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Screening of selected kenyan soybean varieties for resistance to *Phakopsora pachyrhizi* (Soybean rust)

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Abstract

Soybean (Glycine max (L.) Merrill.) is a highly nutritious plant which plays an important role in the world's economy, however soybean rust disease caused by the fungus *Phakopsora pachyrhizi*, is a major challange to the soybean industry. The disease among other constraints has significatly affected crop yields in most soybean growing countries. In this study Seven varieties of soybean (Nyala, Bossier, SB19, Hill, SB8, Gazelle and TGx1987-32F) commoly grown by farmers in Kenya were tested in the green house for resistance to soybean rust. The varieties TGx1987- 32F and SB8 showed resistant reactions characterized by red brown lesion with low level of disease severity, low lesion number, low sporulation level and low area under disease progress curve (AUDPC) value. The other five varieties; Nyala, Bossier, SB19, Hill and Gazelle showed susceptible reactions to soybean rust producing tan lesion with profuse sporulation and high disease severity level. The Soybean varieties with low lesion densities, low disease severity and low sporulation level may be possible sources of rust resistance genes that can be used in breeding programs to produce rust resistant varieties.

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Introduction

The production of soybean in Kenya is affected by numerous biotic and abiotic factors. Some of the constraints include, low yielding varieties, lack of markets, poor agronomic practices, lack of awareness for its potential, competition with other legumes, drought, water logging, and pest and disease attacks (Hartman *et al.,* 2011). Other factors include lack of varieties which are tolerant midseason moisture stress and high yielding varieties tolerant to low phosphorus (FAO, 2005). Among the biotic factors affecting soybean production diseases are of great concern because of their final impact on yield. There are a number of diseases that infect soybean worldwide the most common disease are *Anthracnose,* bacterial blight, bacterial pustule, soybean rust, bean pod mottle virus, brown stem rot, charcoal rot , frog eye leaf spot, soybean cyst nematode and soybean mosaic virus among others (Ploper,1997).

Soybean rust caused *Phakopsora pachyrhizi* as been identified among other diseases as the major challenge to soybean production worldwide. *Phakopsora pachyrhizi* belongs to the fungal phylum *Basidiomycota,* class *Urediniomycetes* and order *Uredinales*, which produce uredinia, on "dome-like" structures that give rise to asexual urediniospores. Hair-like hyaline hyphae called paraphyses grow inside uredinia. Paraphyses and sporophores are base structures for urediniosopore production (Bromfield, 1984). *P. meibomiae* is less aggressive while *P. pachyrhizi* is more aggressive and infects over 95 species of plants from more than 42 genera, including soybean and related *Glycine* species (Bromfield, 1984). The most susceptible host of *P. pachyrhizi* is kudzu (*Pueraria lobata* (Wild.) Ohwi), a weed species that is commonly found in the United States of America. Other common hosts are medic (*Medicago arborea* L.), lupine *(Lupinus hirsutus* L.), sweet clover *(Melilotus officinalis (L.) Lam*), vetch (*Vicia dasycarpa* Ten), common beans (*Phaseolus vulgaris* L.), lima and butter beans *(Phaseolus lunatus* L.), pigeonpea (*Cajanus cajan* (L.) Millsp), garden peas (*Pisum sativum* L.) and cowpeas (*Vigna unguiculata)* (Bromfield, 1984). Soybean rust infection process begins in the low to mid-canopy and moves up the plant. The infection process starts with urediniospores germination to produce a single germ tube that grows across the leaf surface, until an appressorium is formed. Penetration of epidermal cells is direct through the cuticle by an appressorial peg (Miles *et al.*, 2005). During the infection process intracellular invasion of the leaf occurs once hyphae are formed within the mesophyll layer. Within 5 to 7 days volcano shaped uredinia with round ostioles are produced which release urediniospores on the abaxial surface completing the asexual reproduction cycle (Goellner *et al.*, 2010).

The rapid spread of the disease in the continent of Africa has led to major decline in soybean yield (Levy, 2005, Oloka *et al.,* 2008). Losses due to soybean rust can be significantly high. In South Africa losses of 10-80% have been reported and in areas under monocropping system the losses can be as high as 100%. India has experienced losses of 10-90%, Japan 40% and Taiwan has reported losses of 23-90% in (Hartman *et al.,* 1999). It is therefore important that the major production constraints be addressed so as to improve the crop yield to be able to meet the market demands and sustain the production industries. To control the spread of the rust disease chemical fungicides and cultural practices are used howerever the use fungicides to control the disease commercial plantings significantly increases production costs it is therefore not a feasible option in small scale soybean plantings especially in developing countries (Miles *et al.*, 2003). Furthermore the fungicides are expensive and are not very effective at preventing epidemics as Bonde *et al.*, (2006) noted yield losses of up to 50% under severe rust epidemics with chemical control. Other legumes that also form an integral part of the cropping system such as cowpea, pigeon pea and common beans are functional alternative hosts of *P. pachyrhizi* which makes control a great challenge (Anon, 2007; Slaminko *et al.*, 2008). Cultural practices like destruction of alternate hosts, timely irrigation, early planting and growing early maturing cultivars can also reduce the incidence of the disease (Akinsanmi *et al.,* 2001). However, the rapid spread by wind-borne urediniospores and the large number of host species increases chances of soybean rust survival making cultural practices relatively ineffective (Hartman *et al.*, 2005).

Planting of disease resistant cultivars is the most viable way to manage soybean rust disease. To identify rust resistant cultivars soybean plants must be screened for resistance to diverse pathogen populations (Twizeyimana *et al.*, 2007). This study therefore aims at screening selected soybean varieties commonly grown in Kenya for resistance to soybean rust isolates under green house conditions.

Materials and methods

Spore collection and multiplication

Diseased soybean leaves were collected from farmers' fields in Western region of Kenya coordinates 0°30′N 34°35′E/0.500°N 34.583°E/ 0.500.

The leaves were gently rubbed on a wax paper to dislodge the spore. The spores were then transferred into a 2ml vial and tightly capped (JIRCAS, 2016) To obtain soybean rust inoculum and maintain the spore cultures the spores collected from the were bulked and inoculated on susceptible soybean variety Namsoy 1 using detached-leaf method as described by Yamanaka *et al.,* 2010. Disease free mature leaves of the susceptible variety were detached from the plants grown in the screen house.

The leaves were then washed with six changes of sterile distilled water and placed with the abaxial side up in petri dishes containing sterile paper towel moistened with distilled water. Spore suspension of 10^5 ml^{-1} in 0.04% Tween 20 solution was sprayed on the leaves then incubated at 21 ºC for 12h in the dark and then incubated in a growth chamber at 21 ºC under a 12h light photoperiod. Distilled water was added to each petri dish as needed to keep the paper towel moist during the incubation period (9-14 days) to allow for sporulation. After incubation the spores were harvested and stored for subsequent experiments.

Soybean seeds and growth conditions

Seeds of seven varieties of soybean commonly grown in Western Kenya were obtained from Kenya Agricultural and Livestock Research Organization (KALRO) and used in the study. The varieties were Nyala, Bossier, SB19, Hill, SB8, Gazelle and TGX1987-32F (Table 1).

The seeds were pre-germinated in petri dishes for 2 days then grown in 25 cm squared plastic planting pots. Six seeds per pot of each variety of soybean was planted in three different pots in the greenhouse. The pots were laid out in a completely randomized design and the experiments replicated 3 times. After establishment the seedlings were reduced to three plants per pot by thinning. Nitrogen, phosphorus and potassium (NPK) fertilizer was then applied at the rate of 4 g/pot during second trifoliate stage (V2) of growth.

Plant inoculations

The plants were innoculated at the V3 (third trifoliate) growth stage. Stored urediniospores *P. pachyrhizi* isolates were heat shocked at 40°c for 5 minutes then hydrated overnight by floating them in a small plastic weigh boat on sterile distilled water in a petri dish. Urediniospore viability was determined by spraying inoculum of each isolate onto the surface of sterile water agar in petri dishes and determining the percent germination after 24h of incubation at 20°C. To prepare the inoculum urediniospores were suspended in 0.1% Tween 20 (sodium monolaurate) in sterile distilled water, mixed vigorously, and filtering through a 53-µm pore size screen.

The concentration of urediniospore was then adjusted to 5 × 10^5 urediniospores/ ml. The soybean plants were innoculated by applying the inoculum on the abaxial side of the leaves using a hand sprayer (Pham *et al.,* 2009). In order to maintain high relative humidity necessary for infection inoculated plants were covered with polythene bags for 24 hours and temperatures maintained at 22˚C-24˚C

(Twizeyimana, *et al.,* 2007).

Disease detection and ratings

Soybean rust disease severity and resistant reactions were evaluated 14 days after inoculation. Disease severity was assessed on a scale of 1 to 5 based on percentage of leaf area affected, where; 1 = no visible lesions, 2 = 0.1 to 2.5% leaf area affected, 3 = 2.6 to 10% of leaf area affected, 4 = 10.1 to 30% of leaf area affected, and 5 = over 30% of leaf area affected (Miles *et al.,* 2011). Lesion colour, number of lesions per 1cm² and number of spores per lesion was also recorded. Sporulation levels were scored using a scale of 1-5 as described by Miles *et al.,* (2008) where: 1 = no sporulation; 2 = Less than 25% of fully sporulating lesions; 3 = 26% to 50% of fully sporulating lesions; 4 = 51% to 75 % of fully sporulating lesions; 5 = fully sporulating tan coloured lesions. To obtain area under disease progress curve (AUDPC) the disease rating was done twice a day from day 7 after inoculation up to day 21.

Data analysis

To evaluate resistance of soybean varieties to *P. pachyrhizi* the means and standard error of the disease severity lesion number and sporulation level were calculated and analyzed using ANOVA (Excel stat 2015). Area under disease progress curve (AUDPC) values were calculated using the formula below as presented by Kumudini *et al.*, (2008);

$$\text{AUDPC} = \sum_{1}^{n} 1\left[\frac{X_i + X_{i+1}}{2}\right](t_{i+1} - t_i)$$

Where

X_i = the disease severity score at the ith observation;

t_i = the time (day) at the ith observation;

$t_{i+1} - t_i$ = the interval (days) between two consecutive assessments

n = the number of assessments.

Results and discussion

This study has revealed that the soybean varieties tested differed significantly in disease severity, lesion colour, sporulation levels and AUDPC. Lesion colour alone cannot be used to evaluate resistance (Yamanaka *et al.,* 2010), therefore this study combined several factors such as lesion colour sporulation level, disease severity and AUDPC to classify the varieties tested into resistant and susceptible lines.

Reduction in size and number ofurediniospores is also a desirable indicator of resistance when assessing single soybean rust resistance genes (Bonde *et al.,* 2006).

Table 1. Description of the soybean varieties screened for soybean rust resistance.

Variety	Origin	Seed helium colour	Testa colour	Days to physiological maturity	Yield Kgs/Ha
Nyala	KARI Njoro	Dark/brown	Cream	90-160	700-2500
Bossier	KARI Njoro	Brown	Cream	90-115	1800- 2200
SB19	KARI (improved)	Brown	Cream	120-140	950-1500
Hill	KARI Njoro	Brown	Cream	125-155	950-1500
SB8	KARI (improved)	Brown	Cream	90-120	700-2500
Gazelle	KARI Njoro	Cream	Cream	109-165	800-1600
TGx1987-32F	IITA	Brown	Cream	90-120	800-1500

The results showed there was a significant variation in the level of disease severity observed in all the seven varieties ($P<0.05$). The disease severity ranged from 2-5 with Nyala having the highest severity level and TGX1987-32F having the lowest (Figure 1). The severity levels increased significantly from day 7 to day 14 in all the varieties (Figure 2).

The lesions colours were either red brown or tan, no variety showed immune or mixed reactions. Two varieties, SB8 and TGX1987-32F showed red brown lesions (Figure 3) which is a phenotypic characteristics of resistant variety. While Nyala, hill, bossier, SB19 and Gazelle showed Tan lesions (Figure 4) which is a characteristic of susceptible variety.

The varieties with tan lesion had high level of sporulation compared to the varieties with red brown lesions. The sporulation level differed significantly between the varieties (P< 0.01) ranging from 1 to 3.75 (Table 2).

Table 2. Table showing lesion color, AUDPC values and sporulation level.

Variety	Lesion color	Mean sporulation score	AUDPC
Nyala	Tan	3.25	71.19
Hill	Tan	2.75	58.29
Bossier	Tan	3.58	59.97
Gazelle	Tan	3.75	59.50
SB 19	Tan	2.60	49.00
SB 8	Red	1	34.98
TGx1987-32F	Red	1	33.83

The AUDPC values differed significantly among the varieties screened, Nyala which is more susceptible to ASR had high AUDPC values, while TGx1987-32F which showed red brown lesions had the lowest AUDPC value. Soybean varieties with high rust severity level and high sporulation level had the highest AUDPC value (Table 2). From the results of this study varieties SB8 and TGx1987-32F were classified as resistant while Nyala, hill, bossier, Gazelle and SB19 were classified as susceptible.

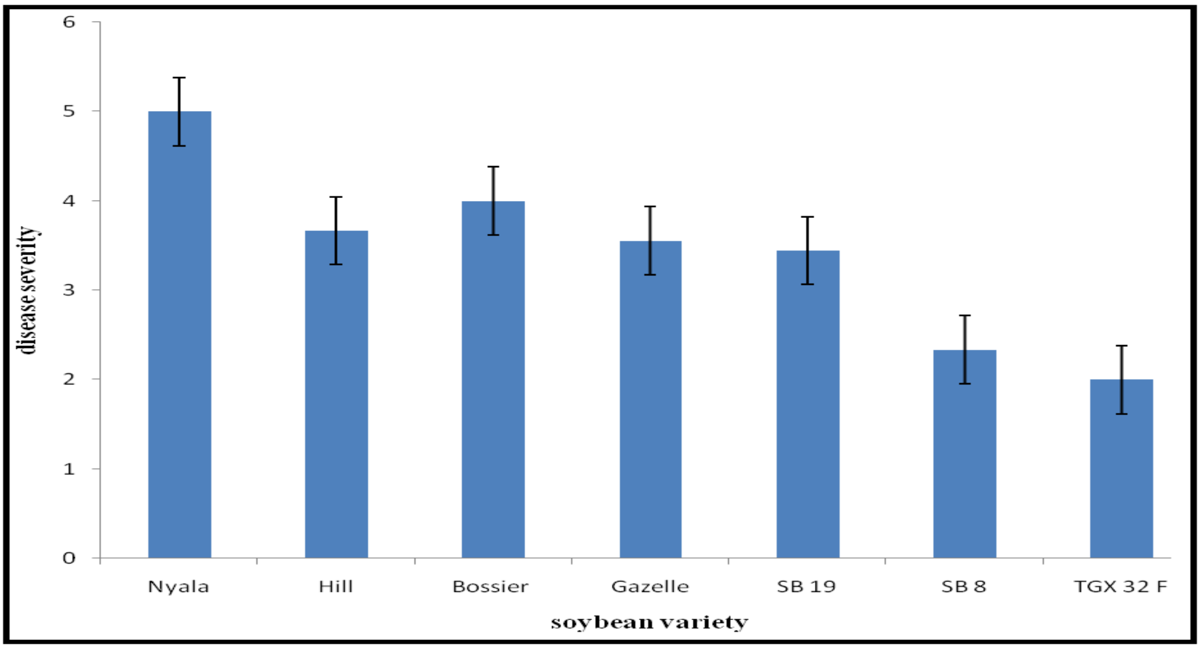

Fig. 1. Soybean rust disease severity level in the seven varieties screened in the green house.

The differences in response to rust fungi can be attributed to the genetic diversity, physiological properties of the soybean varieties and the variation in virulence of different pathotypes of the rust fungi (Twizeyimana *et al.,* 2009, Pham *et al.,* 2009). Variation can also be due the presence of different resistance genes among the soybean accessions which are known to react differently to *P. pachyrhizi* isolates (Garcia *et al.,* 2008). Previous Field and green house evaluations have identified Nyala to be susceptible with rust severity of 9 (1-9 scale) (Wanderi 2012). Despite the high level of susceptibility to ASR Nyala and Gazelle are still recommended for growing in Kenya because of their high yield and short maturity period (Mahasi *et al.,* 2009, Njoroge *et al.,* 2015).

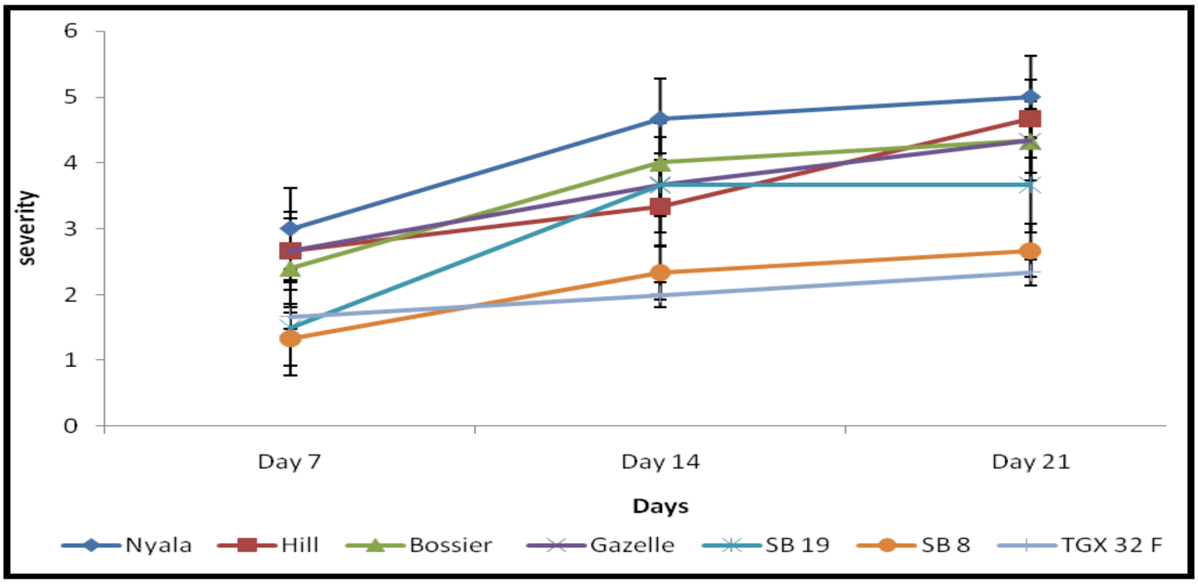

Fig. 2. Average soybean rust disease severity levels recorded at 7 days intervals.

Previous research has shown that soybean genotypes that show the red brown reaction when inoculated with rust fungi have can be associated with single-gene resistance (Hartman *et al.,* 2005a). Identification such resistant genotypes is a key component that will ensure the selection of useful sources of high resistance for breeding programs (Sharma and Duveiller, 2007).

Fig. 3. Soybean leaves with Red brown lesions (TGx1987-32F).

The varieties SB8 and TGx1987-32F showed red brown lesions with low rust severity this type of genotypes with low rust severities may be sources of partial or rate reducing resistance to *P. pachyrhizi* (Miles *et al.,* 2006). Resistance mechanisms identified against *P. pachyrhizi* include specific resistance, partial resistance and tolerance (Hartman *et al.,* 2005a). Partial resistance, expressed as reduced pustule number and increased length of latent period has not been widely used in breeding programs (Hartman *et al.,* 2005a). In breeding it is important to measure the latent period so as identify genotypes with a long latent period and hence a slower rate of rust development (Hartman *et al.,* 2005b). In conclusion the two varieties SB8 and TGx1987-32F are possible sources of rust resistances genes which can be in cooperated into other breeding lines through marker assisted selection resulting in improved yield quality and quantity and disease resistance of the existing breeding lines.

Fig. 4. Soybean leaves with Tan sporulating lesion(Nyala).

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References

Akinsanmi OA, Ladipo JL, Oyekan PO. 2001. First report of soybean rust (Phakopsora pachyrhizi) in Nigeria. Plant Disease **85,** 97.

Anon. 2007. Hosts of Phakopsora pachyrhizi, the Casual Organism of Soybean Rust in South America. Japan International Research Center for Agricultural Sciences (JIRCAS) Newsletter for International Collaboration JIRCAS, Tsukuba, Ibaraki, Japan.

Bonde M, Nester S, Austin C. 2006. Evaluation of virulence of Phakopsora pachyrhizi and Phakopsora meibomiae isolates. Plant Disease **90,** 708–16.

Bromfield KR. 1984. Soybean rust, Monograph (American Phytopathological Society), **11,** American Phytopathological Society. St. Paul, MN.

FAO. 2005. Food and Agricultural Organization. Online
http://fao.org/ag

FAO. 2008. Food and Agricultural Organization. Online
http://fao.org/ag

Garcia A, Calvo E, de Souza Kiihl R, Harada A, Hiromoto D, Vieira L. 2008. Molecular mapping of soybean rust (Phakopsora pachyrhizi) resistance genes: discovery of a novel locus and alleles. Theoretical Applied Genetics **117,** 545-553.

Goellner K, Loehrer M, Langenbach C, Conrath U, Koch E, Schaffrath U. 2010.

Phakopsora pachyrhizi, the causal agent of Asian soybean rust. Molecular Plant Pathology **11,** 169-177.

Hartman GL, Bonde MR, Miles MR, Frederick RD. 2004. Variation of Phakopsora pachyrhizi isolates on soybean. The Proceedings: VII World Soybean Research Conference, Foz do Iguassue, PR, Brazil: 440-446.

Hartman GL, Miles MR, Frederick RD. 2005a. Breeding for resistance to soybean rust. Plant Dis. **89,** 664–666.

Hartman GL, Miles MR, Frederick RD. 2005b. Historical viewpoint and soybean resistance to soybean rust. In Proceedings of the 2005 Illinois Crop Protection Conference, pp. 16 – 20. Available Online at: www.ipm.uiuc.edu/education/proceedings/index.html

Hartman GL, West ED, Herman TK. 2011. Crops that feed the World Soybean worldwide production, use, and constraints caused by pathogens and pests. Food Security **3,** 5-17.

Hartman GL, Sinclair JB, Rupe JC. Eds. 1999. Compendium of soybean diseases (4th ed.). St. Paul: American Phytopathological Society.

JIRCAS. 2016. Laboratory manual for studies on soybean rust resistance available at http://www.jircas.affrc.go.jp/english/manual/soybean_rust/JIRCAS_manual_soybean_rust.pdf

Kumudini S, Prior E, Omielan J, Tollenaar T. 2008. Impact of Phakopsora pachyrhizi infection on soybean leaf photosynthesis and radiation absorption. Crop Science **48,** 2343-2350.

Levy C. 2005. Epidemiological and chemical control of soybean rust in southern Africa. American. Phytopathological journal **89(4),** 669-674.

Njoroge NJ, Owouche JO, Oyoo ME. 2015. Evaluation of soybean [Glycine max(L.)Merr.] genotypes for agronomic and quality traits in Kenya. African Journal of Agricultural Research **10(12),** p 1474- 1479, http://dx.doi.org/10.5897/AJAR2014.9168

Oloka HK, Tukumahabwa P, Sengooba T, Shanmagasundram S. 2008. Reaction of exotic soybean germplasm to Phakopsora pachyrhizi in Uganda. Plant Disease **92(11),** 1493-1496. http://dx.doi.org/10.1094/PDIS-92-11-1493

Pham TA, Miles MR, Frederick RD, Hill CB, Hartman GL. 2009. Differential responses of resistant soybean entries to isolates of Phakopsora pachyrhizi. Plant Disease **93,** 224-228.

Mahasi JM, Vanlauwe B, Mursoy RC, Mbehero P, Mukalama J. 2009. Increasing productivity of soybean in Western Kenya through evaluation and farmers participatory variety selection, pp. 326-334 12th KARI biannual conference, Nairobi, Kenya.

Mahasi JM, Vanlauwe B, Mursoy RC, Mbehero P, Mukalama J. 2011. A sustainable approach to increased soybean production in western Kenya. African crop science conference proceedings **10,** 111-116.

Miles MR, Morel W, Ray JD, Smith JR, Frederick RD, Hartman GL. 2008. Adult plant evaluation of soybean accessions for resistance to Phakopsora pachyrhizi in the field and greenhouse in Paraguay. Plant Disease **92,** 96-102.

Miles MR, Bonde MR, Nester SE, Berner DK, Frederick RD, Hartman GL. 2011. Characterizing resistance to Phakopsora pachyrhizi in soybean. Plant Dis. **95,** 577-581.

Miles MR, Frederick RD, Hartman GL. 2006. Evaluation of soybean germplasm for Resistance to Phakopsora pachyrhizi. Online. Plant Health Progress http://dx.doi.org/10.1094/PHP-2006-0104-01-RS.

Miles MR, Rosenblatt I, Traynor P, Hartman GL. 2005. Severity assessment for soybean rust. Proceedings of the National Soybean Rust Symposium, Nov. 14-16, 2005, Nashville, TN. Plant Management Network. Online publication.

Miles MR, Frederick RD, Hartman GL. 2003 Soybean rust: is the U.S. crop at risk? APSnet Feature, American Phytopathological Society. Online publication.

Ploper LD. 1997. Evolution, impact and current status of soybean diseases in Argentina. In World Soybean Research Conference V: Proceedings, B. Napompeth (Ed.), Kasetsart University Press, p 239-242.

Sharma RC, Duveiller E. 2007. Advancement toward new spot blotch resistant wheat in South Asia. Crop Sci. **47,** 961–968. http://dx.doi.org/10.2135/cropsci2006.03.0201

Slaminko TL, Miles MR, Frederick RD, Bonde MR, Hartman GL. 2008. New legume hosts of Phakopsora pachyrhizi based on greenhouse evaluations. Plant Disease **92,** 767-771.

Twizeyimana M, Ojiambo PS, Sonder K, Ikotun T, Hartman GL, Bandyopadhyay R. 2009. Pathogenic variation of Phakopsora pachyrhizi infecting soybean in Nigeria Phytopathology **99,** 353-361.

Twizeyimana M, Ojiambo PS, Ikotun T, Paul C, Hartman GL, Bandyopadhyay R. 2007 Comparison of field, greenhouse, and detached-leaf evaluations of soybean germplasm for resistance to Phakopsora pachyrhizi. Plant Dis. **91,** 1161-1169.

Wanderi SW. 2012. Genetic analyses for resistance to soybean rust (Phakopsora pachyrhizi) and yield stability among soybean genotypes in Kenya. PhD thesis University of KwaZulu-Natal.

Yamanaka N, Yamaoka Y, Kato M, Lemos NG, Passianotto, ALL, Santos JVM, Benitez ER, Abdelnoor RV, Soares RM, Suenaga K. 2010. Development of classification criteria for resistance to soybean rust and differences in virulence among Japanese and Brazilian rust populations. Tropical Plant Pathology **35,** 153-162.