Virulence Diversity of *Phakopsora pachyrhizi* Isolates From East Africa Compared to a Geographically Diverse Collection

H. M. Murithi, International Institute of Tropical Agriculture (IITA), 34441, Dar es Salaam, Tanzania; and Laboratory of Phytopathology, Wageningen-UR, 6700 AA Wageningen, the Netherlands; **J. S. Haudenschild**, United States Department of Agriculture–Agricultural Research Service and Department of Crop Sciences, University of Illinois, Urbana, 61801; **F. Beed**, AVRDC – The World Vegetable Center, (Kasetsart), Bangkok 10903, Thailand; **G. Mahuku**, International Institute of Tropical Agriculture (IITA) 34441, Dar es Salaam, Tanzania; **M. H. A. J. Joosten**, Laboratory of Phytopathology, Wageningen-UR, 6700 AA Wageningen, the Netherlands; and **G. L. Hartman**, United States Department of Agriculture–Agricultural Research Service and Department of Crop Sciences, University of Illinois, Urbana, 61801

Abstract

Soybean rust, caused by the biotrophic pathogen *Phakopsora pachyrhizi*, is a highly destructive disease causing substantial yield losses in many soybean producing regions throughout the world. Knowledge about *P. pachyrhizi* virulence is needed to guide development and deployment of soybean germplasm with durable resistance against all pathogen populations. To assess the virulence diversity of *P. pachyrhizi*, 25 isolates from eight countries, including 17 isolates from Africa, were characterized on 11 soybean genotypes serving as differentials. All the isolates induced tan lesions with abundant sporulation on genotypes without any known resistance genes and on soybean genotypes with resistance genes *Rpp4* and *Rpp5b*. The most durable gene was *Rpp2*, where 96% of the isolates

induced reddish brown lesions with little or no sporulation. Of the African isolates tested, the South African isolate was the most virulent, whereas those from Kenya, Malawi, and some of the isolates from Tanzania had the lowest virulence. An Argentinian isolate was virulent on most host differentials, including two cultivars carrying multiple resistance genes. Ten distinct pathotypes were identified, four of which comprised the African isolates representing considerable *P. pachyrhizi* virulence. Soybean genotypes carrying *Rpp1b*, *Rpp2*, *Rpp3*, and *Rpp5* resistance genes and cultivars Hyuuga and UG5 were observed to be resistant against most of the African isolates and therefore may be useful for soybean-breeding programs in Africa or elsewhere.

Soybean [*Glycine max* (L.) Merr.] production in eastern Africa has increased, driven by the high demand from feed millers and for human consumption (Murithi et al. 2016; Rusike et al. 2013). This trend is expected to continue as human population increases, and as income and soybean cultivars improve. However, this potential increase in production is under threat from soybean rust, a foliar disease caused by the biotrophic fungus *Phakopsora pachyrhizi* Syd. Soybean rust is one of the most economically important foliar diseases of soybean worldwide. Yield losses due to *P. pachyrhizi* of up to 80% were reported in experimental plots in Taiwan (Hartman et al. 1991), 30 to 75% in Brazil (Yorinori et al. 2005), up to 60% in experimental plots in the United States (Mueller et al. 2009), and a review of the literature predicted potential yield losses in commercial fields in the United States that could exceed 80% (Sikora et al. 2014). In Africa, losses of up to 80% have been reported in Zimbabwe (Levy 2005), and up to 45% in Uganda (Kawuki et al. 2003; Oloka et al. 2008). Leaf lesions caused by *P. pachyrhizi* reduce photosynthetic activity, leading to reduced flowering, fewer pods, smaller seeds, and premature defoliation (Kumudini et al. 2008).

Soybean rust was first reported in Japan in 1902 (Hennings 1903), and spreads by fungal spores transferred by wind currents (Isard et al. 2007). The first confirmed report in Africa was in 1996 in Uganda, shortly after in Kenya and Rwanda in 1998 (Levy 2005), followed by Nigeria (Akinsanmi et al. 2001), South Africa (Pretorius et al. 2001), and Cameroon (Levy 2005). Later, the disease was reported in Ghana (Bandyopadhyay et al. 2007) and the Democratic Republic of Congo (Ojiambo et al. 2007), and most recently in Tanzania (Murithi et al. 2014) and Malawi (Murithi et al. 2015). Favorable temperatures of 15 to 28°C and high humidity during the soybean growing season in most African countries contribute to the establishment and spread of *P. pachyrhizi* (Hartman et al. 2011).

The recent spread of soybean rust in eastern Africa poses a major challenge to the regional soybean sector as most of the soybean

varieties grown are susceptible to this disease (Kawuki et al. 2003; Murithi et al. 2016; Oloka et al. 2008). Effective control measures are required to safeguard soybean production posed by the potential threat of *P. pachyrhizi* in Africa. Disease management measures include early planting (Twizeyimana et al. 2011), use of resistant cultivars (Hartman et al. 2005), and fungicides (Levy 2005; Miles et al. 2007; Mueller et al. 2009). Scouting for soybean rust may also be critical for best management practices and several reviews have outlined their importance (Kelly et al. 2015; Sikora et al. 2014). Although fungicides are widely used in other locations, they are seldom used on soybean in East Africa.

Breeding soybean for resistance to soybean rust is considered one of the best management options to limit yield losses from the disease (Hartman et al. 2005). Soybean rust resistance has been identified in a number of soybean genotypes bearing single resistance genes (Goellner et al. 2010; Hartman et al. 2011). For example, the genes *Rpp1* to *Rpp6* have been mapped to six different loci and are known to confer resistance to specific isolates of the pathogen (Garcia et al. 2008; Li et al. 2012). However, resistance breakdown has been reported across the globe (Hartman et al. 2005; Miles et al. 2008) and new *P. pachyrhizi* pathotypes emerge as a result of selection pressure, making it difficult to develop soybean cultivars with durable resistance to soybean rust.

Isolates of *P. pachyrhizi* collected from various countries have been reported to differ in virulence based on their reactions on differential hosts (Bonde et al. 2006; Miles et al. 2006; Pham et al. 2009). Differential virulence of *P. pachyrhizi* was first demonstrated in Taiwan where six pathotypes were described (Lin 1966). Subsequently, 18 pathotypes were described in Japan (Yamaoka et al. 2002, 2014), three in Brazil (Yamanaka et al. 2010), and three in the United States (Twizeyimana and Hartman 2012). In Africa, seven pathotypes were described in Nigeria (Twizeyimana et al. 2009) and three in Uganda (Tukamuhabwa and Maphosa 2012). A pathotype refers to a class of pathogen isolates whose members express a similar pattern of host-specific virulence toward individual plant clones or species within a specified set, and pathogen virulence in combination with plant resistance may be monitored through pathotyping (Ramstedt et al. 2002).

The virulence of different isolates on host differentials carrying specific resistance genes are commonly used to determine the virulence spectrum of the pathogen. Evaluation of *P. pachyrhizi*

Accepted for publication 21 March 2017.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2017.

virulence is based on three reaction types produced on soybean host differentials: Immune (IM), representing no observable reaction; reddish brown (RB), which represents reddish brown lesions with little or no sporulation and implying an incompatible interaction in which the plant is resistant and the pathogen is avirulent; and tan reaction (TAN), which represents tan lesions with abundant sporulation in which the plant is susceptible and the pathogen is virulent (Bromfield and Hartwig 1980; McLean and Byth 1980).

Virulence of the *P. pachyrhizi* population for any geographic location may be dependent on virulence of prominent pathotypes. Knowledge of virulence dynamics should aid the development of soybean cultivars with effective resistance genes. Currently, only a limited number of *P. pachyrhizi* isolates from Africa have been evaluated for virulence (Bonde et al. 2006; Tukamuhabwa and Maphosa 2012; Twizeyimana et al. 2009). For example, an isolate collected from Zimbabwe produced a TAN reaction on all soybean differentials carrying resistance genes *Rpp1* to 4; in contrast, an isolate from South Africa produced RB reactions on soybean carrying *Rpp1*, *Rpp2*, and *Rpp4* (Bonde et al. 2006). In addition, Ugandan populations were virulent on *Rpp1* and *Rpp4* (Oloka et al. 2008), and virulent on PI 230970 (*Rpp2*) (Maphosa et al. 2013).

With the recent rapid spread of *P. pachyrhizi* in East Africa, it is necessary to characterize the virulence spectrum and evaluate the effectiveness of the various *Rpp* genes against the present *P. pachyrhizi* population in order to guide their deployment in breeding programs. The goal of the study was to characterize the virulence spectrum of rust isolates from Africa in comparison with isolates from other continents and identify soybean resistance genes that would be effective against *P. pachyrhizi* pathotypes in Africa. The specific objectives were to (i) characterize the virulence diversity of *P. pachyrhizi* populations from eastern and southern Africa on a defined set of host differentials, (ii) compare the virulence spectrum of these populations with populations from other African regions and other continents, and (iii) evaluate the effectiveness of multiple resistance genes against the various isolates. The information gained may be useful for guiding screening methods and deployment of cultivars with durable resistance to all predominant pathotypes of *P. pachyrhizi* in eastern Africa.

Materials and Methods

Field surveys and sample collections. A total of 12 soybean fields in central (Dowa, Kasungu, and Mchinji) and southern Malawi (Thyolo) and 31 fields in the southern highlands (Iringa, Mbeya, Morogoro, and Ruvuma regions) of Tanzania were surveyed for the presence of soybean rust in the 2013–14 growing season. Out of the total fields surveyed, soybean rust was observed in 23 fields from which about 10 symptomatic leaf samples were collected from different soybean plants. In addition, 23 samples from different fields in the northwest and northeast regions of Pampa, Argentina, and one sample each from Australia (New South Wales), Kenya (western region), and South Africa (KwaZulu Natal) were sent to the University of Illinois under an APHIS permit. Two samples from the United States (Florida and Texas) and one from Taiwan were included in the study to give a total of 52 samples.

Soybean rust recovery and multiplication of inoculum. To establish cultures of *P. pachyrhizi*, spores of infected leaf samples from each location were used to inoculate the abaxial surface of healthy detached leaves of Williams 82 (Twizeyimana and Hartman 2010), a fully susceptible soybean cultivar lacking resistance genes against all pathotypes of *P. pachyrhizi*. The inoculated leaves were placed on a paper towel saturated with 5 ml sterile distilled water in a 13.7 × 22.0 × 3.2 cm transparent plastic container; an additional 4 ml/container of distilled water was added as needed at 6-day intervals to keep the paper towel saturated. The containers first were placed in a tissue chamber in the dark for 24 h at 23°C, followed by alternating dark and light periods of 12 h each at temperatures between 22 and 25°C and humidity between 65 to 75% (Percival Scientific, Inc., Boone, IA) for 14 days. To obtain sufficient spores, recovered isolates were further multiplied on the cultivar Williams 82 under the same conditions.

Isolate infection types on soybean differentials. Eleven soybean host differentials obtained from the USDA Soybean Germplasm

Collection were selected based on prior knowledge of their resistance to soybean rust and the differential reaction to various *P. pachyrhizi* isolates (Table 1). Seven seeds of each of the 11 differential lines were sown in a plastic tray filled with Sunshine LCI mix (Sun Grow Horticulture Inc., Bellevue, WA) mixed with 10 mg 14:14:14 N/P/K Osmocote fertilizer (Scotts-Sierra Horticultural Products Co., Marysville, OH). Trays were placed in the growth chamber in alternating dark and light periods of 12 h each at temperatures between 22 and 25°C and humidity between 65 and 75% and under rust-free conditions.

After 3 to 4 weeks, leaflets from the 2nd and/or 3rd trifoliolate leaves were used for inoculation. Each container, containing one leaflet from each of the 11 soybean differentials, was inoculated with a single *P. pachyrhizi* isolate. The containers were placed in a randomized complete block design with three replications for each isolate. Inoculation was done by tapping two to three leaves bearing sporulating uredinia from each isolate to dislodge the spores onto the abaxial side of the leaflets of the differential. The leaflets were initially incubated in the dark for a period of 24 h followed by 12 h photoperiods at 22 to 25°C, and humidity of 65 to 75% in a tissue chamber for 14 days.

Pathotype evaluation and analysis. Soybean leaves were examined by means of a stereomicroscope at 80× magnification to determine the infection types induced by each isolate 14 to 17 days after inoculation. The infection types were recorded as IM, RB, or TAN. IM and RB were grouped as resistant response and TAN was regarded as a susceptible response. These categories were used to assign the pathotypes for each isolate. Inoculation tests were repeated and if a reaction was not clearly resistant or susceptible, it was repeated again.

For pathotype classification, the octal nomenclature system was used (Goodwin et al. 1990). TAN reactions were coded as 1, and IM and RB as 0 (Twizeyimana et al. 2009). Isolate virulence data were summarized using the HaGiS (Habgood-Gilmour spreadsheet) V.3.1 program (Herrmann et al. 1999) that converts virulence data into octal format. Briefly, the 11 host differentials were arranged in triplets as follows, first octal digit comprising LD0916057 (*Rpp1*), LD103005 (*Rpp1b*), and PI 417125 (*Rpp2*). The second octal digit included PI 462312 (*Rpp3*), PI 459025B (*Rpp4*), and PI 200526 (*Rpp5a*). The third octal digit consisted of LD1014274 (*Rpp5b*), PI 567102B (*Rpp6*), and cultivar Hyuuga carrying *Rpp3* and *Rpp5* (Kendrick et al. 2011). The fourth octal digit was represented by cultivar UG5. For each collection, the virulence frequency and mean virulence complexity or average number of soybean differentials with which an isolate has a susceptible interaction were calculated.

The Euclidean distance matrix was calculated using R version 3.2.0 in default settings (R Development Core Team 2008). The resulting matrices were entered into a hierarchical clustering function of the software. A dendrogram was generated using the unweighted pair group method with arithmetic mean (UPGMA; 31). The R package pvclust

Table 1. Soybean host differentials used to classify *Phakopsora pachyrhizi* isolates

Genotype	Resistance gene(s)	Reference for gene designation and/or source of plant material
LD09-16057	<i>Rpp1</i>	Diers et al. 2014; McLean and Byth 1980
LD10-30052	<i>Rpp1b</i>	Chakraborty et al. 2009; Diers et al. 2014
PI417125	<i>Rpp2</i>	Hartwig and Bromfield 1983; Laperuta et al. 2008
PI462312	<i>Rpp3</i>	Bromfield and Melching 1982
PI459025B	<i>Rpp4</i>	Hartwig 1986
LD10-14274	<i>Rpp5a</i>	Diers et al. 2014
PI200526	<i>Rpp5b</i>	Garcia et al. 2008
PI567102B	<i>Rpp6</i>	Li et al. 2012
LD10-14205 (Hyuuga)	<i>Rpp3,5</i>	Kendrick et al. 2011; Diers et al. 2014
UG5	<i>Rpp1,3</i>	Not published
Williams 82	Susceptible	Miles et al. 2006

was executed to assess the uncertainty in hierarchical cluster analysis, which calculates *P*-values for each cluster using bootstrap resampling techniques.

Results

Isolate infection types on soybean differentials. All isolates induced a TAN infection type on cultivars LD0916057 (*Rpp1*), PI 459025B (*Rpp4*), and PI 200526 (*Rpp5b*) and on the susceptible soybean cultivar Williams 82 (Table 2), except isolate FL07-1, which produced an IM infection type on *Rpp1*. RB and IM infection types were induced on PI 417125 (*Rpp2*) by all isolates, except for the isolate from South Africa. RB and IM infection types were induced on *Rpp1b* by all isolates except KE-12-01 and FL-07-01, which induced a TAN infection type. ARG-14-01 and FL07-1 induced a TAN infection type on LD1014274 (*Rpp5a*) while all the other isolates induced an RB infection type. All isolates induced an RB infection type on Hyuuga (except AUS-14-01, ARG-14-01, ARG-02, -04, and FL07-1) and on UG5 (except for the four isolates from Argentina).

The Argentinian isolate (ARG-14-01) was the most virulent on 8 of the 11 differentials, including cultivars Hyuuga and UG5 carrying multiple resistant genes. Likewise, the other Argentinian isolates were also virulent on the two cultivars except isolate ARG-14-03, which was avirulent on Hyuuga. The Australian isolate (AUS-14-01) was the least virulent of the isolates from outside Africa, but was virulent on cultivar Hyuuga. FL07-1 from the United States was virulent on Hyuuga, but avirulent on UG5.

The South African isolate SA-14-01 was the most virulent of the African isolates, while the Malawian and 6 of the 12 Tanzanian isolates were the least virulent. Of all the isolates tested, only six from Tanzania were virulent on PI 567102B (*Rpp6*) while none of the African isolates were virulent on cultivars Hyuuga and UG5. There were no virulence differences observed between samples collected from the same field.

Pathotype evaluation and analysis. The 25 isolates were distributed into 10 distinct *P. pachyrhizi* pathotypes (Table 3). Isolates from Kenya and South Africa each had distinct pathotypes, 3212 and 5312, respectively. Tanzanian isolates were grouped into two distinct pathotypes, 1212 and 1232, each comprising 50% of the isolates. One of these pathotypes, 1212, was also associated with all three isolates from Malawi and the single isolate from Taiwan (TW). This was the only pathotype that was common in three different countries while the other pathotypes were distinct to the country of origin. Argentinian isolates ARG-14-02 and ARG-14-04 were grouped into one pathotype (1353), while ARG-14-01, ARG-14-03, FL-07-01 (U.S.A.), TX-13-01 (U.S.A.), and AUS-14-01 (Australia) had distinct pathotypes. The number of virulences on the 11 differentials (virulence complexity) varied from four to eight (Fig. 1). Pathotype 1753 from Argentina had the highest number of virulences (73%) including cultivars Hyuuga and UG5. The least virulent pathotype (1212) from Malawi, Tanzania, and Taiwan was virulent on *Rpp1*, *Rpp4*, and *Rpp5* genes and the susceptible check.

The isolates were clustered into four major groups (Fig. 1). Group I comprised a single isolate (FL07-1) from the United States, while all the isolates from Argentina were grouped into cluster II. The South African isolate was grouped in cluster III. Cluster IV included the Taiwanese and Australian isolates, one isolate from Texas, and all the isolates from Malawi and Tanzania.

Discussion

The recent global spread of *P. pachyrhizi* requires constant monitoring for the presence and changes in virulence to different pathotypes in order to guide the development and deployment of soybean germplasm with durable host resistance. We discovered 10 distinct pathotypes with specific virulence patterns from the 25 isolates of *P. pachyrhizi* from eight countries when tested against a set of 11 differential hosts with known resistant genes. Four pathotypes were described among the 17 isolates collected from Africa, with Kenyan and South African isolates

Table 2. Infection types induced by *Phakopsora pachyrhizi* isolates on the 11 soybean host differentials

Isolates ^a	Differential ^b											TAN (%) ^c
	<i>Rpp1</i>	<i>Rpp1b</i>	<i>Rpp2</i>	<i>Rpp3</i>	<i>Rpp4</i>	<i>Rpp5a</i>	<i>Rpp5b</i>	<i>Rpp6</i>	Hyuuga	UG5	Williams 82	
KE-12-01	TAN	TAN	RB	RB	TAN	RB	TAN	RB	RB	IM	TAN	45
MAL-14-01	TAN	RB	RB	RB	TAN	RB	TAN	RB	RB	IM	TAN	36
MAL-14-02	TAN	RB	RB	RB	TAN	RB	TAN	RB	RB	IM	TAN	36
MAL-14-03	TAN	IM	RB	RB	TAN	RB	TAN	IM	RB	IM	TAN	36
SA-14-01	TAN	IM	TAN	TAN	TAN	RB	TAN	RB	RB	IM	TAN	54
TZ-14-01	TAN	RB	RB	RB	TAN	RB	TAN	TAN	RB	IM	TAN	45
TZ-14-02	TAN	RB	RB	RB	TAN	RB	TAN	TAN	RB	IM	TAN	45
TZ-14-03	TAN	RB	RB	RB	TAN	RB	TAN	TAN	RB	IM	TAN	45
TZ-14-04	TAN	RB	RB	RB	TAN	RB	TAN	TAN	RB	RB	TAN	45
TZ-14-05	TAN	RB	RB	RB	TAN	RB	TAN	TAN	RB	IM	TAN	45
TZ-14-06	TAN	RB	RB	RB	TAN	RB	TAN	TAN	RB	RB	TAN	45
TZ-14-07	TAN	IM	RB	IM	TAN	RB	TAN	IM	RB	RB	TAN	36
TZ-14-08	TAN	IM	RB	RB	TAN	RB	TAN	RB	RB	RB	TAN	36
TZ-14-09	TAN	RB	RB	RB	TAN	RB	TAN	RB	RB	RB	TAN	36
TZ-14-10	TAN	RB	RB	RB	TAN	RB	TAN	RB	RB	RB	TAN	36
TZ-14-11	TAN	RB	RB	RB	TAN	RB	TAN	RB	RB	RB	TAN	36
TZ-14-12	TAN	RB	RB	RB	TAN	RB	TAN	RB	RB	RB	TAN	36
AUS-14-01	TAN	IM	RB	RB	TAN	RB	TAN	IM	TAN	IM	TAN	45
ARG-14-01	TAN	RB	RB	TAN	TAN	TAN	TAN	RB	TAN	TAN	TAN	73
ARG-14-02	TAN	RB	RB	TAN	TAN	RB	TAN	RB	TAN	TAN	TAN	64
ARG-14-03	TAN	RB	RB	TAN	TAN	RB	TAN	RB	RB	TAN	TAN	54
ARG-14-04	TAN	RB	RB	TAN	TAN	RB	TAN	RB	TAN	TAN	TAN	63
FL-07-01	IM	TAN	RB	RB	TAN	TAN	TAN	TAN	TAN	IM	TAN	64
TW-07-01	TAN	RB	IM	RB	TAN	RB	TAN	RB	RB	IM	TAN	36
TX-13-01	TAN	IM	IM	RB	TAN	RB	TAN	IM	RB	IM	TAN	36
RB/IM (%) ^c	4	92	96	80	0	92	0	72	80	84	0	

^a Isolate name consisting of three parts: (i) country or state of origin; AUS = Australia, ARG = Argentina, FL = Florida, KE = Kenya, MAL = Malawi, SA = South Africa, TW = Taiwan, TX = Texas, TZ = Tanzania; (ii) year of collection; 07 (2007), 12 (2012), 13 (2013), 14 (2014); and (iii) isolate number in each location.

^b *Rpp1* = LD0916057, *Rpp1b* = LD1030052, *Rpp2* = PI 417125, *Rpp3* = PI 462312, *Rpp4* = PI 459025B, *Rpp5a* = LD10-14274, *Rpp5b* = PI 200526, *Rpp6* = PI 567102B. LD1014205 (Hyuuga) = *Rpp3* and 5, UG5 = *Rpp1* and 3. TAN = Tan colored infection type with abundantly sporulating uredinia, RB = reddish brown infection type (with little or no sporulation), IM = immune response with no visible symptoms.

^c Percentage of TAN or RB/IM of the isolates and the differentials, respectively.

classified into distinct pathotypes. The three isolates from Malawi and 50% of the Tanzanian isolates were grouped into one common pathotype, suggesting the presence of an identical *P. pachyrhizi* population in those countries. Isolates obtained from a single field did not differ in virulence, which suggested that they belonged to the same *P. pachyrhizi* population. We report for the first-time classification of *P. pachyrhizi* isolates into pathotypes from multiple East African countries. In Nigeria, located in West Africa, seven pathotypes among 116 isolates were reported (Twizeyimana et al. 2009), while three pathotypes among 45 isolates were reported in Uganda (Tukamuhabwa and Maphosa 2012).

In our study, the South African isolate was the most virulent of the African isolates with virulence on six of the host differentials, including differentials with resistance genes *Rpp2* and *Rpp3* that were effective against the pathotype from Kenya, Malawi, and Tanzania. An isolate from South Africa, collected in 2001, was shown to be avirulent on PI 462312 (*Rpp3*) (Bonde et al. 2006), suggesting that a more virulent pathotype in South Africa may have appeared since the initial introduction of *P. pachyrhizi*.

Pathotype 1212 was represented by six Tanzanian, the three Malawian, and the one Taiwanese isolate, which was the least virulent isolate in our study. Soybean cultivation in Malawi and Tanzania occurs only once per year, which implies that the pathogen population size may be reduced due to periods without a host. This may limit gene diversity in the pathogen since only a small subset of genetic diversity from the original population may be present in the new population. The isolate from Taiwan represents one of the older cultures within the collection in this study. It is possible that the culture has lost some of its original virulence due to prolonged storage.

Despite the limited number of isolates tested, four from Argentina were classified into three distinct pathotypes, while those from Australia, Taiwan, and the United States were each grouped into distinct pathotypes, suggesting a broader variation among *P. pachyrhizi* isolates from the Americas used in our study as compared with those from Africa. Pathotype 1732 from Argentina was the most virulent, with virulence on eight differentials including the two cultivars Hyuuga and UG5 with multiple resistance genes. Greater virulence has previously been observed among isolates from Argentina compared with those from Brazil, Japan, and Paraguay (Akamatsu et al. 2013). Twizeyimana and Hartman (2012) reported three distinct pathotypes among 72 *P. pachyrhizi* isolates collected from three different regions in the United States, while Yamaoka et al. (2014) identified six races out of the 26 isolates collected within a single district in Japan. This suggests that *P. pachyrhizi* is highly dynamic across the globe, and underscores the need to screen and deploy effective and novel sources of resistance.

We did not find any isolates that differentiated *Rpp1*, *Rpp4*, and *Rpp5b* genes as all isolates produced TAN lesion types except for FL07-1 from the United States on *Rpp1*. Previous studies on *Rpp1* reported TAN type infections among South American isolates (Akamatsu et al. 2013; Miles et al. 2008; Silva et al. 2008), Thailand (Miles et al. 2011), and Vietnam (Pham et al. 2010). In contrast, *Rpp1* was effective against some isolates from the United States (Paul et al. 2015; Twizeyimana and Hartman 2012) and Japan (Yamanaka et al. 2010; Yamaoka et al. 2014).

Our study showed the ineffectiveness of *Rpp4*, which was reported to confer resistance against rust populations in Brazil (Laperuta et al. 2008; Pham et al. 2009; Silva et al. 2008; Yamanaka et al. 2010), Japan (Yamanaka et al. 2010; Yamaoka et al. 2014), Vietnam (Pham et al. 2010), Nigeria (Twizeyimana et al. 2009), Uganda (Maphosa et al. 2013), and Zimbabwe (Pham et al. 2009). Others have similarly reported the ineffectiveness of *Rpp4* to isolates from Argentina and Paraguay (Akamatsu et al. 2013).

Our study showed the ineffectiveness of *Rpp5b*, which was reported effective against rust from South America and Japan (Akamatsu et al. 2013; Lemos et al. 2011; Yamaoka et al. 2014), and from the United States and Vietnam (Kendrick et al. 2011; Pham et al. 2010; Twizeyimana and Hartman 2012). In Africa, TAN infection types previously have been reported on *Rpp5b* induced by isolates from South Africa and Zimbabwe (Kendrick et al. 2011).

The *Rpp2* gene was highly effective to most isolates but ineffective to one South African isolate that was similar to reports from South America (Akamatsu et al. 2013; Laperuta et al. 2008; Miles et al. 2008; Yamanaka et al. 2010) and Japan (Akamatsu et al. 2013). There are no previous reports on testing of this genotype carrying *Rpp2* against rust isolates from Africa.

The *Rpp3* gene was mostly effective with only five isolates producing a TAN reaction; four from Argentina and the one South African isolate. In other studies, this gene was effective against rust populations in Japan (Yamanaka et al. 2010; Yamaoka et al. 2014), the United States (Paul et al. 2015; Twizeyimana and Hartman 2012), Nigeria (Twizeyimana et al. 2009), Uganda (Maphosa et al. 2013), and Zimbabwe (Pham et al. 2009). In contrast, *Rpp3* was effective against rust isolates from Argentina (Akamatsu et al. 2013), Brazil (Laperuta et al. 2008; Silva et al. 2008), and Vietnam (Pham et al. 2010).

Gene *Rpp1b* was reported ineffective against rust isolates from Vietnam (Pham et al. 2010) and the United States (Paul et al. 2015), but was effective against rust in Nigeria (Twizeyimana et al. 2009), where it conferred complete resistance. This gene was effective against most of the isolates in our study.

Rpp5a gene was effective against rust from Colombia, Hawaii, South Africa, Vietnam, Zimbabwe (Kendrick et al. 2011), and the United States (Paul et al. 2015). This gene also was effective against 92% of the rust isolates in our study.

The *Rpp6* gene discovered in PI 567102B (Li et al. 2012) produced a resistance response to *P. pachyrhizi* populations from Paraguay

Table 3. *Phakopsora pachyrhizi* isolates from samples collected in eight countries, the year of collection, and pathotype designation

Isolate name ^a	Country of origin	Year of collection	Pathotype designation ^b
TZ-14-01	Tanzania	2014	1232
TZ-14-02	Tanzania	2014	1232
TZ-14-03	Tanzania	2014	1232
TZ-14-04	Tanzania	2014	1232
TZ-14-05	Tanzania	2014	1232
TZ-14-06	Tanzania	2014	1232
TZ-14-07	Tanzania	2014	1212
TZ-14-08	Tanzania	2014	1212
TZ-14-09	Tanzania	2014	1212
TZ-14-10	Tanzania	2014	1212
TZ-14-11	Tanzania	2014	1212
TZ-14-12	Tanzania	2014	1212
TW-07-01	Taiwan	2007	1212
MAL-14-01	Malawi	2014	1212
MAL-14-02	Malawi	2014	1212
MAL-14-03	Malawi	2014	1212
AUS-14-01 ^c	Australia	2014	1252
ARG-14-02 ^c	Argentina	2014	1353
ARG-14-01 ^c	Argentina	2014	1753
ARG-14-03 ^c	Argentina	2014	1313
ARG-14-04 ^c	Argentina	2014	1353
TX-13-01 ^d	U.S.A.	2013	1712
FL-07-01 ^d	U.S.A.	2007	2652
KE-12-01	Kenya	2012	3212
SA-14-01 ^c	South Africa	2014	5312

^a The isolate name consists of three parts: (i) country or state of origin; AUS = Australia, ARG = Argentina, FL=Florida, KE = Kenya, MAL = Malawi, SA = South Africa, TW = Taiwan, TX = Texas, TZ = Tanzania; (ii) year of collection; 07 (2007), 12 (2012), 13 (2013), 14 (2014); and (iii) isolate number in each location.

^b Pathotype designation was based on octal nomenclature. Octal digits were assigned as follows: 000 = 0, 100 = 1, 010 = 2, 110 = 3, 001 = 4, 101 = 5, 011 = 6, and 111 = 7. The octal digits in this system are sorted according to the number of plants susceptible to the particular isolate per triplet (Herrmann et al. 1999).

^c Leaf samples provided by Dr. Natalie Moore (NWS, Australia), Dr. Antonio Ivancovich (INRA Argentina), and Dr. Jarvie Antony (Pannar Seed, South Africa).

^d Isolates maintained at the University of Illinois-Urbana, Soybean Pathology Laboratory provided by Yi-Shou Huang in 2007.

(Miles et al. 2008) and the United States (Paul et al. 2015). Despite most of the isolates from our study inducing RB infection types on genotypes containing this gene, half of the isolates from Tanzania induced a TAN infection type. This suggests that its future as a source of resistance may be limited.

Our study, as well as other similar studies suggest an evolving virulence of the *P. pachyrhizi* population and that the *Rpp* genes can easily lose their durability for resistance and therefore may not be uniformly useful for resistance breeding. The breakdown of resistance could be attributed to evolution of local pathogen populations through mutation, recombination, or direct selection on the cultivars deployed (Burdon and Silk 1997; McDonald and Linde 2002).

Gene pyramiding stacks with multiple resistance genes in a single cultivar has been suggested as a method to develop more durable resistance against *P. pachyrhizi* (Goellner et al. 2010; Lemos et al. 2011; Maphosa et al. 2012; Yamanaka et al. 2015; Yamaoka et al. 2014). In the current study, cultivars UG5 and Hyuuga each stacked with two genes had a resistance response to over 80% of the isolates providing evidence that gene stacking may be effective in certain cases. Cultivar UG5 was developed from Uganda and carries *Rpp1* and *Rpp3* (Paul et al. 2015). The cultivar has been reported to consistently confer resistance against soybean rust in Nigeria (Twizeyimana et al. 2009), the United States (Paul et al. 2015; Twizeyimana and Hartman 2012), and Uganda (Maphosa et al. 2013; Oloka et al. 2008). Cultivar UG5 has been deployed in breeding programs in Nigeria and Uganda (Paul et al. 2010; Tukamuhabwa and Maphosa 2012) and appears to be effective as was observed among the African isolates in our study. Despite its effectiveness, isolates from Argentina induced susceptible reactions on this cultivar, suggesting the presence of a more virulent population in South America.

Cultivar Hyuuga, containing *Rpp3* and *Rpp5*, also consistently exhibited resistant reactions against isolates from Africa. Kendrick et al.

(2011) reported the effectiveness of this cultivar among seven of the eight isolates from different countries as well as rust from Brazil (Yamanaka et al. 2013). This cultivar could potentially be deployed in African soybean breeding programs to provide sustainable resistance to soybean rust.

Virulence of AUS-14-01, FL07-1, and three of the Argentinian isolates on cultivar Hyuuga and all the Argentinian isolates on cultivar UG5 suggests stacking with only two genes may not be sufficient to produce durable resistance. Yamanaka et al. (2013) reported the effectiveness of a line carrying three *Rpp* genes (*Rpp1*, *Rpp4*, and *Rpp5*), compared with lines carrying either one (*Rpp2*) or two (*Rpp2* and *Rpp4*) genes against single-lesion rust isolates from Brazil, demonstrating the need to stack multiple genes. Gene stacking has been reported to be effective in providing more durable resistance against bacterial blight of rice (Huang et al. 1997; Singh et al. 2001), rice leaf blast (Fukuoka et al. 2015; Yasuda et al. 2015), and leaf rust and stripe rust of wheat (Charpe et al. 2012).

Although the number of isolates in our study was limited, it is clear that comparable virulence diversity exists among *P. pachyrhizi* populations from Africa, as well as from other continents. Therefore, to obtain a more detailed insight into the extent of this diversity, additional studies involving more isolates are required. The knowledge on *P. pachyrhizi* virulence and the available effective resistance genes from this study is especially important for Africa, where soybean rust has spread rapidly. Our study demonstrates that soybean lines carrying *Rpp2*, *Rpp3*, and *Rpp5a* genes and the cultivars Hyuuga and UG5 are effective against rust in eastern Africa. Information from our study will help breeders to determine what resistance genes should be used in their breeding programs. The current virulence diversity of *P. pachyrhizi* populations and its anticipated increase necessitate the continuous monitoring of virulence changes and screening for more sources of novel resistance to guide targeted breeding to safeguard soybean production.

Fig. 1. Cluster dendrogram of the 25 *Phakopsora pachyrhizi* isolates from eight different countries based on their virulence on the differential set of soybean. Cluster analysis was performed using R software version 3.2.0 (R Development Core Team 2008). The distance matrix was entered into a hierarchical clustering function of the software and the dendrogram was constructed using the minimum variance (Ward's method). Group I consist of an isolate from the United States (FL07-1), group II consists of isolates from Argentina (ARG-14-1-4). A South African isolate (SA-14-01) was grouped in III. The remaining isolates were grouped in IV (AUS = Australia, KE = Kenya, MAL = Malawi, TX = Texas, TW = Taiwan, and TZ = Tanzania).

Acknowledgments

We acknowledge the funding and support by the International Institute of Tropical Agriculture (IITA) toward this research through the CGIAR funded CRP 3.5 and the Lukas Brader Scholarship fund. We thank Dr. Antonio Ivanovich (INRA, Argentina), Dr. Jarvie Antony (Pannar Seed, South Africa), Dr. Yi-hsiou Huang (Dow AgroSciences, Taiwan), and Dr. Natalie Moore (NWS, Australia) for providing infected leaf samples for use in this study, and Dr. R. Nelson (USDA – Soybean Germplasm Collection Center, Urbana, Illinois) and Dr. Brian Diers (University of Illinois) for providing soybean seed. We also thank Jesse Stewart for a technical assistance with evaluations. The lead author is grateful to Biosciences East and Central Africa (BeCA) for supporting his attendance at their 2015 Paper-writing Workshop that stimulated preparation of this article.

Literature Cited

- Akamatsu, H., Yamanaka, N., Yamaoka, Y., Soares, R. M., Morel, W., Ivanovich, A. J. G., and Suenaga, K. 2013. Pathogenic diversity of soybean rust in Argentina, Brazil, and Paraguay. *J. Gen. Plant Pathol.* 79:28-40.
- Akinsami, O., Ladipo, J., and Oyekan, P. 2001. First report of soybean rust (*Phakopsora pachyrhizi*) in Nigeria. *Plant Dis.* 85:97.
- Bandyopadhyay, R., Ojiambo, P. S., Twizeyimana, M., Asafo-Adjei, B., Frederick, R. D., Pedley, K. F., and Hartman, G. L. 2007. First report of soybean rust caused by *Phakopsora pachyrhizi* in Ghana. *Plant Dis.* 91:1057.
- Bonde, M. R., Nester, S. E., Austin, C. N., Stone, C. L., Frederick, R. D., Hartman, G. L., and Miles, M. R. 2006. Evaluation of virulence of *Phakopsora pachyrhizi* and *P. meibomia* isolates. *Plant Dis.* 90:708-716.
- Bromfield, K. R., and Hartwig, E. 1980. Resistance to soybean rust and mode of inheritance. *Crop Sci.* 20:254-255.
- Bromfield, K. R., and Melching, J. S. 1982. Sources of specific resistance to soybean rust. *Phytopathology* 72:709.
- Burdon, J. J., and Silk, J. 1997. Sources and patterns of diversity in plant-pathogenic fungi. *Phytopathology* 87:664-669.
- Chakraborty, N., Curley, J., Frederick, R. D., Hyten, D. L., Nelson, R. L., Hartman, G. L., and Diers, B. W. 2009. Mapping and confirmation of a new allele at *Rpp1* from soybean PI 594538A conferring RB lesion-type resistance to soybean rust. *Crop Sci.* 49:783-790.
- Charpe, A., Koul, S., Gupta, S. K., Singh, A., Pallavi, J. K., and Prabhu, K. V. 2012. Marker assisted gene pyramiding of leaf rust resistance genes Lr 9, Lr 24 and Lr 28 in a bread wheat cultivar HD 2329. *J. Wheat Res.* 4:20-28.
- Diers, B. W., Kim, K., Frederick, R. D., Hartman, G. L., Unfried, J., Schultz, S., and Cary, T. 2014. Registration of eight soybean germplasm lines resistant to soybean rust. *J. Plant Regist.* 8:96-101.
- Fukuoka, S., Saka, N., Mizukami, Y., Koga, H., Yamanouchi, U., Yoshioka, Y., Hayashi, N., Ebana, K., Mizobuchi, R., and Yano, M. 2015. Gene pyramiding enhances durable blast disease resistance in rice. *Sci. Rep.* 5:7773.
- Garcia, A., Calvo, E. S., Kiihl, R. A. D., Harada, A., Hiromoto, D. M., and Vieira, L. G. E. 2008. Molecular mapping of soybean rust (*Phakopsora pachyrhizi*) resistance genes: discovery of a novel locus and alleles. *Theor. Appl. Genet.* 117:545-553.
- Goellner, K., Loehrer, M., Langenbach, C., Conrath, U., Koch, E., and Schaffrath, U. 2010. *Phakopsora pachyrhizi*, the causal agent of Asian soybean rust: Pathogen profile. *Mol. Plant Pathol.* 11:169-177.
- Goodwin, S., Allard, R. W., and Webster, R. K. 1990. A nomenclature for *Rhynchosporium secalis* pathotypes. *Phytopathology* 80:1330-1336.
- Hartman, G. L., Hill, C. B., Twizeyimana, M., Miles, M. R., and Bandyopadhyay, R. 2011. *Phakopsora pachyrhizi*, the cause of soybean rust. *CAB Reviews. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour.* 6:1-13.
- Hartman, G. L., Miles, M. R., and Frederick, R. D. 2005. Breeding for resistance to soybean rust. *Plant Dis.* 89:664-666.
- Hartman, G. L., Wang, T. C., and Tschanz, A. T. 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 4th major gene conferring resistance to soybean rust. *Crop Sci.* 26:1135-1136.
- Hartwig, E. E., and Bromfield, K. R. 1983. Relationships among three genes conferring specific resistance to rust in soybeans. *Crop Sci.* 23:237-239.
- Hennings, P. 1903. Some new Japanese Uredinales. IV. *Hedwigia* 42:107-108.
- Herrmann, A., Löwer, C. F., and Schachtel, G. A. 1999. A new tool for entry and analysis of virulence data for plant pathogens. *Plant Pathol.* 48:154-158.
- Huang, N., Angeles, E. R., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kumaravadevel, N., Bennett, J., and Khush, G. S. 1997. Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. *Theor. Appl. Genet.* 95:313-320.
- Isard, S. A., Russo, J. M., and Arriati, A. 2007. The integrated aerobiology modeling system applied to spread of soybean rust into Ohio River valley in September 2006. *Aerobiologia* 23:271-282.
- Kawuki, R. S., Adipala, E., and Tukamuhabwa, P. 2003. Yield loss associated with soybean rust (*Phakopsora pachyrhizi* Syd.) in Uganda. *J. Plant Pathol.* 151:7-12.
- Kelly, H. Y., Dufault, N. S., Walker, D. R., Isard, S. A., Schneider, R. W., Giesler, L. J., Wright, D. L., Marois, J. J., and Hartman, G. L. 2015. From select agent to an established pathogen: The response to *Phakopsora pachyrhizi* (soybean rust) in North America. *Phytopathology* 105:905-916.
- Kendrick, M. D., Harris, D. K., Ha, B. K., Hyten, D. L., Cregan, P. B., Frederick, R. D., Boerma, H. R., and Pedley, K. F. 2011. Identification of a second Asian soybean rust resistance gene in Hyuuga soybean. *Phytopathology* 101:535-543.
- Kumudini, S., Godoy, C. V., Board, J. E., Omielan, J., and Tollenaar, M. 2008. Mechanisms involved in soybean rust-induced yield reduction. *Crop Sci.* 48:2334-2342.
- Laperuta, L. D. C., Arias, C. A. A., Ribeiro, A. S., Rachid, B. F., Pierozzi, P. H. B., Toledo, J. F. F., Pipolo, A. E., and Carneiro, G. E. S. 2008. New genes conferring resistance to Asian soybean rust. Alleic testin for the *Rpp2* and *Rpp4* loci. *Pesqui. Agropecu. Bras.* 43:1741-1747.
- Lemos, N. G., Braccini, A., Abednour, R. V., Oliveira, M. C., Suenaga, K., and Yamanaka, N. 2011. Characterization of genes *Rpp2*, *Rpp4* and *Rpp5* for resistance to soybean rust. *Euphytica* 182:53-64.
- Levy, C. 2005. Epidemiology and chemical control of soybean rust in Southern Africa. *Plant Dis.* 89:669-674.
- Li, S., Smith, J. R., Ray, J. R., and Frederick, R. D. 2012. Identification of a new soybean rust resistance gene in PI567102B. *Theor. Appl. Genet.* 125:133-142.
- Lin, S. Y. 1966. Studies on the physiologic races of soybean rust fungus, *Phakopsora pachyrhizi* Syd. *J. Taiwan Agric. Res.* 51:24-28.
- Maphosa, M., Talwana, H., and Tukamuhabwa, P. 2012. Enhancing soybean rust resistance through *Rpp2*, *Rpp3* and *Rpp4* pair wise gene pyramiding. *Afr. J. Agric. Res.* 7:4271-4277.
- Maphosa, M., Talwana, H., and Tukamuhabwa, P. 2013. Assessment of comparative virulence and resistance in soybean using field isolates of soybean rust. *J. Agric. Sci.* 5:249-257.
- McDonald, B. A., and Linde, C. 2002. Pathogen population genetics, evolutionary potential and durable resistance. *Annu. Rev. Phytopathol.* 40:349-379.
- McLean, R. J., and Byth, D. E. 1980. Inheritance of resistance to rust (*Phakopsora pachyrhizi*) in soybeans. *Aust. J. Agric. Res.* 31:951-956.
- Miles, M. R., Bonde, M. R., Nester, S. E., Berner, D. K., Frederick, R. D., and Hartman, G. L. 2011. Characterizing resistance to *Phakopsora pachyrhizi* in soybean. *Plant Dis.* 95:577-581.
- Miles, M. R., Frederick, R. D., and Hartman, G. L. 2006. Evaluation of soybean germplasm for resistance to *Phakopsora pachyrhizi*. *Plant Health Prog.* Online. doi:10.1094/PHP-2006-0104-01-RS
- Miles, M. R., Levy, C., Morel, W., Mueller, T., Steinlage, T., van Rij, N., Frederick, R. D., and Hartman, G. L. 2007. International fungicide efficacy trials for the management of soybean rust. *Plant Dis.* 91:1450-1458.
- Miles, M. R., Morel, W., Ray, J. D., Smith, J. R., Frederick, R. D., and Hartman, G. L. 2008. Adult plant evaluation of soybean accessions for resistance to *Phakopsora pachyrhizi* in the field and greenhouse in Paraguay. *Plant Dis.* 92:96-105.
- Mueller, T. A., Miles, M. R., Morel, W., Marios, J. J., Wright, D. L., Kemerait, R. C., Levy, C., and Hartman, G. L. 2009. Effect of fungicide and timing of application on soybean rust severity and yield. *Plant Dis.* 93:243-248.
- Murithi, H. M., Beed, F., Madata, C., Haundenschild, J., and Hartman, G. L. 2014. First report of *Phakopsora pachyrhizi* on soybean causing rust in Tanzania. *Plant Dis.* 98:1586.
- Murithi, H. M., Beed, F., Soko, M., Haundenschild, J., and Hartman, G. L. 2015. First report of *Phakopsora pachyrhizi* causing rust on soybean in Malawi. *Plant Dis.* 99:420.
- Murithi, H. M., Beed, F., Tukamuhabwa, P., Thomma, B. P. H. J., and Joosten, M. H. A. J. 2016. Soybean production in eastern and southern Africa and threat of yield loss due to soybean rust caused by *Phakopsora pachyrhizi*. *Plant Pathol.* 65:176-188.
- Ojiambo, P. S., Bandyopadhyay, R., Twizeyimana, M., Lema, A., Frederick, R. D., Pedley, K. F., Stone, C. L., and Hartman, G. L. 2007. First report of rust caused by *Phakopsora pachyrhizi* on soybean in the Democratic Republic of Congo. *Plant Dis.* 91:1204.
- Oloka, H. K., Tukamuhabwa, P., Sengooba, T., and Shanmugasundram, S. 2008. Reaction of exotic soybean germplasm to *Phakopsora pachyrhizi* in Uganda. *Plant Dis.* 92:1493-1496.
- Paul, C., Bowen, C. R., Bandyopadhyay, R., Tefera, H., Adeleke, R., Sikora, E., Pegues, M. D., and Hartman, G. L. 2010. Registration of three soybean germplasm lines resistant to *Phakopsora pachyrhizi* (soybean rust). *J. Plant Regist.* 4:244-248.
- Paul, C., Frederick, R. D., Hill, C. B., Hartman, G. L., and Walker, D. R. 2015. Comparison of pathogenic variation among *Phakopsora pachyrhizi* isolates collected from the United States and international locations, and identification of soybean genotypes resistant to the U.S. isolates. *Plant Dis.* 99:1059-1069.
- Pham, T. A., Hill, C. B., Miles, M. R., Nguyen, B. T., Vu, T. T., Vuong, T. D., VanToai, T. T., Nguyen, H. T., and Hartman, G. L. 2010. Evaluation of soybean for resistance to soybean rust in Vietnam. *Field Crops Res.* 117:131-138.
- Pham, T. A., Miles, M. R., Frederick, R. D., Hill, C. B., and Hartman, G. L. 2009. Differential responses of resistant soybean entries to isolates of *Phakopsora pachyrhizi*. *Plant Dis.* 93:224-228.
- Pretorius, Z. A., Kloppers, F. J., and Frederick, R. D. 2001. First report of soybean rust in South Africa. *Plant Dis.* 85:1288.
- R Development Core Team. 2008. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, <http://www.R-project.org>

- Ramstedt, M., Hurtado, S., and Åström, B. 2002. Pathotypes of *Melampsora* rust on *Salix* in short-rotation forestry plantations. *Plant Pathol.* 51:185-190.
- Rusike, J., van den Brand, G., Boahen, S., Dashiell, K., Kantengwa, S., Ongoma, J., Mongane, D. M., Kasongo, G., Jamagani, Z. B., Aidoo, R., and Abaidoo, R. 2013. Value chain analyses of grain legumes in N2Africa: Kenya, Rwanda, eastern DRC, Ghana, Nigeria, Mozambique, Malawi and Zimbabwe. Wageningen, Netherlands: N2Africa. <http://www.n2africa.org/content/value-chain-analyses-grain-legumes-n2africa>. Accessed 15 March 2017.
- Sikora, E. J., Allen, T. W., Wise, K. A., Bergstrom, G., Bradley, C. A., Bond, J., Brown-Rytlewski, D., Chilvers, M., Damicone, J., DeWolf, E., Dorrance, A., Dufault, N., Esker, P., Faske, T. R., Giesler, L., Goldberg, N., Golod, J., Gomez, I. R. G., Grau, C., Grybauskas, A., Franc, G., Hammerschmidt, R., Hartman, G. L., Henn, A., Herselman, D., Hollier, C., Isakeit, T., Isard, S., Jacobson, B., Jardine, D., Kemerait, B., Koenning, S., Langham, M., Malvick, D., Markell, S., Marois, J. J., Monfort, S., Mueller, D., Mueller, J., Mulroony, R., Newman, M., Osborne, L., Padgett, G. B., Ruden, B. E., Rupe, J., Schneider, R., Schwartz, H., Shaner, G., Singh, S., Stromberg, E., Sweets, L., Tenuta, A., Vaiciunas, S., Yang, X. B., Young-Kelly, H., and Zidek, J. 2014. A coordinated effort to manage soybean rust in North America: a success story in soybean disease monitoring. *Plant Dis.* 98:864-875.
- Silva, D. C. G., Yamanaka, N., Brogin, R. L., Arias, C. A. A., Nepomuceno, A. L., Di Mauro, A. O., Pereira, S. S., Nogueira, L. M., Passianoto, A. L. L., and Abdelnoor, R. V. 2008. Molecular mapping of two loci that confer resistance to Asian rust in soybean. *Theor. Appl. Genet.* 117:57-63.
- Singh, S., Sidhu, J. S., Huang, N., Vikal, Y., Li, Z., Brar, D. S., Dhaliwal, H. S., and Khush, G. S. 2001. Pyramiding three bacterial blight resistance genes (xa5, xa13 and Xa21) using marker-assisted selection into indica rice cultivar PR106. *Theor. Appl. Genet.* 102:1011-1015.
- Tukamuhabwa, P., and Maphosa, M. 2012. State of knowledge in breeding for resistance to soybean rust in the developing world. *FAO Plant Production and Protection Paper*, 204. FAO, Rome, Italy.
- Twizeyimana, M., and Hartman, G. L. 2010. Culturing *Phakopsora pachyrhizi* on detached leaves and urediniospore survival at different temperatures and relative humidities. *Plant Dis.* 94:1453-1460.
- Twizeyimana, M., and Hartman, G. L. 2012. Pathogenic variation of *Phakopsora pachyrhizi* isolates on soybean in the United States from 2006 to 2009. *Plant Dis.* 96:75-81.
- Twizeyimana, M., Ojiambo, P. S., Hartman, G. L., and Bandyopadhyay, R. 2011. Dynamics of soybean rust epidemics in sequential plantings of soybean cultivars in Nigeria. *Plant Dis.* 95:43-50.
- Twizeyimana, M., Ojiambo, P. S., Sonder, K., Ikotun, T., Hartman, G. L., and Bandyopadhyay, R. 2009. Pathogenic variation of *Phakopsora pachyrhizi* infecting soybean in Nigeria. *Phytopathology* 99:353-361.
- Yamanaka, N., Lemos, N. G., Akamatsu, H., Yamaoka, Y., Kato, M., Silva, D. C. G., Passianotto, A. L., do Santos, J. V. M., Benitez, E. R., Abdelnoor, R. V., Soares, R. M., and Suenaga, K. 2010. Development of classification criteria for resistance to soybean rust and differences in virulence among Japanese and Brazilian rust populations. *Trop. Plant Pathol.* 35:153-162.
- Yamanaka, N., Lemos, N. G., Uno, M., Akamatsu, H., Yamaoka, Y., and Abdelnoor, R. V. 2013. Resistance to Asian soybean rust in soybean lines with the pyramided three *Rpp* genes. *Crop Breed. Appl. Biotechnol.* 13:75-82.
- Yamanaka, N., Morishita, M., Mori, T., Lemos, N. G., Hossain, M. M., Akamatsu, H., and Yamaoka, Y. 2015. Multiple *Rpp*-gene pyramiding confers resistance to Asian soybean rust isolates that are virulent on each of the pyramided genes. *Trop. Plant Pathol.* 40:283-290.
- Yamaoka, Y., Yamanaka, N., Akamatsu, H., and Suenaga, K. 2014. Pathogenic races of soybean rust *Phakopsora pachyrhizi* collected in Tsukuba and vicinity in Ibaraki, Japan. *J. Gen. Plant Pathol.* 80:184-188.
- Yamaoka, Y., Yukiyoishi, F., Makoto, K., Keizo, K., Kengo, Y., and Hiroshi, H. 2002. Pathogenic races of *Phakopsora pachyrhizi* on soybean and wild host plants collected in Japan. *J. Gen. Plant Pathol.* 68:52-56.
- Yasuda, N., Mitsunaga, T., Hayashi, K., Koizumi, S., and Fujita, Y. 2015. Effects of pyramiding quantitative resistance genes pi21, Pi34, and Pi35 on rice leaf blast disease. *Plant Dis.* 99:904-909.
- Yorinori, J. T., Paiva, W. M., Frederick, R. D., Costamilan, L. M., Bertagnolli, P. F., Hartman, G. L., Godoy, C. V., and Nunes, J., Jr. 2005. Epidemics of soybean rust (*Phakopsora pachyrhizi*) in Brazil and Paraguay from 2001 to 2003. *Plant Dis.* 89:675-677.