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Registration of Three Soybean Germplasm Lines Resistant to *Phakopsora pachyrhizi* (Soybean Rust)

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ABSTRACT

Soybean rust, caused by *Phakopsora pachyrhizi* Sydow, is one of the most important foliar diseases of soybean [*Glycine max* (L.) Merr.]. Development of rust-resistant lines is one objective of many soybean breeding programs. Three soybean germplasm lines designated as TGx 1987–76F (Reg. No. GP-369, PI 657702), TGx 1987–118F (Reg. No. GP-370, PI 657703), and TGx 1987–129F (Reg. No. GP-368, PI 657701) with resistance to *P. pachyrhizi* and to *Xanthomonas axonopodis* pv. *glycines* (Nakano) Vauterin, Hoste, Kersters & Swings (bacterial pustule) were selected from lines developed by the International Institute of Tropical Agriculture (IITA) in collaboration with the USDA-ARS at the University of Illinois. These lines were derived from a tropical soybean rust-resistant cultivar UG5, and a rust-susceptible, high-yielding elite breeding line, TGx 1805–31F. A total of 297 F_7 lines were evaluated for rust resistance under greenhouse conditions using whole inoculated plants, in the laboratory using detached leaves, and under field conditions in Nigeria and Alabama (287 lines). Based on assessments from these various tests, these three lines have combinations of high levels of rust and bacterial pustule resistance, good agronomic traits, and adaptable maturity to the southern U.S. soybean breeding programs. Breeders will find the three lines useful as rust-resistant parental material in rust-prone areas.

Soybean rust (caused by *Phakopsora pachyrhizi* Sydow) is one of the most important foliar diseases of soybean (Miles et al., 2003). The release of three soybean [*Glycine max* (L.) Merr.] germplasm lines — TGx 1987–76F (Reg. No. GP-369, PI 657702), TGx 1987–118F (Reg. No. GP-370, PI 657703), and TGx 1987–129F (Reg. No. GP-368, PI 657701) — will provide breeders with improved lines that combine high levels of soybean rust and bacterial pustule [caused by *Xanthomonas axonopodis* pv. *glycines* (Nakano) Vauterin, Hoste, Kersters & Swings] resistance, good agronomic traits, and adaptable maturity to the southern United States and in Nigeria. These three lines were selected from 297 F_7 lines developed by the International Institute of Tropical Agriculture (IITA) in collaboration with the USDA-ARS at the University of Illinois. The tropical soybean rust-resistant cultivar UG5 and a rust-susceptible, high-yielding elite breeding line, TGx 1805–31F, are the parents of these three lines.

Soybean rust was first found in the continental United States in 2004 (Schneider et al., 2005). Because of its potential threat to soybean production there, a USDA sponsored monitoring system was implemented starting in 2005 (USDA, 2009). The discovery and development of soybean rust-resistant germplasm had been a research priority in the USA even before the rust appeared in the continental United States in 2004. Sponsored by the United Soybean Board, research aimed at discovering new sources of resistance began in 2001 (Miles et al., 2006) and found numerous potential sources, some of which have been further screened under field conditions in Nigeria (Twizeyimana et al., 2008), Paraguay (Miles et al., 2008), and the USA (Walker et al., 2008), as well as challenged with six single-spore purified *P. pachyrhizi* isolates from the United States (Paul and Hartman, 2009).

Soybean rust was first reported in Nigeria in 2001 (Akinsanmi et al., 2001) and has been endemic in Nigeria since then (Twizeyimana et al., 2009). Working together in Nigeria, the IITA and USDA-ARS at the University of Illinois collected and analyzed the pathogenic variability of *P. pachyrhizi* isolates (Twizeyimana et al., 2009) and evaluated soybean rust-resistant germplasm (Twizeyimana et al., 2008). The

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Abbreviations: IITA, International Institute of Tropical Agriculture; MG, maturity group.

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IITA has a long history of working with soybean germplasm as part of the institute's global mandate to improve the productivity and uses of soybean in Africa (Dashiell et al., 1991; Tefera et al., 2009). When soybean rust became a concern in Nigeria, the IITA started a program to develop rust-resistant breeding lines (Adeleke et al., 2006). On the basis of earlier evaluations conducted at the IITA (Twizeyimana et al., 2008), an accession with unknown pedigree from Uganda, UG5, was found to have a high level of resistance, as was previously reported (Kawuki et al., 2003). UG5 was crossed with a rust-susceptible, high-yielding, and early-maturing elite breeding line, TGx 1805–31F. F_7 generation lines from this cross were evaluated in a preliminary yield trial in 2008 in Ibadan. At the same time, F_7 generation lines from this cross were tested in the United States for rust and bacterial-pustule resistance and adaptability.

Methods

Line Development

The cross TGx 1805–31F × UG5 was made to transfer rust resistance from UG5, a bacterial pustule–susceptible line, to an elite breeding line in June of 2003. TGx 1805–31F is a medium maturing (106 d), bacterial pustule resistant line that was a top-performing entry in advanced trials at three locations in Nigeria in 1999, and since then it has been made available to national programs for testing (IITA, 1999). In Nigeria, soybean genotypes are considered early if they mature less than 100 d after planting (Tefera et al., 2009). This line was found later to be susceptible to soybean rust. Since TGx 1805–31F has not been released in Nigeria, TGx 1485–1D (a cultivar released in Nigeria in 1990) that is also susceptible to rust was used as a check. UG5 is adapted to conditions in Central Africa and is reported to have high levels of rust resistance in Uganda (Kawuki et al., 2003) and Nigeria (Twizeyimana et al., 2008) but is susceptible to bacterial pustule and frogeye leaf spot (data not presented).

Seed of the derived breeding lines from TGx1805–31F × UG5 were obtained following the pedigree breeding method. More than 100 F_1 seeds were obtained from the cross and then bulked. All F_1 seeds were planted, and the F_2 seeds were harvested from each F_1 plant. A portion of the F_2 seeds was sown as several 1-m-long row plots. From F_2 to the F_6 generation, individual plants with low levels of leaf disease severity (bacterial pustule, frogeye leaf spot, and rust), good plant appearance, and high pod density were selected (IITA, 2004). Seeds from the selected individual plants were planted as progeny rows, and one or more single plant selections were performed from progeny rows if desirable plants were present. Plants not selected were discarded. Seeds from all plants of the selected F_6 progeny rows were bulked to obtain F_7 lines. A sample of 297 F_7 lines was sent to the University of Illinois at Urbana in 2007. TGx 1987–76F, TGx 1987–118F, and TGx 1987–129F were derived from three separate F_2 plants.

Field Performance in Nigeria

Sixty-five lines were evaluated for agronomic performance in Ibadan, Nigeria (7°23′ N/3°55′ E). The trial was laid out in randomized complete block design with three replications. Each plot consisted of two 4-m-long rows spaced 0.75 m apart. Seeds were planted by hand on 10 July 2008. Plots were manually weeded when necessary. The plots received basal application of 100 kg ha^{-1} 15:15:15 fertilizer and 50 kg ha^{-1} of triple super phosphate. Data were recorded for grain yield, time from sowing to flowering and maturity, and plant height at the R6 stage, when pod-filling was complete (Fehr et al., 1971). Lodging and pod shattering scores were taken 10–14 d after plant maturity (R8 stage). Severe rust occurs naturally in most years in Ibadan, but the incidence of rust occurred late in the season in 2008.

Rust Resistance in Illinois

A total of 297 F_7 lines were evaluated for rust resistance in the greenhouse using a single-spore purified *P. pachyrhizi* isolate (FL07–1) from Gadsden County, FL in 2007. Three checks were also included in this trial, for a total of 300 entries. The trial was replicated twice using a randomized complete block design with seven plants per experimental unit, although the number was sometimes less if germination was poor. Plants were inoculated when 2 wk old with a suspension of urediniospores (approximately 10^5 spores per mL) until runoff, incubated for 24 h under mist conditions at 100% relative humidity, and kept in the greenhouse at 20–22°C. Plants were rated 16–18 d after inoculation by counting the number of lesions and sporulating uredinia in three 1.3-cm^2 regions on two leaflets per plant. Area was converted to 1.0 cm^2 for analysis and presentation. Data were analyzed by PROC ANOVA (JMP, SAS Institute).

With the combination of greenhouse and field evaluations in Alabama (see details for field evaluation below), 20 lines were selected that had no or low numbers of red-brown lesions. In the fall of 2008, three additional isolates (AL07, IL07, and NC07) were inoculated on soybean seedlings of these 20 lines plus the three check lines (UG5, resistant, and TGx 1485–1D and 'Williams 82', susceptible) in the greenhouse as previously described. In addition, detached leaves were inoculated in a completely randomized design with two replications using the drop inoculation technique (Paul and Hartman 2009); a total of six inoculation sites per leaflet with 20-µL inoculation drops were placed on each side of the leaflet midrib at equal distances from each other to challenge these selected lines and checks with an additional six isolates (IL07–1, LA08, VA07, MS07, OK07, and TX08). A single leaflet represented an experimental unit. Numbers of lesions and sporulating uredinia were recorded in each drop-inoculated area.

Field Performance in Alabama

Two hundred eighty-seven lines, along with parents and adapted checks, were planted at the Gulf Coast Research and Extension Center, 8300 State Highway 104, Fairhope, AL (30°31′22″N/87°54′11″W). Two blocks of replicated hill plots consisting of five seeds were planted on 11 June 2008. Plots were spaced 61.0 cm apart in 96.5-cm rows. Parents were included three times in each replication. Adapted checks were the Delta and Pine Land Company cultivars

Table 1. Agronomic performance of rust-resistant lines TGx 1987–76F, TGx 1987–118F, and TGx 1987–129F and a rust-susceptible check, TGx 1485–1D under rust-free conditions in Ibadan, Nigeria, in 2008.

Line	Yield	Flower	Maturity	Height	Lodging†	Shattering‡	Seed weight
	kg ha^{-1}	d	d	cm	1–5	1–5	g 100-seeds^{-1}
TGx 1987–76F	1248	45	91	45	1.0	2.5	12.7
TGx 1987–118F	1005	39	91	37	1.0	2.3	15.0
TGx 1987–129F	1733	45	91	54	2.0	2.3	13.0
TGx 1485–1D	1444	42	93	36	1.0	1.5	12.5
LSD (0.05)	585	1.9	3.5	12.6	0.4	0.8	1.7
CV (%)	29	3	2	16	31	17	7

†Extent of plant lodging evaluated 10 to 14 d after plant maturity (R8 stage) on a scale of 1 to 5, where 1 = no plant lodging and 5 = all plants lodged.

‡Extent of pod shattering evaluated at 10 to 14 d after plant maturity (R8 stage) on a scale of 1 to 5, where 1 = none of the pods split open to release seeds and 5 = all pods split open naturally to release seeds.

'DP5915RR' and 'DP6568RR'. Maturity, pod shattering, and rust reaction information was collected.

Characteristics

Based on field performance in Ibadan, Nigeria, grain yield of the three selected lines (TGx 1987–76F, TGx 1987–118F, and TGx 1987–129F) did not significantly differ from the released variety TGx 1485–1D (Table 1). The seed weights of the selected lines ranged from 12.7 to 15.0 g per 100 seeds, maturity was around 90 d, heights varied from 37 to 54 cm, and the lines were rated resistant to bacterial pustule and frogeye leaf spot (all had ratings of 1 on a scale of 1 to 5, with 1 being most resistant).

The full pedigrees of the three registered lines are

- TGx 1987–76F = [[(TGM 685 × TGM 210–1-2363) × {(TGM 80 × TGM 737) × TGM 737}] × [{(TGM 80 × TGM 737) × TGM 737} × (TGM 393 × TGM 297–2)] × UG5]-74–1-3–2-3-B
- TGx 1987–118F = [[(TGM 685 × TGM 210–1-2363) × {(TGM 80 × TGM 737) × TGM 737}] × [{(TGM 80 × TGM 737) × TGM 737} × (TGM 393 × TGM 297–2)] × UG5]-7–1-6–2-2-B
- TGx 1987–129F = [[(TGM 685 × TGM 210–1-2363) × {(TGM 80 × TGM 737) × TGM 737}] × [{(TGM 80 × TGM 737) × TGM 737} × (TGM 393 × TGM 297–2)] × UG5]-6–1-4–2-3-B

The parent TGM 80 is equivalent to the cultivar 'Bossier' (PI 567789), while that of TGM 393 is the cultivar 'Hampton' (PI 614156). Pedigrees of the other parents of the three registered lines were not traceable.

Based on the rust evaluations at Illinois with the FL07–1 isolate, the average number of sporulating uredinia per cm^2 was 0 for TGx 1987–76F and TGx 1987–118F, and 0.4 for TGx 1987–129F, which was far below the 3.0 and 5.7 uredinial means for the susceptible checks (Table 2). With the additional isolates tested both in the greenhouse (three isolates; data not presented) and using detached leaves (six isolates), all three lines and UG5 had red-brown lesions, while TGx 1485–1D and Williams 82 had tan lesions. In the greenhouse, the average over isolates of uredinia per lesion was 0 for the three lines and UG5 but was 2 and 3 for TGx 1485–1D and Williams 82, respectively. For the detached leaf evaluations using six isolates, there were no sporulating uredinia in the three selected lines and UG5, while TGx 1485–1D and Williams 82 had a range from 3 to 15 sporulating uredinia per inoculation drop (Table 3).

On the basis of agronomic characteristics and reactions to *P. pachyrhizi* in the field in Alabama, the selected lines matured between 136 and 137 d after planting and were earlier than UG5 and the other U.S. checks, indicating that their maturity would be classified as Maturity Group (MG) V or earlier (Table 4). Two leaflets per plant were used to count

Table 2. Evaluation of rust-resistant lines TGx 1987–76F, TGx 1987–118F, and TGx 1987–129F and a rust-resistant check, UG5, and rust-susceptible checks, TGx 1485–1D and Williams 82, challenged with FL07–1 (collected from Gadsden County, FL, 2007), under greenhouse-inoculation conditions in Urbana, IL, in 2007.

		Number of		
Line†	Lesion type	Lesions ‡	Uredinia§	Uredinia
		cm^{-2}	cm^{-1}	lesion^{-1}
TGx 1987–76F	red-brown	0.1	0	0
TGx 1987–118F	red-brown	0.5	0	0
TGx 1987–129F	red-brown	1.1	0.4	0.4
UG5	red-brown	0.2	0	0
TGx 1485–1D	tan	2.8	3.8	1.5
Williams 82	tan	0.9	5.7	6.5
Trial mean		0.78	0.65	0.57
SE		0.04	0.07	0.06
Range		0–2.9	0–8.2	0–6.5

†There was a total of 300 lines in this trial, each replicated twice with seven plants per replication.

‡For each plant, lesions were counted on each two leaflets at three different areas per leaflet.

§Sporulating uredinia counts were made 16 to 18 d after inoculation.

Table 3. Lesion types and number of lesions on a detached leaf from selected germplasm resistant lines and their parents inoculated with six *Phakopsora pachyrhizi* isolates.

	Isolates†											
	IL07–1		LA08–1		MS07–1		OK07–1		TX07–1		VA07–1	
Line	Lesion type	No. of uredinia‡	Lesion type	No. of uredinia	Lesion type	No. of uredinia	Lesion type	No. of uredinia	Lesion type	No. of uredinia	Lesion type	No. of uredinia
TGx 1987–76F	RB§	0	RB	0	RB	0	RB	0	RB	0	RB	0
TGx 1987–118F	RB	0	RB	0	RB	0	RB	0	RB	0	RB	0
TGx 1987–129F	RB	0	RB	0	RB	0	RB	0	RB	0	RB	0
UG5	NR¶	0	RB	0	NR	0	RB	0	NR	0	RB	0
TGx 1485–1D	tan	3	tan	3	tan	4	tan	4	tan	6	tan	6
Williams 82	tan	6	tan	15	tan	4	tan	12	tan	10	tan	13

†IL07–1 collected in Massac County, Illinois 2007; LA08–1 collected in East Baton Rouge Parish, LA, 2008; MS07–1 collected in Isola County, MS, 2007; OK07–1 collected in Tulsa County, OK, 2007; TX07–1 collected in Hidalgo County, TX, 2007; and VA07–1 collected in Chesapeake County, VA, 2007.

‡Mean sporulating uredinial counts based on a 20 µl spore suspension (50 spores per drop) on the surface of a detached leaf.

§RB = red-brown lesion.

¶No response.

the number of uredinia with a dissecting scope (SMZ1000, Nikon, Tokyo, Japan) at 10× magnification. Leaflets of the test lines and UG5 were scanned to find uredinia present on the entire leaflet. However, uredinia for the susceptible entries (local checks and TGX1485–1D) were much more abundant so sampling was done by taking three 1.3-cm^2 regions on two leaflets per plant; this data was converted to mean number of uredinia per cm^2 leaf area. The selected lines all had red-brown lesions with no sporulation, while all the U.S. checks had tan lesions with abundant sporulating uredinia (Table 4).

Based on field performance (Alabama and Nigeria) and greenhouse and detached-leaf assays, we were able to narrow our selection to three lines. The selected lines were determinant and matured similarly to a MG V or VI cultivar for the southern United States. The relative maturities of the checks used were from 5.9 to 6.5. All three lines have purple flowers, gray pubescence, a yellow seed coat, and a light brown hilum. Among the three lines, TGx 1987–76F was intermediate in yield and height, TGx 1987–118F was the shortest, and TGx 1987–129F produced the highest yield and was the tallest. All three lines showed bacterial pustule resistance compared to its bacterial pustule susceptible parent UG5.

Discussion

We report the development and release of three soybean breeding lines that combine resistance to soybean rust and bacterial pustule, have good agronomic traits, and have a maturity adaptable to the southern U.S. These three lines are TGx 1987–76F, TGx 1987–118F, and TGx 1987–129F. The Ugandan germplasm UG5 contributed the rust resistance trait (Kawuki et al., 2003), while the IITA breeding line TGx 1805–31F (IITA, 1999) provided other adaptation traits to these three lines. In contrast to UG5, the three registered lines were resistant to bacterial pustule, a trait that came from TGx 1805–31F (IITA, 1999). There were few rust lesions on leaves and the lesions had few or no uredinia, indicating that few, if any, rust spores were produced on the leaves of these three lines when they were inoculated with the isolates used in our evaluations. Therefore, rust development on these lines would be slow in the absence of sufficient numbers of spores to cause significant secondary infection in the field (Hartman et al., 2005). TGx 1987–76F, TGx 1987–118F, and TGx 1987–129F were resistant to

Table 4. Agronomic performance of selected F6 soybean lines evaluated for resistance to Phakopsora pachyrhizi in the field in Fairhope, AL in 2008.

Line	Maturity†	Shattering‡	Lesion type§	Mean uredinia¶	Bacterial pustule#
	d	1–5			1–5
TGx 1987–76F	137	1.5	red-brown	0	1.3
TGx 1987–118F	136	1.0	red-brown	0	1.6
TGx 1987–129F	137	1.5	red-brown	0	1.3
UG5	142	1.0	red-brown	1	4.9
TGx 1485–1D	127	1.0	tan	18	1.4
Prichard (check)	150 (8.5)	1.0	tan	9	1.3
DP5915RR	145(5.9)	1.0	tan	6	3.3
DP6568RR	146 (6.5)	1.0	tan	9	3.0
DP7870RR	148 (7.8)	1.0	tan	20	4.5
LSD (0.05)	11	ns			1.6

†Numbers given in parentheses are days to maturity from date of planting and relative maturities of the checks.

‡Scale of 1 to 5, where 1 = no shattering and 5 = shattered completely.

§Field reaction: T = tan lesion (susceptible); RB = red-brown lesion (resistant).

¶Mean uredinia cm^{-2} for TGx 1485–1D and checks. For TGx 1987–76F, TGx 1987–118F, TGx 1987–129F, and UG5, two entire leaflets were used for the count of uredinia.

#Bacterial pustule severity recorded on a scale of 1 to 5, where 1 = no disease and 5 = more than 25% leaf area damaged.

P. pachyrhizi isolates collected from eight states (AL, FL, IL, LA, MS, NC, OK, and VA), suggesting that rust resistance in these lines is effective against *P. pachyrhizi* isolates collected from widely dispersed geographic locations in the United States. The public release of the three rust-resistant germplasm lines in adapted background will be of value to soybean breeding programs that aim to develop rust-resistant soybean cultivars.

Availability

Seeds of lines have been deposited in the National Center for Genetic Resources Preservation at Fort Collins, CO. Small quantities of seed for research and breeding purposes can be obtained from the corresponding author or from Glen Hartman. However, if these germplasm lines are used to develop new breeding lines or cultivars, appropriate acknowledgment should be made. All lines are covered by the International Treaty on Plant Genetic Resources for Food and Agriculture and therefore will only be distributed under the terms of the Standard Material Transfer Agreement (SMTA) from the treaty.

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