Screening US Soybean Germplasm for Rust Resistance

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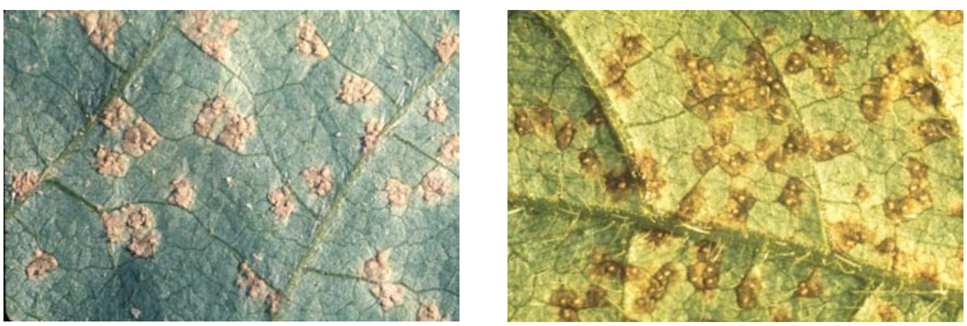
Abstract

Soybean rust (*Phakopsora pachyrhizi* Sydow), which has been known as one of the most destructive foliar diseases of soybean in the world, was discovered in the continental United States in November 2004. Rapid spread of the pathogen in Southern and Midwest states increases the potential threat to soybean production in the country. Great research efforts have been made in collaborations of national and international institutions aimed at the evaluation of the USDA soybean germplasm collection and exotic germplasm for resistance to the disease. Over 16,500 soybean accessions were initially evaluated in a two-tiered inoculation program at the USDA-ARS BSL-3 containment facility, Ft. Detrick. Of these, 805 accessions with RB lesion or low disease severity were selected for re-evaluations. Subsequently, field and net house tests conducted in Vietnam and Paraguay in two consecutive seasons identified many accessions consistently exhibiting resistant reactions to rust. Other field and greenhouse tests of 778 accessions conducted in Georgia identified many PIs with varied levels of resistance. To further confirm the new sources of resistance, these resistant accessions were tested with ten isolates of rust, including three newly purified isolates from the US. The results showed that these soybean accessions had resistant reactions (RB lesions) or low severity to certain isolates, suggesting that they may have additional resistant genes other than four known dominant *Rpp* genes or partial resistance. These accessions can be potential sources of resistance for the development of new resistant variety in soybean breeding programs.

1. Introduction

Soybean rust, caused by the fungus *Phakopsora pachyrhizi* Sydow, has been known in Australia, Asia, Africa and South America, and has now become established in North America. Significant yield losses varying from 40 to 90% have been reported in Asian countries when environmental conditions were conducive for disease development (Sinclair and Hartman, 1999). The rapid aerial spread of the pathogen and the potential for high risk of severe yield losses makes this potentially the most destructive foliar disease of soybean (Hartman et al., 2005). In the United States, the disease was first reported in Hawaii in 1994 (Killgore et al., 1994). In November 2004, soybean rust was first observed and officially confirmed near Baton Rouge, Louisiana, (Schneider et al., 2005). By November 2005 soybean rust was confirmed in eight southern states (FL, GA, AL, MS, SC, NC, LA, and TX) (http://www.sbrusa.net/). During 2006, soybean rust was found infecting more than 260 counties in 15 states (AL, AR, FL, GA, IL, IN, KY, LA, MO, MS, NC, SC, TN, TX) (http://www.sbrusa.net/) by November 10. This indicates that future soybean production in the US could be at a high risk for potential rust epidemics which will have a major impact on soybean production and production costs.

Although chemical control has been recommended as the first line of defense, breeding for resistant varieties with greater yield stability and discovery of additional single-gene or partial resistance will be an economically effective approach for long-term rust management (Hartman, et al., 2005). Genetic studies of resistance to soybean rust have identified four single dominant genes, Rpp_1, Rpp_2, Rpp_3, and Rpp_4, (McLean et al., 1980; Bromfield et al., 1980; Hartwig and Bromfield, 1983; Hartwig, 1986). These genes condition specific resistance to a limited set of rust isolates. When inoculated with rust isolates, soybean genotypes with Rpp_1 or the other genes produce red-brown (RB) lesions (Fig. 1), which were considered to be a resistant lesion type; in contrast, soybean genotypes producing TAN lesions (Fig. 1) are fully susceptible. However, single-gene resistance in soybean has not been found durable soon after the resistant sources were identified. Plant introductions (PI) or cultivars introduced with the *Rpp* genes, were found producing susceptible TAN lesions in the field (Kochman, 1977; Bromfield, 1984). It was believed that the fungus *P. pachyrhizi* had considerable variation in virulence among isolates and was able to effectively overcome single-gene resistance in soybean (Hartman et al., 2005). Thus, an alternative approach for the identification of resistant sources was an urgent need for soybean rust management.



TAN lesion RB lesion

Figure 1. Soybean rust lesion types: TAN = fully susceptible reaction, RB = resistant reddish brown lesions with defined margins (Miles et al., 2006).

Partial resistance to soybean rust was reported in soybean (Wang and Hartman, 1992). It was suggested that these soybean genotypes developed fewer lesions throughout the season or had smaller lesions and longer latent periods resulting in reduced pustule numbers over time (Miles et al., 2003). As a result, it could effectively reduce the need for multiple fungicide applications (Hartman et al., 2005). However, identification and utilization of partial resistance and development of the methodology for its use has been limited, especially under field conditions. Use of partial resistance may be time-consuming and difficult to incorporate into breeding programs. Moreover, dealing with yield stability in a breeding program may not be an easy task, because it requires yield comparisons of breeding material in soybean plots with and without fungicide applications under severe rust epidemics.

In collaborative efforts of USDA and universities with support from the United Soybean Board (USB), evaluations of the USDA soybean germlasm for resistance to *P. pachyrhizi* was initiated at the USDA-ARS Foreign Disease-Weed Science Research Unit (FDWSRU), Ft. Detrick. At the same time a set of accessions identified from the literature was evaluated in field trials in Africa, Asia and South America. In summer 2005, additional soybean accessions were evaluated in the field in South Georgia and Florida, where soybean rust was previously reported. The objective of the present report is to summarize the results of national and international research collaboration aiming to the identification and evaluation of resistant sources of the US soybean germplasm for specific and partial resistance that can be useful for soybean breeding programs in the US.

2. Preliminary Screening of the US Soybean Germlasm Collection

Since the first report of soybean rust in Hawaii in 1994, the USDA-ARS has renewed its support for soybean rust research in the US and has expanded its international programs as well. Initially, more than 16,500 soybean accessions in the USDA germplasm collection, along commercial and public cultivars were screened for resistance to *P. pachyrhizi* at the USDA-ARS FDWSRU Biosafety Level 3 containment greenhouses (Miles et al., 2006). Soybean seedlings (14-18 days old) of these accessions were inoculated with a mixture of four isolates, one each from Thailand, Brazil, Paraguay, and Zimbabwe. A 1-5 disease severity scale (Fig. 2) was used to score lesions of infected leaves 14 days post inoculation.

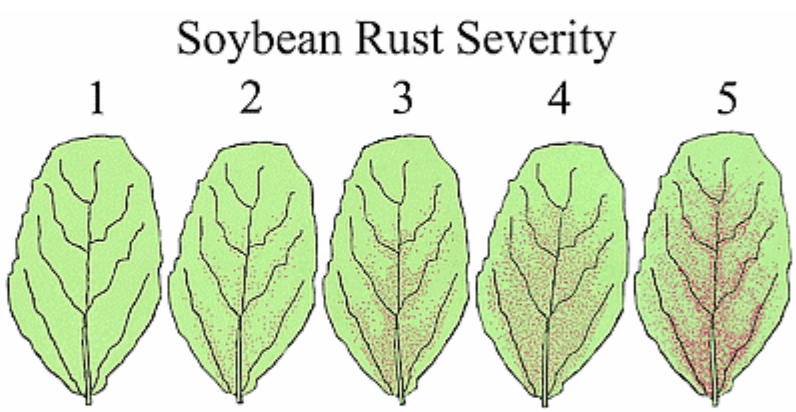


Figure 2. A 1-5 severity scale used for the visual assessment of soybean rust in the preliminary evaluations of the USDA soybean germplasm collection. 1 = no visible lesions, 2 = few scattered lesions present, 3 = moderate number of lesions on at least part of the leaf, 4 = abundant number of lesions on at least part of the leaf, and 5 = prolific lesion development over most of the leaf (Miles et al, 2006).

The germplasm evaluations were performed in a two-round procedure. The results of the first evaluation showed that the majority of accessions (87%) had a disease severity varying from 3 to 5 and the remaining (13%) was rated as 2 or less for severity. The RB lesion was also recorded in 1,237 accessions (7.4%) that had disease severity being rated from 1 to 5. Based on the categorizations, a total of 3,215 accessions (19%) with severity scored of 2 or less and RB lesion at any disease severity level were selected for a second evaluation. None of the US commercial cultivars evaluated were found to be resistant to the mixture of rust isolates. Subsequently, the selected accessions were re-evaluated with three replicates using the same inoculation technique and disease severity rating. The results showed that 1,864 accessions (58%) were rated between 3.0 and 3.9, and more than 400 accessions (13%) were rated from 1.0 to 2.9. Over 500 accessions (17%) had RB lesion distributed across all disease severity levels. From these evaluations, a subset of 805 accessions, which had a mean severity of 2.7 or less or had RB lesion, were selected as potential sources of resistance (Fig. 3)

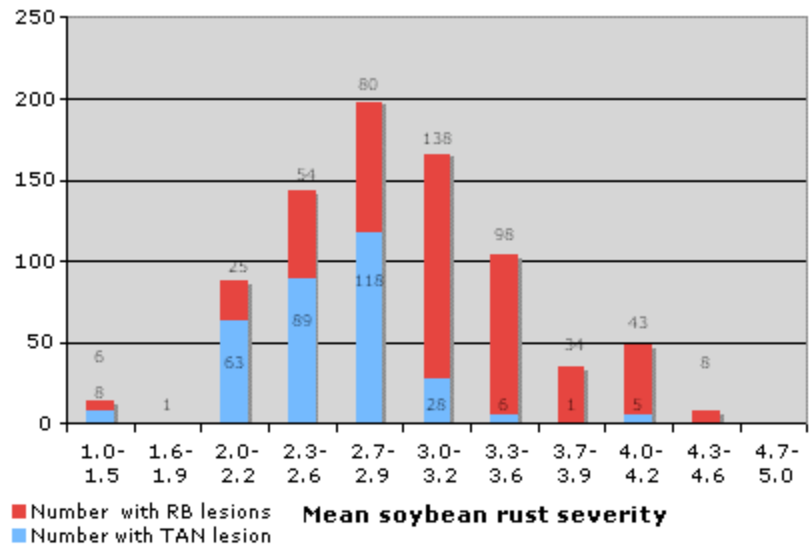


Figure 3. Frequency distribution of 805 soybean accessions with either TAN or RB lesion type in the second preliminary evaluation (Miles et al., 2006).

3. International Cooperation for Rust Resistant Germplasm Evaluations

In addition to efforts to screen the USDA soybean germplasm collection at the USDA-ARS FDWSRU containment facility at Ft. Detrick, international collaborations were initiated to re-evaluate selected soybean accessions in foreign countries where rust epidemics often occur. In 2005, in a collaboration of University of Illinois, University of Missouri, and USDA-ARS and Vietnam Agriculture Sciences Institute (VASI), Vietnam, a USDA-FAS-funded research was conducted at VASI to evaluate US and Vietnamese soybean germplasm for resistance to rust (Fig. 4A). Initially, 57 US soybean accessions, including plant introductions (PIs) and public varieties, were tested along with susceptible and resistant checks. Plants were inoculated twice at the V6 and R1 growth stages with a spore suspension (5×10^4 spores/ml) of a local unpurified isolate of the fungus. Disease severity was assessed at different times from growth stages R2 to R6, using a 1-4 severity scale, where 1 = no symptoms and 4=heavily number of pustules (>500). Lesion types and sporulation were recorded. Area under disease progress curve (AUDPC) was also computed for quantification of partial resistance.

A B

Figure 4. Selected US soybean accessions and local checks were re-evaluated in the field in 2005- 2006 at the VASI, Vietnam (Panel A) (Vuong et al., 2005) and CRIA, Paraguay (Panel B) (Miles et al., 2003).

In the spring of 2006, a subset of 39 US accessions selected from the 2005 field and net house evaluations were re-evaluated for their resistance to soybean rust. The AUDPCs, lesion types, and sporulation of US soybean accessions were compared with those of local checks (Table 1). Several PIs, such as PI 437323, PI 459025B, and PI 230970, had RB lesion type, low sporulation, and disease progress remained as low as the local resistant check, DT2000 (PI 635999). In addition to DT2000, two other local cultivars, Vang Ha Giang and Cao Bang, and the line GC84058-5 also consistently showed RB lesions and lower rust severity across the tests (Vuong et al., 2005).

In addition to 57 US soybean accessions initially studied in 2005 at VASI, 25 additional PIs from maturity groups (MGs) III to VI, which had previously reported to have resistance to rust (Dr. Roger Boerma, pers. comm.), were also evaluated in the spring of 2006 at the National Plant Protection Institute (NPPI), Vietnam. Spore inoculation method and disease severity assessment were performed as previously described. Preliminary results showed that most of these soybean accessions had TAN lesions and greater sporulation. A few accessions had RB or mixed lesion type, but greater AUDPC values not different from the susceptible check (data not shown). Among these accessions, a few accessions, such as PI 605781A, PI 605830A, which originated from Vietnam, were also reported to be resistant to rust when evaluated in Georgia in 2005 (Walker et al., 2006). However, it was recommended that these accessions need to be re-evaluated to confirm the results.

In Paraguay, 544 selected soybean accessions from MGs III through IX were evaluated for rust resistance in the field and greenhouse trials at Centro Regional de Investigación Agrícola (CRIA) in Capitán Miranda, Paraguay, during the 2005-06 growing season (Fig. 4B). A 1 to 9 scale was employed for disease severity rating. The results showed that rust severities ranged from 1.0 (resistant) to 9.0 (susceptible) in each of the maturity groups. Of these, two accessions, PI 587886 and PI 594754, were immune in both the field and greenhouse evaluations. No soybean rust was found on any of the trifoliates evaluated.

The results of field and greenhouse re-evaluations in Vietnam and Paraguay suggest that PIs and cultivars with RB lesions and low AUDPC and severity can be potential sources of resistance to *P. pachyrhizi* in soybean breeding programs.

4. Evaluations of Rust Resistant Germplasm in the USA

Since soybean rust was reported in the southern states, efforts have also been made to re-evaluate selected accessions in the US. In 2005, 778 soybean accessions from the preliminary screen were evaluated in the field at Attapulgus, Georgia (Drs. Roger Boerma and Dan Phillips, pers. comm.). Artificial lighting was employed to synchronize flowering among the accessions, which ranged from MGs 000 to X. A 1-5 scale indicating disease severity and distribution in the canopy (and therefore different from the scale used by Miles et al., 2006) was used to rate accessions was used to rate accessions. Out of 778 accessions tested, 328 selected accessions were assayed in a greenhouse in early 2006 with a pooled isolates of *P. pachyrhizi* from several locations in Georgia. Of these, a subset of 72 accessions showed varied levels of resistance with RB or low severity in the greenhouse tests. Although many appeared to have TAN lesions in the field evaluations (data not shown), subsequent experience suggests that some older RB lesions may have been misclassified. The University of Georgia investigators suggested that further field and greenhouse re-evaluations are needed to confirm the resistance of these accessions (Walker et al., 2006).

In 2006, an additional set of 244 accessions from MGs III through X were evaluated for rust resistance in a field trial at the University of Florida's North Florida Research and Education Center, in Quincy, FL. Soybean rust severities ranged from resistant to susceptible in each of the maturity groups. No immune response was observed in any of the lines. Data from this location, a location in Alabama, Paraguay, and in the containment facility at Ft. Detrick, Maryland, are being compared.

Due to the lack of the durability of known resistance genes (*Rpp*), the identification of new sources of resistance in soybean germplasm has become urgent. As part of the effort, several soybean accessions previously identified as resistant in Vietnam (Table 1) and Paraguay (unpublished data), along with other Vietnamese local cultivars and the sources of the four known genes, were further tested with ten rust isolates from various soybean growing regions of the world, including isolates from the southern states of the US. The test was conducted at the USDA-ARS FDWSRU BSL-3 containment facility, Ft. Detrick. Overall, the susceptible checks, Williams 82 and DT12, had TAN lesions to all ten of the isolates. Most accessions varied in reaction type from immune, RB, mixed, to TAN across isolates. The exception was PI 459025B, which had RB lesion types with all isolates. Three accessions, PI 594754**,** PI 605833 and PI 587905, identified as resistant in Paraguay, had RB reactions with low sporulation to nine isolates, but had TAN lesion to the isolate from Louisiana (LA04-1). Vietnamese cultivars and the Asian Vegetable Research and Development Center (VRDC) breeding lines, DT2000, DT95, and GC84058-18-4, had RB reactions to many isolates, including the isolate LA04-1, which have high degree of virulence. Between the two accessions identified as resistant in Vietnam, PI 437323 (Table 1) had RB lesions to the four most virulent isolates (TW72-1, TH01-1, PG01-2 and LA04-1). The PI 594754 identified as resistant in Paraguay was immune to the isolate IN73-1, similar to the immune reaction of PI 200492 (Rpp_1), suggesting that the PI 594754 may carry the Rpp_1 gene. These findings reflect the fact that the identified accessions have specific resistance to certain rust isolates. The results also showed that the three newly purified rust isolates in the U.S. may have a high degree of virulence based on the lesion types observed in accessions, especially the isolate AL04-3, which showed intermediate severity and caused TAN reactions on 12 out of 18 accessions studied.

Conclusions

With the support of the USDA-ARS, USDA-FAS, United Soybean Board (USB), and NCSB, great efforts of national and international cooperation have been initiated to evaluate the USDA soybean germplasm collection along with privately developed and public varieties for resistance to soybean rust. Although none of commercial or public varieties were found to be resistant, a number of soybean PIs exhibited resistant reaction lesions or low disease severity levels to rust. Subsequent re-evaluations of these selected PIs in the field in Vietnam and Paraguay identified several PIs with consistent resistance reactions to local isolates of rust.

When the accessions identified as resistant in the field were evaluated in seedling screens conducted at the USDA-ARS BSL-3 containment facility, Ft. Detrick, against ten rust isolates, including three isolates recently collected in the US, physiological specialization or race specificity was identified. The pattern of the resistant reaction in these accessions differed across isolates from that of the accessions that were the sources of the four known genes. It suggests that these PIs may carry new sources of resistance genes that are different from the four known resistant genes (*Rpp*). Some of these PIs have been used to develop genetic populations for molecular research and soybean breeding programs of the University of Illinois, the University of Missouri, and the University of Georgia.

Although fungicide application is recommended for chemical control, breeding for genetic resistance will be an economically effective approach for a long-term management of soybean rust. The availability of simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers for soybean (Cregan et al., 2006), coupled with high-throughput genomic genotyping technologies, such as Illumina BeadStation, ABI 7900 HT qRT-PCR provide soybean researchers effective tools to facilitate genetic mapping and marker-assisted breeding.

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Table 1. Area under disease progress curve, lesion type, and sporulation level of 39 US soybean plant introductions and public varieties re-evaluated in spring 2006 at NPPI, Hanoi, Vietnam. The accessions were blocked by three maturity group ranges.

Accession	**AUDPC**	**LSD test**	**Lesion type**	**Sporulation**	(a)
MG III-IV					
Williams 82	365.1	a	TAN	5	
PI407730	358.4	ab	TAN	5	
Rend	332.8	ab	TAN	5	
PI068494	330.1	ab	TAN	5	
PI567565	319.6	b	TAN	5	
PI088452	264.8	c	TAN	5	
Pana	264.3	c	TAN	5	
PI561287A	257.4	c	TAN	5	
Vang Ha Giang	192.8	d	Mixed	3	√
PI437323	180.6	de	RB	2	√
PI549017	173.7	de	TAN	5	
DT2000 (b)	161.5	de	RB	2	√
GC84058-5	143.4	e	RB	2	√
LSD.05	***43.5***				
MG V-VII					
PI429329	476.3	a	TAN	4	
PI459025F(*Rpp4*)	448.7	a	TAN	5	
PI628859	434.8	ab	TAN	5	
PI427241	380.4	bc	TAN	5	
PI548484	340.6	cd	TAN	5	
Bragg	339.2	cd	TAN	5	
PI548463	320.1	de	TAN	5	
PI561287B	306.2	de	TAN	5	
PI200492(Rpp1)	298.1	def	TAN	5	
PI208437	285.9	defg	TAN	5	
PI518759	282.5	efg	TAN	5	
PI594172A	272.2	efgh	TAN	5	
PI085089	243.2	fgh	TAN	5	
PI459025B(*Rpp4*)	233.5	gh	RB	2	√
PI398998	230.0	gh	RB	2	√
PI319525	222.4	h	TAN	5	
DT2000 (b)	161.5	i	RB	2	√
PI230970 (*Rpp2*)	146.0	i	Mixed	3	√
LSD.05	***56.4***				
MG VIII-IX					

PI206258	597.7	a	TAN	4	
Jupiter-R	494.6	b	TAN	4	
PI240667A	477.6	b	TAN	5	
PI189402	433.8	bc	TAN	4	
PI164885	373.4	cd	TAN	4	
PI417089A	348.6	de	TAN	4	
PI417317	310.5	def	TAN	5	
PI594538A	292.0	ef	TAN	5	
PI462312(*Rpp3*)	274.9	ef	TAN	5	
PI423972	245.3	fg	Mixed	3	√
Cao Bang	185.8	gh	Mixed	3	√
DT2000 (b)	161.5	h	RB	2	√
LSD.05	***78.1***				

(a) The marked accessions were selected as potential sources of resistance to rust.
(b) The local check DT2000 was officially designated as PI635999 in 2006.