

REVIEW

Effectiveness of Resistance Genes to the Soybean Rust Pathogen *Phakopsora pachyrhizi*

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Abstract

Rust is the most damaging fungal disease in soybean-producing areas. Until 1990, the disease was restricted to Asia and Australia. It later spread to Africa and South America, and finally to North America. Studies on the effectiveness of resistance genes were initiated in an area where the disease had newly occurred. In this review, the effectiveness of seven known resistance genes (*Rpp1* to *Rpp6* and *Rpp1-b*) is compared among regions. The variety carrying *Rpp6* was generally resistant to most isolates worldwide. Varieties carrying *Rpp1* and *Rpp3* showed resistance in the United States (US), but not in other regions. The resistance of soybean varieties carrying the *Rpp2* gene against soybean rust was shown to be overcome by highly variable pathogen populations. The performance of *Rpp4* was of moderate resistance in several soybean-producing areas worldwide. In contrast, after 2006, varieties carrying *Rpp1-b* showed resistance in South America, but not in the US. The effectiveness of *Rpp5* depends on the variety of soybean in which this gene occurs. In future studies, near-isogenic differential lines with each resistance gene adapted to a region will be more useful in obtaining information on pathogenic variation than the current differential varieties.

Discipline: Plant disease

Additional keywords: differentials, fungal disease, *Glycine max*, *Rpp* gene

Introduction

Soybean (*Glycine max* (L.) Merr.) is an important crop globally because it provides not only oil and protein for human consumption but also feed for animals. Rust is the most economically damaging disease affecting soybean-producing areas worldwide. The reduction of yield due to rust can reach up to 80% under conditions conducive to the disease (Yorinori et al. 2005). The planting of resistant soybean varieties is the easiest strategy to control the disease, and does not entail additional expenses. Therefore, the selection and development of resistant varieties became a topic of research after, and sometimes before occurrence of the disease. As past studies focused on the selection of resistant germplasms in specific regions, most compared differences in resistance among varieties. Therefore, this review compares the effectiveness of resistance genes among geographical regions. Other recently published review articles on the study of soybean rust (Kawuki et al. 2003b, Hartman et

al. 2011, Kelly et al. 2015, Rosa et al. 2015, Murithi et al. 2016) have also discussed the differential effectiveness of resistance genes.

History of soybean rust

Since soybean rust was first recorded in Japan in 1902, the disease subsequently spread to other Asian countries and Australia. In the 1990s, the disease occurred in Uganda and several other African countries until 2000 (Murithi et al. 2016). It later spread to Paraguay and Brazil—the largest soybean-producing areas in South America—by 2001 and within 3 years had spread to most soybean-producing areas in South America (Yorinori et al. 2005). In November 2004, the disease spread to the United States (Schneider et al. 2005). Soybean rust was reported in Mexico by 2005 (Cárcamo Rodríguez, et al. 2006) and in Cuba by 2006 (Perez-Vicente et al. 2010).

Two pathogens causing soybean rust have been

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identified: *Phakopsora pachyrhizi* and *P. meibomia*. Although both were considered the same pathogen in the past, a morphological separation was recently recognized (Ono et al. 1992) and distinguished using species-specific DNA primers (Frederick et al. 2002). *P. pachyrhizi* is a virulent pathogen that causes soybean rust more readily than *P. meibomia*. *P. pachyrhizi* and *P. meibomia* were named Asian soybean rust and American soybean rust, respectively, because *P. pachyrhizi* was found in Asia and Australia, whereas *P. meibomia* was identified in Central and South America until 2000 (Ono et al. 1992). *P. pachyrhizi* is distributed worldwide as the primary pathogen responsible for severe soybean rust damage.

Resistant soybean varieties

The planting of soybean varieties resistant to soybean rust is desirable from the standpoints of cost reduction and ease of practice. Up until now, seven resistance genes have been discovered: *Rpp1* to *Rpp6* and *Rpp1-b* (Hartwig & Bromfield 1983, Hartwig 1986, Garcia et al. 2008, Chakraborty et al. 2009, Ray et al. 2009, Li et al. 2012). Table 1 lists the varieties or lines known to possess resistance genes.

When varieties without resistance genes are infected with soybean rust, they produce tan color lesions (TAN), usually with abundant sporulation. When those with resistance genes are infected with incompatible races, they produce reddish-brown lesions (RB) with less sporulation. In some combinations, immune reactions (IM) with no visible lesions are observed (Bromfield 1984).

Method of evaluation

In pioneering studies conducted in the 1970s and 1980s, the color of lesions was the most important trait of soybean rust resistance. Bromfield (1984) classified the resistance and susceptibility of soybean varieties using the lesion color and uredinial number per lesion. According to his classification, however, the final judgement of resistance or susceptibility coincided with judgement based solely on lesion color. Yamanaka et al. (2010, 2015b) used the presence or absence of lesions, uredinial number per lesion, and sporulation levels to evaluate resistance. Several recent studies have evaluated resistance using not only lesion color but also quantitative traits (Bonde et al. 2006, Walker et al. 2011, 2014).

Past studies in fields, greenhouses or incubators have evaluated the responses of varieties and lines to soybean rust. In field evaluations, reaction types (IM, TAN, RB), disease severity, lesion density, and sporulation were

evaluated under natural infection or artificial inoculation conditions. In greenhouses or incubators, seedlings and detached leaflets are inoculated with purified isolates originating from a single lesion, single uredinium, or single spore or bulk isolates, and then evaluated for disease density, reaction types, uredinial number per lesion, and sporulation.

Major resistance genes to soybean rust

(1) *Rpp1*

Some varieties of soybean (i.e., PI 200492, PI 368039, PI 594760B) are known to possess the *Rpp1* gene. Although McLean & Byth (1980) reported that the PI 368039 variety contains *Rpp1* and an additional resistance gene, the other gene remains unknown.

Out of nine isolates collected after the inoculation of plants of PI 200492 in Taiwan, Lin (1966) observed no symptoms and no uredinia with six and seven isolates, respectively. However, the other two isolates produced uredinia. PI 200942 was shown to produce TAN reactions to all isolates collected at five locations in Taiwan (Yeh 1983). PI 200492 was immune or resistant by inoculation to isolates collected in India in 1973, Australia in 1979, and Hawaii in 1994 and 1989. However, this variety produced TAN reactions to isolates collected in Taiwan in 1972 and 1980, and in Thailand in 2001 (Bromfield 1984, Bonde et al. 2006, Pham et al. 2009, Ray et al. 2009, Paul et al. 2015). In Japan, although the majority of soybean and kudzu (*Pueraria lobata*) isolates collected from 1993 to 1997 induced susceptible reactions on PI 200492 (Yamaoka et al. 2002), isolates collected in 2007 to 2009 induced resistance reactions (Yamaoka et al. 2014). PI 200492 showed varied reactions in field experiments conducted in Vietnam from 2005 to 2009 (Pham et al. 2010).

In Africa, PI 200492 produced TAN reactions by inoculation with isolates collected in Zimbabwe and South Africa in 2001 (Bonde et al. 2006, Pham et al. 2009, Ray et al. 2009, Paul et al. 2015). In Uganda, low disease severity was observed in field trials conducted in 2005 and 2006 (Oolka et al. 2008). In Nigeria, 116 isolates collected in 2005 showed less virulence to PI 200492, except in four cases that did show virulence; moreover, the soybean varieties produced RB reactions in half of the isolates and mixed reactions in the other half (Twizeyimana et al. 2009, 2011).

The *Rpp1* gene in PI 200492 was effective against soybean rust in Brazil in 2001/2002, but ineffective in 2002/2003 (Yorinori 2008). Varieties containing *Rpp1* (i.e., PI 200492, PI 368039) were susceptible to most of the bulk populations of rust collected in Argentina,

Brazil, and Paraguay from 2007 to 2010 (Akamatsu et al. 2013).

In the US, rust isolates that induced TAN reactions in the PI 200492 variety were identified in 2004, but have induced immune or RB reactions since 2006 (Li

& Young 2009, Pham et al. 2009, Ray et al. 2009). The varieties PI 200492 and PI 547875 were found to be highly or substantially resistant in field trials conducted in the southeastern US from 2006 to 2009 (Walker et al. 2011); however, PI 547875 was shown to be less resistant

Table 1. Soybean varieties carrying resistant genes.

Plant line or variety	Original Name	Origin	Resistance gene	Reference
PI 200492	Komata	Japan	<i>Rpp1</i>	McLean & Byth (1980)
PI 368039	Tainung No. 4	Taiwan	<i>Rpp1</i>	McLean & Byth (1980)
PI 547875	L85-2378	USA	<i>Rpp1</i>	Walker et al. (2011)
PI 561356	Jin Yun Dou	China	<i>Rpp1</i>	Kim et al. (2012)
PI 594177	Himeshirazu	Japan	<i>Rpp1</i>	Yamanaka et al. (2015a)
PI 594760B	Gou Jiao Huang Dou	China	<i>Rpp1, rpp1</i> ¹	Garcia et al. (2011)
Xiao Jing Huang	Xiao Jing Huang	China	<i>Rpp1</i>	Yamanaka et al. (2015a)
PI 587886	Bai Dou	China	<i>Rpp1-b</i>	Ray et al. (2009)
PI 587855	Jia Bai Jia	China	<i>Rpp1-b</i>	Yamanaka et al. (2016)
PI 587880A	Huang Dou	China	<i>Rpp1-b</i>	Ray et al. (2009)
PI 587905	Xiao Huang Dou	China	<i>Rpp1-b</i>	Hossain et al. (2015)
PI 594538A	Min Hou Bai Sha Wan Dou	China	<i>Rpp1-b</i>	Chakraborty et al. (2009)
PI 594767A	Zhao Ping Hei Dou	China	<i>Rpp1-b</i>	Hossain et al. (2015)
PI 197182	Raub 16.1422	Malaysia	<i>Rpp2</i>	Laperuta et al. (2008)
PI 224270	Hougyoku	Japan	<i>Rpp2</i>	Garcia et al. (2008)
PI 230970	No. 3	Japan	<i>Rpp2</i>	Hartwig & Bromfield (1983)
PI 230971	No.4	Japan	<i>Rpp2</i>	Laperuta et al. (2008)
PI 417125	Kyushu 31	Japan	<i>Rpp2</i>	Laperuta et al. (2008)
PI 416764	Akasaya	Japan	<i>Rpp3</i>	Hossain et al. (2015)
Iyodaizu B	Iyodaizu B	Japan	<i>Rpp2</i>	Yamanaka et al. (2015a)
PI 462312	Ankur	India	<i>Rpp3</i>	Hartwig & Bromfield (1983)
PI 567099A	MARIF 2740	Indonesia	<i>Rpp3</i>	Ray et al. (2011)
PI 628932	FT-2	Brazil	<i>Rpp3</i>	Brogin (2005)
D86-8286	D86-8286	USA	<i>Rpp3</i>	Bonde et al. (2006)
PI 459025	Bing Nan	China	<i>Rpp4</i>	Hartwig (1986)
PI 459025B	Bing Nan	China	<i>Rpp4</i>	Hartwig (1986)
PI 200487	Kinoshita	Japan	<i>Rpp5</i>	Garcia et al. (2008)
PI 200456	Awashima Zairai	Japan	<i>Rpp5</i>	Garcia et al. (2008)
PI 200526	Shiranui	Japan	<i>Rpp5</i>	Garcia et al. (2008)
PI 471904	Orba	Indonesia	<i>Rpp5</i>	Garcia et al. (2008)
PI 567102B	MARIF 2767	Indonesia	<i>Rpp6</i>	Li et al. (2012)
UG-5	UG-5	Uganda	<i>Rpp1/Rpp3</i>	Paul et al. (2015)
PI 506764	Hyuuga	Japan	<i>Rpp3/Rpp5</i>	Kendrick et al. (2011)

1: Dominance or recessiveness is affected by genetic background (Garcia et al. 2011).

in a field in Florida, but remained resistant in Georgia in 2012 (Walker et al. 2014). Paul et al. (2013) confirmed that Florida isolates collected in 2011 and 2012 induced susceptible reactions on PI 200492 and PI 547875. In a field trial conducted in Mexico, the soybean varieties PI 200492 and L85-2378 (PI 547875) were shown to be resistant (Peña-del-Rio et al. 2014).

The resistance gene *Rpp1* of the PI 200492 variety was shown to be relatively effective in the US after 2006, but this was not the case in other regions.

(2) *Rpp1-b*

Rpp1-b was identified in 2009 (Chakraborty et al. 2009, Ray et al. 2009) as an *Rpp1* allele. Since then, the resistance gene as an *Rpp1* allele or a different gene close to *Rpp1* has been found in several varieties of soybean (i.e., PI 587855, PI 587880A, PI 587886, PI 587905, PI 594538A, PI 594767A) (Ray et al. 2009, Hossain et al. 2015, Yamanaka et al. 2015a, Yamanaka et al. 2016). Yamanaka (2015a) discussed that the resistance gene of PI 587886 may be different from *Rpp1-b* of PI 594538A, PI 594767A and PI 587905 on the basis of mapping with simple sequence repeat markers. In this review, PI 587886 is discussed in this section. These varieties were also assessed for resistance against soybean rust in several studies.

PI 587886, PI 587905 and PI 594767A produced IM or RB lesions through inoculating with isolates collected in India in 1973, Australia in 1979, Taiwan in 1980, Hawaii in 1994, and Thailand in 2001 (Pham et al. 2009, Ray et al. 2009). In Japan, in addition to the above-mentioned varieties, PI 587880A produced resistance reactions by inoculation with isolates collected from 2007 to 2009, although susceptible reactions were identified in PI 587886 (Akamatsu et al. 2013, Yamaoka et al. 2014).

PI 594538A showed the least disease severity and no sporulation in field trials conducted in Nigeria in 2005 and 2006, and was confirmed IM by inoculation using 116 isolates collected from three regions in Nigeria in 2005 (Twizeyimana et al. 2008, 2009).

A South African isolate collected in 2001 induced intermediate lesions with high sporulation on soybean varieties of PI 587880A, PI 587886, PI 587905, PI 594538A and PI 594767A (Bonde et al. 2006). Brazilian and Paraguayan isolates of soybean rust collected in 2001 induced susceptible and resistant reactions on PI 587886 and PI 587905, respectively (Pham et al. 2009). Akamatsu et al. (2013) reported that most of the 59 Argentinean, Brazilian, and Paraguayan isolates collected in 2007-2010 induced resistance reactions on PI 587855, PI 587880A, PI 587905 and PI 594767A, whereas susceptible reactions were produced on PI 587886.

In the US, soybean varieties carrying the *Rpp1-b*

gene produced resistant RB and TAN reactions by inoculation with Alabama and Louisiana isolates collected in 2004, respectively (Pham et al. 2009, Ray et al. 2009). A bulk isolate collected from kudzu in Mississippi in 2006 induced a mixture of TAN and RB lesions with a moderate level of sporulation on PI 587880A, and TAN with a moderate level of sporulation on PI 594767 (Li & Young 2009). Paul et al. (2015) reported that PI 587880A and PI 594538A inoculated with 24 isolates collected in the central and southern US in 2007 and 2008 produced TAN reactions, except for two isolates on PI 587880A. Walker et al. (2014) observed a high rust index (based on disease severity and sporulation) on soybean varieties PI 587880A, PI 594538A and PI 594767A in Florida and Georgia in 2012.

Soybean varieties carrying *Rpp1-b* are generally susceptible in the continental US, but resistant in the Eastern Hemisphere and in South America, with the exception being that South American populations of soybean rust induce susceptible reactions in PI 587886.

(3) *Rpp2*

Bromfield (1984), Bonde et al. (2006), and Pham et al. (2009) reported that isolates collected in Taiwan in 1972 and 1980, India in 1973, the Philippines in 1977, Australia in 1979, and Thailand in 2001 induced RB lesions to PI 230970 possessing *Rpp2* genes (Hartwig & Bromfield 1983). In contrast, another Taiwanese isolate collected in 1980 induced TAN reactions (Bonde et al. 2006, Pham et al. 2009). PI 230970 was susceptible to more than half of the rust isolates collected from soybean, but resistant to rust isolates collected from kudzu in Japan in the 1990s (Yamaoka et al. 2002).

A Zimbabwean isolate (ZM01-1) collected in 2001 elicited RB reactions with less sporulation in a susceptibility assessment on PI 230970 (Pham et al. 2009, Kim et al. 2012). Bonde et al. (2006) inoculated PI 230970 with the same isolate, and observed intermediate reactions between RB and TAN. In Uganda, no symptoms were observed on PI 230970 in three crop seasons in 2005 and 2006 (Oloka et al. 2008), and field trials conducted at five locations showed that PI 230970 was resistant (Maphosa et al. 2013). In South Africa, PI 230970 produced RB lesions with less sporulation (Bonde et al. 2006). In Nigeria, RB reactions were predominant by inoculation with 116 soybean rust isolates collected in 2005 (Twizeyimana et al. 2009).

A Brazilian isolate collected in 2001 induced lesions of an intermediate color (i.e., between TAN and RB) (Bonde et al. 2006), as well as RB lesions (Pham et al. 2009). Yorinori (2008) reported that PI 230970 was resistant in 2002, but became susceptible in 2003. A Paraguayan isolate collected in 2001 induced RB lesions

to PI 230970 (Bonde et al. 2006, Pham et al. 2009). In field experiments conducted in Paraguay in 2005/2006, PI 230970 showed low disease severity at 106 days after planting, but relatively high disease severity at 129 days after planting (Miles et al. 2008). In South America, a minority of rust populations collected in Argentina, Brazil, and Paraguay from 2007 to 2010 induced resistance to PI 230970 (Akamatsu et al. 2013).

In the southeastern US, although PI 230970 was generally resistant in 2006 but showed susceptibility to moderate resistance in 2008, it was highly resistant in Louisiana in 2008 (Walker et al. 2011). PI 230970 showed intermediate resistance in Florida and Georgia in 2009, 2011, and 2012 (Walker et al. 2014). PI 230970 showed resistance in a field assay conducted in Mexico in 2007 (Peña-del-Rio et al. 2014).

PI 230971 produced RB reactions by inoculation with 34 out of 50 isolates collected in Taiwan (Yeh 1983); it showed a similar reaction profile to PI 230970 in Japan (Yamaoka et al. 2002). PI 417125 showed resistance to less than half of the soybean rust samples in Japan from 2007 to 2009 (Yamaoka et al. 2014), in South America from 2007 to 2010 (Akamatsu et al. 2013), and in the southeastern US from 2006 to 2012, except in Louisiana in 2007 and 2008 (Walker et al. 2011, 2014). PI 417125 was resistant in Mexico (Peña-de- Rio et al. 2014).

PI 224270 was found to carry a recessive resistance gene at the *Rpp2* region, and showed resistance that varied among locations in the southeastern US fields in 2009 (Walker et al. 2014).

Pathogenic variations of soybean rust that induced reactions ranging from susceptibility to resistance have been reported as mentioned above. The resistance of soybean varieties carrying the *Rpp2* gene against soybean rust was shown to be overcome by highly variable pathogen populations.

(4) *Rpp3*

PI 462312 produced TAN reactions by inoculation with 48 out of 50 isolates collected in Taiwan (Yeh 1983). PI 462312 was susceptible to isolates collected in Taiwan in 1972 and 1980, the Philippines in 1977, and Thailand in 2001, but was resistant to isolates collected in India in 1973, Australia in 1979, and Hawaii in 1994 (Bromfield 1984, Bonde et al. 2006, Pham et al. 2009, Paul et al. 2015). However, PI 462312 was resistant to isolates collected in Australia in 1979 and in Hawaii in 1994 (Bromfield 1984, Bonde et al. 2006, Pham et al. 2009, Paul et al. 2015). In Japan, 15 out of 22 isolates collected from soybean in the 1990s induced susceptible reactions on PI 462312. However, only four out of 21 isolates and none of four isolates collected in Japan from kudzu in the 1990s and in 2007, respectively, induced susceptible

reactions (Yamaoka et al. 2002, 2014). All of the Japanese isolates collected from soybean and kudzu in 2007 to 2009 induced resistance reactions (Yamaoka et al. 2014).

Oloka et al. (2008) observed less disease severity on PI 462312 in 2005, but increased disease severity in 2006 in field trials conducted in Uganda. Artificial inoculation with bulk isolates collected from five regions of Uganda in 2011 produced RB reactions on PI 462312; however, PI 462312 was generally less susceptible than the plot average across five regions in field trials conducted in 2010 and 2011 (Maphosa et al. 2013). Twizeyimana et al. (2009, 2011) reported that all 116 Nigerian isolates were less virulent to PI 462312 and induced RB reactions. Bonde et al. (2006) reported that a South African isolate induced resistance reactions.

In Argentina, PI 462312 was susceptible to all soybean rust samples collected from 2007 to 2010, whereas PI 462312 was susceptible to the majority of samples from Brazil and to half the samples from Paraguay (Akamatsu et al. 2013). Bonde et al. (2006) reported mixed lesions of TAN and RB lesions by inoculation with Brazilian and Paraguayan isolates.

Three US isolates collected in 2004 in Alabama and Louisiana induced RB and TAN lesions, respectively, on PI 462312 (Pham et al. 2009). US isolates collected in 2007 and 2008 only induced RB lesions (Paul et al. 2015). Although PI 462312 showed resistance to soybean rust in field trials conducted in the southeastern US from 2006 to 2008, except in Quincy County in Florida in 2008 (Walker et al. 2011), it was shown to be relatively resistant in Attapulgus, Georgia in 2012 (Walker et al. 2014).

Bonde et al. (2006) reported that an *Rpp3*-carrying line of soybean (D86-8289) showed a different reaction profile from PI 462312 when exposed to isolates of soybean rust collected from India, Thailand, Taiwan, Brazil, and Paraguay. Li & Young (2009) reported mixed reactions of TAN and RB with less disease severity on PI 567099A carrying the recessive *rpp3* gene.

PI 416764 was resistant to all 26 isolates collected in Japan from 2007 to 2009 (Yamaoka et al. 2014). In South America, PI 416764 was susceptible to the majority of rust samples collected in Argentina and Brazil, and to half of the rust samples collected in Paraguay from 2007 to 2010 (Akamatsu et al. 2013).

In Asian countries, the *Rpp3* gene of PI 462312 was not effective against soybean rust before 2000. However, it became resistant in Japan in 2007 to 2009. In South America, it was relatively susceptible from 2007 to 2010. In contrast, this resistance gene was relatively effective in Africa and the US, although susceptibility reportedly depended on the area and year.

(5) *Rpp4*

PI 459025 and PI 459025B are varieties of soybean known to contain the *Rpp4* gene (Hartwig 1986). PI 459025 was resistant to five isolates of soybean rust collected in India, the Philippines, and Taiwan from 1973 to 1980 (Bromfield 1984). Bonde et al. (2006) and Pham et al. (2009) confirmed RB reactions by inoculation with isolates collected from India, the Philippines, Taiwan, and Thailand, with few exceptions. All 45 Japanese isolates of soybean rust collected from soybean, *G. soja*, and kudzu from 1993 to 1997 induced resistance reactions in PI 459025, except for one isolate (Yamaoka et al. 2002), whereas 21 out of 26 isolates collected in 2007 to 2009 induced resistance reactions (Yamaoka et al. 2014). Australian isolates collected in 1979 and Hawaiian isolates collected in 1994 induced TAN and RB reactions, respectively (Bonde et al. 2006).

Isolates collected in Zimbabwe and South America in 2001 produced RB and intermediate lesions between RB and TAN, respectively, on PI 459025B (Bonde et al. 2006, Pham et al. 2009, Kim et al. 2012). Bulk isolates collected from five regions of Uganda in 2011 produced RB reactions on PI 459025 by artificial inoculation, and PI 459025 was generally less susceptible than the plot average across five regions in field trials conducted in 2010 and 2011 (Maphosa et al. 2013). Twizeyimana et al. (2009, 2011) reported that 112 of 116 Nigerian isolates were less virulent to PI 459025, manifesting reactions of RB or mixed lesions of RB and TAN.

Although only a minority resistance reaction was obtained by inoculation with rust samples collected in Argentina from 2007 to 2010, resistance was induced with about half of the rust samples collected in Brazil and Paraguay (Akamatsu et al. 2013). In addition, Bonde et al. (2006) and Pham et al. (2009) reported resistant reactions using Brazilian and Paraguayan isolates.

Pham et al. (2009) also reported that PI 459025B produced RB lesions by inoculation with three isolates collected in Alabama and Louisiana in 2004. Li & Young (2009) found that PI 459025 produced RB lesions and showed less disease severity, but high sporulation by inoculating with a Mississippi isolate collected from kudzu in 2006. PI 459025B was shown to be susceptible to rust in a field trial conducted in Quincy, Florida in 2008 (Walker et al. 2011); however, this variety showed moderate resistant in 2011 to 2012 (Walker et al. 2014).

The performance of *Rpp4* in PI 459025 and PI 459025B was of moderate resistance in several soybean-producing areas worldwide.

(6) *Rpp5*

The soybean varieties carrying the *Rpp5* gene against soybean rust demonstrated variable performance. In Japan, PI 200526 showed resistance to all 26 isolates

belonging to six races collected in Japan from 2007 to 2009 (Yamaoka et al. 2014). PI 200526 also showed resistance reactions to a majority of rust samples collected from Argentina, Brazil, and Paraguay in 2007 to 2010 (Akamatsu et al. 2013).

When PI 200526 was inoculated with soybean and kudzu isolates of rust collected from the southeast, southern, and central regions of the US from 2006 to 2009, this variety produced TAN reactions with relatively high sporulation (Twizeyimana & Hartman 2012, Paul et al. 2015). In the southeastern US, PI 200487 possessing the *Rpp5* gene showed variable resistance reactions depending on the field trials in 2006 to 2012 (Walker et al. 2011, Walker et al. 2014). PI 200487 was resistant in Quincy, Florida in 2007 and 2009, but less resistant in 2011 and 2012, and susceptible in 2008. PI 200487 was relatively resistant in Fairhope, Alabama in 2007 and in Blackville, South Carolina in 2008, but susceptible in Bossier City, Louisiana in 2007. In Attapulgus, Georgia, PI 200487 was less resistant in 2008, but resistant in 2012.

PI 471904 showing incomplete dominant resistance was inoculated with 24 US isolates of rust collected in 2007 and 2008, with immune or resistance reactions being observed (Paul et al. 2015). PI 471904 showed resistance in Quincy, Florida in 2009, relative resistance in 2011 and 2012, and was resistant in Attapulgus, Georgia in 2012 (Walker et al. 2014).

PI 200456 is an additional soybean variety possessing a recessive *rpp5* allele; it was shown to be susceptible to rust at Quincy and Attapulgus in the US in 2012 (Walker et al. 2014).

The effectiveness of the *Rpp5* gene against rust differed among varieties. Paul et al. (2015) showed PI 200526 to be susceptible, whereas PI 471904 was resistant against inoculation with US isolates. Walker et al. (2011, 2014) also showed different levels of resistance performance according to varieties possessing the *Rpp5* gene in the field. The two reports have suggested that the *Rpp5* gene is not effective against most strains of the rust pathogen in the southeastern, southern, and central regions of the US, but is nevertheless effective against some strains. The difference in effectiveness of *Rpp5* among varieties may be attributable to the differences in genetic background other than *Rpp5*. The effectiveness of the *Rpp5* gene is dependent on the region and may be affected by the varieties possessing this gene.

(7) *Rpp6*

The *Rpp6* gene was recently identified in an Indonesian variety of soybean (i.e., PI 567102B) (Li et al. 2012), and has shown resistance reactions to rust in several studies.

Rust isolates collected in India in 1973, in Taiwan in

1980, in South Africa in 2001, and in Zimbabwe in 2001 induced RB reactions on PI 567102B by inoculation (Paul et al. 2015).

In Paraguay, PI 567102B showed low disease severity in a field trial conducted in 2005/2006 before its resistance gene was identified (Miles et al. 2008). Although disease severity was also low in the 2006/2007 crop season, mixed TAN and RB reactions were observed within the variety, and some lesions also produced urediniospores. In Brazil, half of the samples from 2010 to 2015 induced resistance reactions (Kato et al. 2015).

In the US, PI 567102B showed resistance in field trials conducted in Alabama in 2007 and South Carolina in 2008, and was relatively resistant in Florida and Louisiana in 2008; however, it showed susceptible reactions in Georgia in 2008 (Walker et al. 2011). A total of 24 isolates collected in Alabama, Arkansas, Florida, Georgia, Illinois, Louisiana, Mississippi, Oklahoma, and Texas in 2007 and 2008 induced RB or hypersensitive reactions in PI 567102B (Paul et al. 2015). Li & Young (2009) reported that PI 567102B showed RB reactions with low disease severity and no sporulation by inoculation with Mississippi bulk isolates collected from kudzu in 2006.

Rpp6 was relatively resistant to rust in most soybean-producing areas worldwide, although some susceptible reactions are evident.

Multiple Rpp genes

Some varieties or lines of soybean are known to possess multiple resistance genes. PI 368039 possessed the *Rpp1* gene as well as an unknown resistance gene (McLean & Byth 1980). Fourteen of the 24 isolates collected from soybean in the 1990s and none of the 26 isolates collected in the 2000s in Japan induced susceptible reactions on PI 368039 (Yamaoka et al. 2002, 2014). PI 506764 (Hyuuga soybean) possesses both the *Rpp3* and *Rpp5* genes (Kendrick et al. 2011), and was shown to be resistant to 67 US isolates (Twizeyimana et al. 2012). UG-5 possesses both the *Rpp1* and *Rpp3* genes (Paul et al. 2015), and was shown to be resistant in field trials conducted in Uganda from 2000 to 2002 (Kawuki et al. 2003a) and in 2010 and 2011 (Maphosa et al. 2013).

Pyramiding of resistance genes is an effective strategy to develop resistant varieties. Maphosa et al. (2012) showed that pair-wise combinations of *Rpp2*, *Rpp3*, and *Rpp4* produced more resistance against rust than the parents containing a single Rpp gene. Yamanaka et al. (2013) also showed the enhancement of resistance in a pyramided line possessing *Rpp2* + *Rpp4* + *Rpp5* using Brazilian and Japanese isolates in 2007 and 2008.

Conclusions

Pathogenic differences of *P. pachyrhizi* were observed temporally and geographically. These differences are mostly derived from genetic diversity in the virulence or avirulence genes of the strains being studied. However, other factors may affect pathogenic differences.

Genetically purified isolates by single urediniospore isolation or successive mono-uredinial isolation are useful for studies on reactions in a combination of soybean varieties and pathogen isolates. There are possibly several races in a field population of soybean rust because mixed reactions to rust were reported (Bonde et al. 2006, Miles et al. 2008, Li & Young 2009, Twizeyimana et al. 2009, 2011). In the process of purification, the rust population would have less diversity. To evaluate the effectiveness of resistance genes and select resistant varieties, bulk isolates of soybean rust may also be more useful than genetically purified ones.

As described in the sections on *Rpp1* and *Rpp5*, the effectiveness of these genes depends on soybean varieties that carry the resistance genes. An interaction of other genes with the resistance genes or differences in genetic background among the varieties may affect the effectiveness of the resistance gene. Some differential varieties of soybean belong to different maturity groups. Early maturing differentials can escape a rust epidemic. However, because soybean rust generally develops more rapidly at older growth stages (Sinclair & Hartman 1999), early maturing differential varieties may show higher disease severity than late maturing ones when both varieties are evaluated simultaneously. Near-isogenic differential lines with each resistance gene adapted to a region are thus required for future study.

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