of resistance will allow for future mapping work. Planting of rust resistant soybeans in the Southeastern USA should increase grower profits by reducing the need for costly fungicide applications.