Published online January 22, 2007

Soybean Rust Resistance Derived from *Glycine tomentella* in Amphiploid Hybrid Lines

M. E. Patzoldt, R. K. Tyagi, T. Hymowitz,* M. R. Miles, G. L. Hartman, and R. D. Frederick

ABSTRACT

Soybean rust (SBR), caused by the fungal pathogen *Phakopsora pachyrhizi* Syd., has the potential to cause significant economic yield loss in U.S. soybean production. Four single dominant resistance genes have been identified in soybean [*Glycine max* (L.) Merr.] that only confer specific resistance to a few rust isolates that have been tested. Additional resistance genes have been identified in wild perennial relatives, including *G. tomentella* Hayata (accession PI 483218, 2*n* = 78). Intersubgeneric hybrids have been created between *G. max* (cv. Altona) and this *G. tomentella* accession. Amphiploid hybrid lines (2*n* = 118) were the result of this hybridization and when further backcrossed to *G. max* (cv. Clark 63), derived fertile lines (2*n* = 40) were also generated. Both sets of progeny were screened at the USDA-ARS facility at Ft. Detrick, MD, to determine if the resistance to SBR was inherited in the subsequent populations. The amphiploid hybrid clones still retained the genetic SBR resistance that was found in the *G. tomentella* parent. However, the derived fertile lines were susceptible. These lines were not screened for SBR resistance following each backcross, which may explain this occurrence. Reinstituting the backcross procedure, while testing for SBR resistance at every generation, could move the SBR resistance gene(s) from *G. tomentella* to the cultivated soybean *G. max*.

SOYBEAN RUST is a foliar soybean disease capable of causing significant economic yield loss (Bromfield, 1984; Ogle et al., 1979; Sinclair, 1989). SBR was first found in North America in 2004 (Schneider et al., 2005). Four major resistance genes, *Rpp1* through *4*, that confer specific resistance to *P. pachyrhizi* have been identified in *G. max* (Bromfield and Hartwig, 1980; Hartwig and Bromfield, 1983; Hartwig, 1986). However, isolates of *P. pachyrhizi* have been identified that are capable of causing a susceptible reaction type (TAN lesions) despite the presence of SBR resistance genes (Hartwig, 1986, Miles et al., 2003). No general resistance or tolerance genes of SBR have been identified (Miles et al., 2006). The *Rpp1*-mediated resistance response does not yield any visible macroscopic symptoms following inoculation with the *P. pachyrhizi* isolates Australia 79-1, India 73-1, Hawaii 94-1, and Hawaii 98-1, while other isolates result in a susceptible reaction type with TAN lesions. The *Rpp1* resistant reaction type has been referred to as an "immune response." The resistant response that is elicited by the *Rpp2*, *Rpp3*, and *Rpp4* genes when inoculated with some isolates consists of a few lesions that are reddish-brown (RB) in color and typically produce only small amounts of urediniospores which have been referred to as the RB reaction type (Bromfield et al., 1980). Application of fungicide is currently the only method to manage SBR (Hartman et al., 1991; Miles et al., 2003). Screening the known germplasm of *G. max* and *G. soja* has not identified any accession with broad-spectrum resistance to all of the isolates of *P. pachyrhizi* tested to date (Miles et al., 2006).

Additional sources of genetic resistance to this disease have been identified in the wild perennial *Glycine* species, including *G. tomentella* (Burdon and Marshall, 1981; Burdon, 1986; Hartman et al., 1992). There are significant difficulties in utilizing wild perennials as a potential source of SBR resistance since the ploidy level between the two species are not necessarily comparable. A successful hybridization between a *G. tomentella* (accession PI 483218, 2*n* = 78) and a *G. max* (cv. Altona, 2*n* = 40) was accomplished and four seeds resulted from this cross (Singh et al., 1990). This *G. tomentella* (PI 483218) was previously identified as one of the SBR resistant perennial accessions (Hartman et al., 1992).

One amphiploid seed (2*n* = 118) was grown out for further backcrossing to the cultivated soybean. The backcross scheme continued for four generations until the ploidy level was reduced to 2*n* = 40. This scheme resulted in fertile plants that resembled the *G. max* backcross parent Clark 63 and contained genetic material from the perennial parent (Singh et al., 1993). Two of the remaining hybrid seeds did not successfully germinate. Ten years later, the remaining single amphiploid hybrid seed germinated and was manipulated under tissue culture to produce multiple plantlets (Tyagi et al., 2005). These plantlets were grown out and were maintained in the greenhouse by clonal propagation. A total of 32 cloned plants from this single seed were available for screening for SBR resistance.

Since the *G. tomentella* parent used to create these two populations exhibited resistance to soybean rust (Hartman et al., 1992) the intersubgeneric infertile amphiploid hybrids (2*n* = 118) and the derived fertile lines (2*n* = 40) were tested for SBR resistance at the USDA-ARS, Foreign Disease-Weed Science Research Unit (FDWSRU) Biosafety Level 3 Plant Pathogen Containment Facility at Ft. Detrick MD (Melching et al., 1983). The objective of this experiment was to determine if the resistance to SBR identified in the *G. tomentella* parent was transferred to either population.

M.E. Patzoldt and T. Hymowitz, Dep. of Crop Sciences, Univ. of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801; R.K. Tyagi, National Bureau of Plant Genetic Resources, New Delhi, India; M.R. Miles and G.L. Hartman, USDA-ARS, and Dep. of Crop Sciences, Univ. of Illinois, 1101 W. Peabody Dr., Urbana, IL 61801; R.D. Frederick, USDA-ARS, Foreign Disease-Weed Science Research Unit, 1301 Ditto Ave., Ft. Detrick, MD 21702. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the USDA or the University of Illinois and does not imply its approval to the exclusion of other products or vendors that may also be suitable. Received 18 May 2006. *Corresponding author (soyui@uiuc.edu).

Published in Crop Sci. 47:158–161 (2007).
Crop Breeding & Genetics
doi:10.2135/cropsci2006.05.0328

677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: SBR, Soybean rust.

MATERIALS AND METHODS

The amphiploid cloned plants were propagated from the mother plant by taking cuttings with at least three nodes on them. Stems were dipped in sterile deionized water and then dipped into a root promoting hormone powder Rhizopon AA no. 2 (Phytotronics, Earth City, MO) and placed into test tubes containing sterile deionized water. Cuttings were placed under growth lights in the lab at 25°C with a 16-h photoperiod. After 2 to 3 wk, when the roots were well developed and lengthened, the cuttings were transplanted into 4-cm Jiffy Disks (Jiffy Products of America, Norwalk, OH) and placed back under growth lights. When roots were visibly growing through the Jiffy Disk containers, plants were transplanted to 10-cm^2 plastic pots containing a 1:1:1 sand:perlite:soil mixture. Plants were watered daily as needed and fertilized once with time-release fertilizer beads (Osmocote, Scotts-Sierra Horticultural Products, Marysville, OH) and also bimonthly with liquid fertilizer (Scotts Miracle Grow, Marysville, OH). Plants were supported by long bamboo stakes and were trimmed back as needed to keep the plants from growing into each other. Pesticides were applied as needed to keep the plants healthy. There were three cuttings (pots) of each amphiploid hybrid clone, and three pots of each check genotype line. Check resistant *G. max* genotypes included PI 200492 (*Rpp1*), PI 230970 (*Rpp2*), and PI 459025B (*Rpp4*), which typically have a mean SBR severity rating of 2 to 3 and RB type lesions with some *P. pachyrhizi* isolates. The more susceptible checks were *G. max* cvs. Williams and Jack, which have a comparable mean SBR severity rating and have mixed lesion type with the *P. pachyrhizi* isolates used in this study. Three pots of each cloned hybrid parent line, *G. tomentella* parent PI 483218 and the *G. max* cv. Altona, were also included. Pots were then randomly arranged into trays with 10 plants per tray.

The clones were driven to the USDA-ARS, FDWSRU at Ft. Detrick, MD, where they were placed in a greenhouse in isolation from other plants. Once the clones were confirmed to have no detectible live pest infestations, the plants were trimmed back to pot size and transferred inside the BSL-3 Plant Pathogen Containment Facility for inoculation. *Phakopsora pachyrhizi* urediniospores were removed from liquid nitrogen storage, heat shocked at 40°C for 5 min, and rehydrated by incubating the spores in a small plastic weigh boat over water in an enclosed Petri plate. The inoculum consisted of four *P. pachyrhizi* isolates in equal portions, all collected in 2001 from Thailand (TH01-1), Brazil (BZ01-1), Paraguay (PG01-2), and Zimbabwe (ZM01-1). The urediniospores were added to distilled water containing 0.01% (v/v) Tween 20 (Sigma, St, Louis, MO), mixed, and filtered through a 53-μm nylon screen to remove any debris and clumps of spores. The urediniospores were quantified with a hemocytometer and adjusted with distilled water to a final concentration of 20 000 spores per mL. The individual pots were inoculated with 10 mL of the spore mixture with an atomizer at 138 KPa. Pots were returned to trays, and the trays placed overnight in a dew chamber at 20 to 22°C. The next day, the trays were transferred to the greenhouse at 20 to 25°C and placed on a bench that was misted for 1 min at 20-min intervals. Disease ratings were taken 14 and 28 d post inoculation (dpi). Because of the small leaf size of the plants and to eliminate the possibility that plants had escaped inoculation, a second inoculation was performed on these same plants using previously described methods. The plants were again trimmed back before the second inoculation.

For the derived fertile lines experiment, seeds were shipped to the FDWSRU at Ft. Detrick, MD, and randomly planted in 72-cell flats (27 × 52 cm) in Sunshine LC1 soil-less mix (Sun Grow Horticulture Products, Belleview, WA). Two seeds were sown in each cell and then thinned to 1 plant after 10 d. The 842 derived fertile lines for this test came from the experiment conducted by Singh et al. (1993). Of the 842 lines planted, about half did not germinate for this test. Of the 423 lines that did germinate, there were 1 to 5 plants to rate. Some of these seed stocks were over 10 yr old, so reduced germination was expected. The outside cells were planted with *G. max* cv. Williams, as border rows. Twenty-five plants of the check *G. max* genotypes, PI 200492 (*Rpp1*), PI 230970 (*Rpp2*), PI 459025B (*Rpp3*), and cvs. Williams and Jack were planted randomly within the flats. Plants were inoculated at approximately the V1 growth stage between 14 and 17 d after planting. The inoculum was prepared as described above, and each flat was atomized at 138 KPa with 40 mL of inoculum concentration of 60 000 spores per mL. The flats were placed in dew chambers at 20 to 22°C overnight before being placed back in the greenhouse under 16-h photoperiod. Supplemental light was provided by 1000 W Metalarc lights (Sylvania, Danvers, MA) spaced 0.6 m apart 1.2 m above the bench.

The disease rating scheme was identical in both experiments. Plants were assigned a score from 1 to 5 on the basis of overall SBR severity (Miles et al., 2006). A score of 1 = no visible lesions, 2 = a few scattered lesions, 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. Lesions were examined under 3.5× magnification with an optical glass binocular magnifier (OptiVISOR, Donegan Optical, Lenexa, KS) to determine lesion type, TAN or RB. TAN lesions are considered a typical susceptible response, while RB lesions are associated with the presence of rust resistance genes. Reactions were rated as MIXED if both lesion types appeared on the same leaf.

RESULTS

Amphiploid Hybrid Clones

Amphiploid plants derived from the *G. max* and *G. tomentella* cross are normally female sterile and male fertile. Thus seeds are not produced. However, in work done by Singh et al. (1993), four seeds were obtained of which one seed was used to create derived fertile lines. The remaining three seeds were kept in cold storage. In 2002, attempts were made to germinate the remaining three seeds of which only one seed germinated (Tyagi et al., 2005).

The amphiploid clones exhibited resistance to SBR both at 14 and 28 dpi. The same protocol was followed and ratings were again taken at 14 and 28 dpi for the second inoculation experiment. In those plants that did show lesion development (have a mean SBR severity rating of 2 or 3), the lesions were all of the RB type (Table 1). The *G. max* cvs. Clark63 and Altona exhibited similar disease response, in both SBR mean severity score and lesion type, as the check *G. max* cvs. Williams and Jack, indicating a susceptible response (Table 1). The *G. tomentella* parent had a lower disease severity scores in both tests than the more resistant check genotypes. The few lesions that were noted on *G. tomentella* were all of the RB type indicating that this parent is the source of resistance in these plants. All check *G. max* genotypes exhibited higher rust severity scores than the hybrid plants or the *G. tomentella* parent. The

Table 1. Soybean rust (SBR) ratings from cloned amphiploid hybrid plants that resulted from hybridization between *G. max* (cv. Altona, 2*n* = 40) and *G. tomentella* (PI 483218, 2*n* = 78) from data collected at the USDA Agricultural Research Service, Foreign Disease-Weed Science Research Unit Biosafety Level 3 Plant Pathogen Containment Facility, Ft. Detrick, MD.

	First Inoculation†		Second Inoculation†	
Parents	SBR‡ mean	Lesion§ type	SBR mean	Lesion type
Altona	2.0	Tan	2.3	Tan
PI 483218	1.3	RB	1.0	RB
No. of clones				
25	1.0	None	1.0	None
7	1.3–2.0	RB	1.5–3.0	RB
Checks¶				
Clark63	3.3	Mix	3.3	Mix
Jack	3.0	Mix	3.3	Mix
Williams	2.5	Tan	2.5	Tan
PI 200492	2.6	RB	3.3	RB
PI 230970	2.5	RB	2.5	RB
PI 459025B	2.6	Mix	2.7	Mix

† Ratings taken at 28 d post inoculation.
‡ Mean SBR severity based on lesion number where: 1 = no visible lesions, 2 = a few scattered lesions, 3 = moderate number of lesions on at least part of the leaf, 4 = abundant lesions on at least part of leaf, and 5 = prolific lesion development over most of leaf.
§ Description of lesion type where a resistant response has reddish-brown (RB) lesions susceptible response has Tan (TAN) lesions and Mix indicates both lesion types on a single leaf.
¶ Check genotypes.

check plants showed lesions that were either TAN, RB, or MIXED.

Derived Fertile Lines

The 423 out of 842 derived fertile lines that germinated were inoculated once and rated at both 14 and 28 dpi. All of these lines showed similar disease severity scores to the five check *G. max* genotypes for mean SBR severity (data not shown). Five seeds from each derived fertile line were planted. However, germination was uneven with one seed germinated in some and up to all five seed germinated in others.

DISCUSSION

The perennial *Glycine* species have been shown to contain resistance genes to a number of fungal soybean diseases (Riggs et al., 1998; Kenworthy 1989; Hartman et al., 1992, 2000; Schoen et al., 1992) and parasitic nematodes (Riggs et al., 1998). However, these potentially useful genes from the *Glycine* perennials have not been incorporated into soybean cultivars, mostly because of difficulties in attempting hybridizations between plants with different ploidy levels. However, hybridization has been successfully demonstrated between a *G. max* (2*n* = 40) cultivar and a perennial *G. tomentella* (2*n* = 78) (Singh et al., 1990). Repeated backcrossing to *G. max* resulted in fertile lines from this cross that developed and have the same ploidy level and general appearance of the *G. max* parent, while retaining genetic material from the perennial parent (Singh et al., 1993). While the procedures required to undertake such an ambitious project are difficult and quite time consuming, they are by no means impossible. This gene pool of useful traits has been somewhat under utilized by the soybean breeding community, but because of the narrow genetic base of modern soybean cultivars, that trend will likely reverse itself.

The experiment with the cloned amphiploid hybrid plants confirmed that the *G. tomentella* PI 483218 does have resistance to SBR and that the resistance found in this perennial was successfully transferred during the original hybridization process. However, the subsequent loss of that resistance in the derived fertile line progenies indicates that SBR resistance was lost during the backcrossing and chromosome reduction and stabilization. The amphiploid plants used in this study are derived from three different genomes (Singh et al., 1993). During the plantlet proliferation process in tissue culture, chromosome pairing during cell division may have been abnormal. Thus, some amphiploid plantlets lost resistance to SBR. This is an interesting area to examine in future studies. However, since the resistance in *G. tomentella* appears to be higher than what is currently available based on inoculations, it will probably be worth repeating the backcross methods to *G. max* with testing for SBR resistance with every generation. The procedure for creating a clone is neither terribly difficult nor time consuming, so this would be easy enough to test for SBR resistance while waiting for the backcross plants to flower. Another reason these amphiploid plants are potentially very important is that they also contain immunity, or at least a very high level of resistance, to soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) (G. Noel, personal communication). Unfortunately, this SCN resistance was not maintained during the original backcrossing effort (Singh et al., 1993), as the derived fertile lines have been shown to have susceptibility to SCN (Brucker, 2004). Given that SCN has a significant negative impact on yield (Wrather et al., 2003), and SBR has the potential to do so, it would make sense to repeat the backcrossing of the amphiploid hybrid to *G. max* and test for resistance to both pests at each generation so they are not lost again.

ACKNOWLEDGMENTS

The authors would like to thank JoAnn Garrett and Sheila Diepold for conducting the rust inoculation experiments at Ft. Detrick, MD. We thank Dr. Leina Mary Joseph for assistance in manuscript preparation. Funding for these experiments was provided in part by the United Soybean Board Projects 4235 and 5235 and by the Illinois Agricultural Experiment Station.

REFERENCES

Bromfield, K.R. 1984. Soybean Rust. Monogr. 11. American Phytopathological Society, St. Paul, MN.

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

Bromfield, K.R., J.S. Melching, and C.H. Kinsolver. 1980. Virulence and aggressiveness of cultures of *Phakopsora pachyrhizi* causing soybean rust. Phytopathology 70:17–21.

Brucker, E.A. 2004. The effect of the soybean gene *Rhg1* on reproduction of *Heterodera glycines* in the field and greenhouse and associated effects on agronomic traits. M.S. thesis. Univ. of Illinois, Urbana-Champaign.

Burdon, J.J. 1986. The potential of Australian native *Glycine* species as sources of resistance to soybean leaf rust (*Phakopsora pachyrhizi*).

p. 823–832. *In* B. Nampompeth and S. Subhadrabandu (ed.) New frontiers in breeding researches. Faculty of Agriculture, Kasetsart University, Bangkok.

Burdon, J.J., and D.R. Marshall. 1981. Evaluation of Australian native species of *Glycine* for resistance to soybean rust. Plant Dis. 65:44–45.

Hartman, G.L., M.E. Gardner, T. Hymowitz, and G.C. Naidoo. 2000. Evaluation of perennial *Glycine* species for resistance to soybean fungal pathogens that cause sclerotinia stem rot and sudden death syndrome. Crop Sci. 40:545–549.

Hartman, G.L., T.C. Wang, and T. Hymowitz. 1992. Sources of resistance to soybean rust in perennial *Glycine* species. Plant Dis. 76: 396–399.

Hartman, G.L., T.C. Wang, and A.T. Tschanz. 1991. Soybean rust development and the quantitative relationship between rust severity and soybean yield. Plant Dis. 75:596–600.

Hartwig, E.E. 1986. Identification of a fourth major gene conferring resistance to soybean rust. Crop Sci. 26:1135–1136.

Hartwig, E.E., and K.R. Bromfield. 1983. Relationship among three genes conferring specific resistance to rust in soybean. Crop Sci. 23:237–239.

Kenworthy, W.J. 1989. Potential genetic contributions of wild relatives to soybean improvement. p. 883–888. *In* A.J. Pascale (ed.) World Research Conference IV. Proc., Buenos Aires. 5–9 Mar. 1989. Argentina. Assoc. Soja, Buenos Aires.

Melching, J.S., K.R. Bromfield, and C.H. Kingsolver. 1983. The plant pathogen containment facility at Frederick, Maryland. Plant Dis. 67:717–722.

Miles, M.R., R.D. Frederick, and G.L. Hartman. 2003. Soybean rust: Is the U.S. crop at risk? Online. APS*net* Feature, American Phytopathological Society, June 2003.

Miles, M.R., R.D. Frederick, and G.L. Hartman. 2006. Evaluation of soybean germplasm for resistance to *Phakopsora pachyrhizi*. Online. Plant Health Progress doi:10.1094/PHP-2006–0104–01-RS.

Ogle, H.J., D.E. Byth, and R. McLean. 1979. Effect of rust (*Phakopsora pachyrhizi*) on soybean yield and quality in southeastern Queensland, Australia. Aust. J. Agric. Res. 30:883–893.

Riggs, R.D., S. Wang, R.J. Singh, and T. Hymowitz. 1998. Possible transfer of resistance to *Heterodera glycines* from *Glycine tomentella* to soybean. Supp. J. Nematol. 30(4S):547–552.

Schneider, R.W., C.A. Hollier, H.K. Whitam, M.E. Palm, J.M. McKenny, J.R. Hernandez, L. Levy, and R. Devries-Paterson. 2005. First report of soybean rust caused by *Phakopsora pachyrhizi* in the continental United States. Plant Dis. 89:774.

Schoen, D.J., J.J. Burdon, and A.H.D. Brown. 1992. Resistance of *Glycine tomentella* to soybean leaf rust *Phakopsora pachyrhizi* in relation to ploidy level and geographical distribution. Theor. Appl. Genet. 83:827–832.

Sinclair, J.B. 1989. Threats to production in the tropics: Red leaf blotch and leaf rust. Plant Dis. 73:604–606.

Singh, R.J., K.P. Kollipara, and T. Hymowitz. 1990. Backcross-derived progeny from soybean and *Glycine tomentella* Hyata intersubgeneric hybrids. Crop Sci. 30:871–874.

Singh, R.J., K.P. Kollipara, and T. Hymowitz. 1993. Backcross (BC_2–BC_4)-derived fertile plants from *Glycine max* and *G. tomentella* intersubgeneric hybrids. Crop Sci. 33:1002–1007.

Tyagi, R.K., J.J. Zou, and T. Hymowitz. 2005. High frequency plant regeneration from a single amphiploid hybrid seed of *Glycine max* × *G. tomentella* through tissue culture. Indian J. Genet. Plant Breed. 65:107–111.

Wrather, J.A., S.R. Koenning, and T.R. Anderson. 2003. Effect of diseases on soybean yields in the United States and Ontario (1999 to 2002). Available at: Plant Health Progress www.plantmanagementnetwork.org/pub/php/review/2003/soybean/ verified 30 Nov. 2006.