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Evaluation of soybean genotypes for resistance against the rust-causing fungus *Phakopsora pachyrhizi* in East Africa

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Abstract

Soybean rust, caused by the biotrophic fungus *Phakopsora pachyrhizi*, is the most important foliar disease of soybean (*Glycine max*) worldwide. Deployment of resistant soybean cultivars is the best option for managing this disease. Genes conferring resistance to *P. pachyrhizi* have been identified, but pathotypes of the rust fungus overcoming these resistance genes have also been found. To identify novel resistance genes, soybean genotypes from both local and international sources were screened at multiple locations in Tanzania and Uganda in 2016 and 2017. The results from this screening revealed that infection types, disease severities, and sporulation levels varied among the genotypes and locations. The majority of the genotypes had tan-coloured (TAN) lesions and developed moderate sporulation, implying susceptibility, while only seven of the 71 lines had reddish-brown (RB) lesions and showed low disease severities in all of the screening environments. We identified seven genotypes that were the most resistant to rust in the most locations over the two years. These genotypes will be useful for further studies and, ultimately, for rust management, as they show broad resistance to various pathotypes of the rust fungus.

KEYWORDS

infection types, *Phakopsora pachyrhizi*, resistance, soybean genotypes, susceptibility

1 | INTRODUCTION

Soybean (*Glycine max*) is an important legume crop, as it is a major source of protein and oil in Africa (Hartman et al., 2011). Soybean is used as a component in livestock feed, but also for human consumption in the form of soymilk, tofu, soybean oil, and as a vegetable (Ali, 2010). Besides its importance as a food/feed source, soybean cultivation is important for the improvement of soil quality, as it leads to the fixation of nitrogen into the soil, leading to improved soil fertility. Hence, it is a preferred crop for intercropping and rotation with nonleguminous crops (Sanginga, 2003). More than 2.1 million tonnes of soybeans were produced in Africa in 2016, representing a 67% increase since 2007 (FAOSTAT, 2018). Soybean production has intensified in eastern and southern Africa, a trend that is expected to continue. For instance, in Malawi, soybean production has more than tripled since 2005, while the production area increased by about 50% within the same period (FAOSTAT, 2018). Soybean production in Tanzania is concentrated in the southern highlands, and the production area and quantity has doubled over the past 10 years (FAOSTAT, 2018). Similar trends have been observed in Kenya, Rwanda, and Uganda (Murithi et al., 2016). Average yields range between 0.8 and 1.2 t/ha, while the yield potential is predicted to

range between 2.5 and 4 t/ha (FAOSTAT, 2018). The relatively low productivity of soybean is largely due to abiotic factors (soil fertility, drought, and poor nodulation) and biotic ones, such as diseases and insect pests (Wrather et al., 1997).

Rust, caused by the biotrophic fungus *Phakopsora pachyrhizi*, is one of the most damaging foliar diseases of soybean. The disease is native to Asia but has spread to Australia, India (Goellner et al., 2010), and Africa where it was first reported in Uganda in 1996 (Levy, 2005). It subsequently spread to Brazil in 2002 (Yorinori et al., 2005) and to the USA in 2004 (Schneider et al., 2005). Its introduction into Africa probably occurred through urediniospores blowing from western India to the African east coastal areas by moist north-east monsoon winds (Levy, 2005). The fungus spread rapidly and was reported after its introduction into Uganda on soybean in South Africa in 2001 (Pretorius et al., 2001), in western Cameroon in 2003 (Levy, 2005), and in Ghana and the Democratic Republic of Congo in 2007 (Bandyopadhyay et al., 2007; Ojiambo et al., 2007). The disease was also confirmed in Ethiopia, Malawi, and Tanzania (Murithi et al., 2014, 2015; Tesfaye et al., 2017). A second species causing rust on soybean, *Phakopsora meibomiae*, has not been reported in Africa or elsewhere outside the Americas (Hartman et al., 2011). *P. meibomiae* is much less aggressive than *P. pachyrhizi* and therefore does not pose a threat to soybean yield.

Within 7 to 9 days after penetration of the leaves of a susceptible soybean plant, *P. pachyrhizi* forms uredinia that erupt through the epidermis and release numerous urediniospores (Goellner et al., 2010). The uredinia form loosely woven to compact masses of mycelium in the palisade or spongy mesophyll and visibly disrupt the epidermis of the soybean leaves (Marchetti et al., 1979). Temperature and moisture play a vital role in soybean rust establishment and epidemics. The optimum temperature for spore germination ranges from 17 to 29 °C (Bonde et al., 2012), with a relative humidity higher than 85% and moisture on the leaf surface for a period of 6–12 hr (Melching et al., 1989). Therefore, the climate conditions in central and south-eastern Africa favour the infection of soybean by *P. pachyrhizi* throughout the year (Pivonia & Yang, 2004). The leaf tissue around the first uredinia turns from pale green to reddish-brown or purple, and later to dark brown (Goellner et al., 2010). Severe infection results in premature plant defoliation (Kumudini et al., 2008), leading to yield losses normally ranging between 18% and 55%, but losses can be as high as 80%, as has been reported in Uganda and Zimbabwe (Levy, 2005; Oloka et al., 2008).

The use of fungicides is currently the most widely employed method for the management of soybean rust disease, although fungicides are not easily accessible to many smallholder farmers in developing countries. If available, their use significantly increases production costs, can cause environmental risks, and can result in fungicide resistance of the pathogen, especially when single-site mode fungicides are used over a long time. Such resistance has been reported in South America and efforts are now directed to combine single-site fungicides with different modes of action, or with multisite fungicides (Godoy et al., 2016). Nevertheless, it is increasingly recognized that deployment of resistant soybean cultivars is a better disease control method because it is economical, safe, environmentally friendly, and complements other control methods. At least 200 germplasm accessions and breeding lines with resistance to soybean rust have been screened and seven resistance loci, designated *Rpp* (for resistance to *P. pachyrhizi*) have been characterized. These resistance loci comprise *Rpp1* (McLean & Byth, 1980), *Rpp2* (Bromfield & Hartwig, 1980), *Rpp3* (Bromfield & Melching, 1982), *Rpp4* (Hartwig, 1986), *Rpp5* (Garcia et al., 2008), *Rpp6* (Li et al., 2012), and *Rpp7* (Childs et al., 2018). None of these resistance genes is effective against all currently known soybean rust pathotypes (Childs et al., 2018).

Resistance or susceptibility of soybean to *P. pachyrhizi* is determined by the infection types that eventually develop upon challenge with the fungus. Both reddish-brown (RB) and immune (IM) infection types imply that the interaction between the particular soybean accession and the rust fungus is incompatible (Goellner et al., 2010). In this case the sporulation levels are low, the soybean plant is resistant, and *P. pachyrhizi* is avirulent. The formation of tan-coloured (TAN) lesions with abundant sporulation implies compatibility, with the soybean accession being susceptible and the fungal pathotype being virulent (Goellner et al., 2010). It should be noted that even highly effective single *Rpp* genes generally provide partial resistance, which is characterized by the development of RB lesions having one to three uredinia and showing low levels of sporulation. Several studies have been conducted to identify effective genes that can be used in breeding programmes to provide durable resistance. For instance, over 16,000 soybean genotypes were screened in 2006 in the USA, using a mixture of four different rust isolates sourced from Brazil, Paraguay, Thailand, and Zimbabwe. Out of these genotypes, about 805 were identified as a potential source of resistance (Miles et al., 2006). In 2008, 530 genotypes, which were a subset of the 805 genotypes, were screened in Paraguay under field conditions and about 16 of these genotypes were found to be resistant (Miles et al., 2008). In the USA, 64 resistant genotypes were identified among 576 genotypes evaluated at seven locations (Walker et al., 2011). Pham et al. (2009) identified about 10 resistant genotypes out of the 63 that were tested in Vietnam. In Africa, screening of soybean genotypes for resistance to the local *P. pachyrhizi* population has been conducted in only a few countries. Out of the 178 genotypes developed at the International Institute of Tropical Agriculture (IITA) and tested at three different locations in Nigeria, three breeding lines that showed low rust severities across the three locations were identified (Twizeyimana et al., 2008). In the same study, only three resistant genotypes were identified out of the 101 genotypes sourced from the United States Department of Agriculture (USDA), and tested at a single location in Nigeria (Twizeyimana et al., 2008). In the 2005 and 2006 soybean-growing seasons, 25 soybean genotypes sourced from the World Vegetable Center (WorldVeg) were tested in Uganda. Out of these, 10 resistant genotypes were identified and among them was accession PI 230970 (carrying *Rpp2*) that was found to be highly effective when compared with other genotypes carrying either the *Rpp1* and *Rpp3* or *Rpp4* genes (Oloka et al., 2008). In South Africa, all 26 soybean cultivars tested from 2003 to 2005 were found to be susceptible to the rust fungus (McLaren, 2008). Due to the high variability among *P. pachyrhizi* pathotypes, which includes shifts in virulence, resistant soybean varieties that were,

for example, commercially available in Brazil, rapidly succumbed to the pathogen (Godoy et al., 2016). Furthermore, *P. pachyrhizi* has a broad host range and appears to evolve different pathotypes, even in the absence of selection pressure exerted by the extensive deployment of particular soybean resistance genes against this fungus. Continuous screening of germplasm for resistance to soybean rust is important, as it will aid in the identification of effective resistance genes to be used in breeding programmes.

The objective of this study was to identify soybean genotypes that are resistant to *P. pachyrhizi* at multiple locations in Tanzania and Uganda for their use to manage soybean rust.

2 | MATERIALS AND METHODS

2.1 | Establishment of soybean genotypes and experimental design

A total of 71 soybean genotypes (Tables 1 and 2) were evaluated during the 2016 growing season, and a subset of the genotypes that showed some level of resistance were further evaluated in the 2017 growing season. Germplasm was obtained from the International Institute of Tropical Agriculture (IITA, Ibadan, Nigeria), AVRDC (currently the World Vegetable Center, Arusha, Tanzania), and the United States Division of Agriculture (USDA Soybean Germplasm Collection, Urbana, Illinois, USA). Furthermore, local cultivars were included (Table 1). Field experiments were established at Iringa (08.1183°S, 35.4110°E, 1,737 m a.s.l) and Mikumi (36.8993°S, 07.4797°E, 725 m a.s.l) in Morogoro and at Suluti (10.5441°S, 36.0776°E, 894 m a.s.l.) in Ruvuma, Tanzania. In Uganda, the experiments were established at Ngetta (02.2974°N, 32.9120°E, 1,073 m a.s.l) in Lira and at Mubuku (00.2234°N, 30.1314°E, 1,005 m a.s.l.) in Kasese.

The soybean genotypes were evaluated using a randomized complete block design (RCBD) with two replications. In 2016, plots consisted of three rows of 1 m in length for each accession, with 50 cm spacing between the rows and 5 cm within the rows. In 2017, plots consisted of four rows of 5 m in length with the same spacing as in 2016. A row of the highly susceptible variety Soya 2 was planted around the blocks in Tanzania, while Wonder soya was used in Uganda to increase the amount of rust inoculum. At all locations common cultural practices, including weeding, were applied, but fungicides were not used.

2.2 | Evaluation of disease severity and reactions of the various genotypes to *P. pachyrhizi*

The infection type, disease severity, and sporulation levels were scored for all genotypes. Disease severity (the percentage of leaf area affected by soybean rust) was evaluated based on a modified nine-point disease severity scale (Walker et al., 2011; Table 3). Evaluations were conducted between the R4 (pod-forming) and R6 (seed-filling) soybean growth stages. At all locations, three leaflets

TABLE 1 Resistance gene(s) present in the soybean genotypes evaluated for resistance to *Phakopsora pachyrhizi* in the Mikumi region of Tanzania in the 2016 growing season

	Genotype[a,b]	Donor[c]	Resistance gene[d]
1	AGS 339	AVRDC	Unknown
2	AGS 423	AVRDC	Unknown
3	AGS 459	AVRDC	Unknown
4	AGS 461	AVRDC	Unknown
5	GC 4051321	AVRDC	Unknown
6	Line 8	ARI Uyole	Unknown
7	TZA 448	AVRDC	Unknown
8	PI 200492	USDA	*Rpp1*
9	PI 200526	USDA	*Rpp5*
10	PI 230970	USDA	*Rpp2*
11	PI 459025B	USDA	*Rpp4*
12	PI 567102B	USDA	*Rpp6*
13	PI 594538A	USDA	*Rpp1b*
14	SC Saga	SeedCo	Unknown
15	SC Sequel	SeedCo	Unknown
16	SC Squire	SeedCo	Unknown
17	Soya 2	ARI-Uyole	Unknown
18	TGx 1987 8F	IITA	*Rpp1*, *Rpp3*
19	TGx 1987 10F	IITA	*Rpp1*, *Rpp3*
20	TGx 1987 14F	IITA	*Rpp1*, *Rpp3*
21	TGx 1987 31F	IITA	*Rpp1*, *Rpp3*
22	TGx 1987 32F	IITA	*Rpp1*, *Rpp3*
23	TGx 1987 34F	IITA	*Rpp1*, *Rpp3*
24	TGx 1987 62F	IITA	*Rpp1*, *Rpp3*
25	TGx 1987 64F	IITA	*Rpp1*, *Rpp3*
26	TGx 1988 3F	IITA	*Rpp1*, *Rpp3*
27	TGx 1989 5F	IITA	*Rpp1*, *Rpp3*
28	TGx 1989 11F	IITA	*Rpp1*, *Rpp3*
29	TGx 1989 19F	IITA	*Rpp1*, *Rpp3*
30	TGx 1989 20F	IITA	*Rpp1*, *Rpp3*
31	TGx 1989 21F	IITA	*Rpp1*, *Rpp3*
32	TGx 1989 40F	IITA	*Rpp1*, *Rpp3*
33	TGx 1989 41F	IITA	*Rpp1*, *Rpp3*
34	TGx 1989 42F	IITA	*Rpp1*, *Rpp3*
35	TGx 1989 45F	IITA	*Rpp1*, *Rpp3*
36	TGx 1989 48FN	IITA	*Rpp1*, *Rpp3*
37	TGx 1989 49FN	IITA	*Rpp1*, *Rpp3*
38	TGx 1989 53FN	IITA	*Rpp1*, *Rpp3*
39	TGx 1989 68F	IITA	*Rpp1*, *Rpp3*
40	TGx 1989 75FN		*Rpp1*, *Rpp3*
41	TGx 1990 110FN	IITA	*Rpp1*, *Rpp3*
42	TGx 1990 114FN	IITA	*Rpp1*, *Rpp3*
43	TGx 1990 2F	IITA	*Rpp1*, *Rpp3*

(Continues)

TABLE 1 (Continued)

	Genotype[a,b]	Donor[c]	Resistance gene[d]
44	TGx 1990 3F	IITA	*Rpp1*, *Rpp3*
45	TGx 1990 5F	IITA	*Rpp1*, *Rpp3*
46	TGx 1990 15F	IITA	*Rpp1*, *Rpp3*
47	TGx 1990 40F	IITA	*Rpp1*, *Rpp3*
48	TGx 1990 46F	IITA	*Rpp1*, *Rpp3*
49	TGx 1990 52F	IITA	*Rpp1*, *Rpp3*
50	TGx 1990 55F	IITA	*Rpp1*, *Rpp3*
51	TGx 1990 57F	IITA	*Rpp1*, *Rpp3*
52	TGx 1990 67F	IITA	*Rpp1*, *Rpp3*
53	TGx 1990 78F	IITA	*Rpp1*, *Rpp3*
54	TGx 1990 80F	IITA	*Rpp1*, *Rpp3*
55	TGx 1990 95F	IITA	*Rpp1*, *Rpp3*
56	TGx 1990 97F	IITA	*Rpp1*, *Rpp3*
57	TGx 1991 10F	IITA	*Rpp1*, *Rpp3*
58	TGx 1993 4FN	IITA	*Rpp1b*
59	TGx 1995 5FN	IITA	*Rpp1b*

[a]TGx: Tropical *Glycine max* crosses; Soya 2 and Line 8 are susceptible checks.

[b]AVRDC materials can be obtained through https://avrdc.org/seed/seeds/. The genotypes from the USDA are available at the University of Illinois and can be accessed through https://npgsweb.ars-grin.gov/gringlobal/search. IITA materials are available through the germplasm unit at Ibadan, Nigeria.

[c]AVRDC: Asian Vegetable Research Development Centre (currently known as the World Vegetable Centre), Arusha, Tanzania; USDA: United States Department of Agriculture, USA; SeedCo, Zimbabwe; ARI: Agricultural Research Institute, Tanzania; IITA: International Institute of Tropical Agriculture, Nigeria.

[d]*Rpp*: Resistance to *P. pachyrhizi*.

each from the bottom, middle, and top canopy of five randomly selected plants in each plot were rated separately per replication. For rating sporulation levels, a hand lens was used in the field. Disease severity of the entire plant was based on the mean severity of the nine leaflets per plant.

Three infection types were used for distinguishing compatible and incompatible reactions among soybean genotypes infected by *P. pachyrhizi* (Bromfield et al., 1980). Both RB and IM infection types signify incompatibility between the soybean accession and the rust fungus. Sporulation levels of RB and TAN lesions were recorded based on a 0 to 3 scale, in which 0 = no sporulation, 1 = 1–10 lesions with spores (little), 2 = 11–15 lesions with spores (moderate), and 3 = >15 lesions with spores (abundant) (Yamanaka et al., 2010). The sporulation level of each accession was based on the average of three ratings.

2.3 | Data analysis

Analysis of variance (ANOVA) for soybean rust severity was conducted using PROC GLIMMIX in SAS v. 9.3 (SAS Institute). A Bartlett

TABLE 2 Resistance gene(s) present in the soybean genotypes evaluated for resistance to *Phakopsora pachyrhizi* in Ngetta and Mubuku regions of Uganda in the 2016 growing season

	Genotype[a]	Donor[b]	Resistance gene[c]
1	AGS 339	AVRDC	Unknown
2	AGS 3829	AVRDC	Unknown
3	Hyuuga	USDA	*Rpp3*, *Rpp5*
4	Maksoy 1N	Makerere	Unknown
5	Maksoy 2N	Makerere	Unknown
6	Maksoy 3N	Makerere	Unknown
7	Maksoy 4N	Makerere	Unknown
8	PI 200492	USDA	*Rpp1*
9	PI 200526	USDA	*Rpp5*
10	PI 594538A	USDA	*Rpp1b*
11	SC Saga	SeedCo	Unknown
12	SC Sequel	SeedCo	Unknown
13	SC Squire	SeedCo	Unknown
14	TGx 1987 8F	IITA	Unknown
15	TGx 1987 10F	IITA	*Rpp1*, *Rpp3*
16	TGx 1987 14F	IITA	*Rpp1*, *Rpp3*
17	TGx 1987 31F	IITA	*Rpp1*, *Rpp3*
18	TGx 1987 32F	IITA	*Rpp1*, *Rpp3*
19	TGx 1987 34F	IITA	*Rpp1*, *Rpp3*
20	TGx 1987 62F	IITA	*Rpp1*, *Rpp3*
21	TGx 1987 64F	IITA	*Rpp1*, *Rpp3*
22	TGx 1988 3F	IITA	*Rpp1*, *Rpp3*
23	TGx 1988 5F	IITA	*Rpp1*, *Rpp3*
24	TGx 1989 11F	IITA	*Rpp1*, *Rpp3*
25	TGx 1989 19F	IITA	*Rpp1*, *Rpp3*
26	TGx 1989 21F	IITA	*Rpp1*, *Rpp3*
27	TGx 1989 40F	IITA	*Rpp1*, *Rpp3*
28	TGx 1989 41F	IITA	*Rpp1*, *Rpp3*
29	TGx 1989 42F	IITA	*Rpp1*, *Rpp3*
30	TGx 1989 48FN	IITA	*Rpp1*, *Rpp3*
31	TGx 1989 49FN	IITA	*Rpp1*, *Rpp3*
32	TGx 1989 53FN	IITA	*Rpp1*, *Rpp3*
33	TGx 1989 68FN	IITA	*Rpp1*, *Rpp3*
34	TGx 1989 75FN	IITA	*Rpp1*, *Rpp3*
35	TGx 1990 2F	IITA	*Rpp1*, *Rpp3*
36	TGx 1990 3F	IITA	*Rpp1*, *Rpp3*
37	TGx 1990 5F	IITA	*Rpp1*, *Rpp3*
38	TGx 1990 15F	IITA	*Rpp1*, *Rpp3*
39	TGx 1990 21F	IITA	*Rpp1*, *Rpp3*
40	TGx 1990 40F	IITA	*Rpp1*, *Rpp3*
41	TGx 1990 52F	IITA	*Rpp1*, *Rpp3*
42	TGx 1990 55F	IITA	*Rpp1*, *Rpp3*
43	TGx 1990 57F	IITA	*Rpp1*, *Rpp3*
44	TGx 1990 67F	IITA	*Rpp1*, *Rpp3*

(Continues)

TABLE 2 (Continued)

	Genotype[a]	Donor[b]	Resistance gene[c]
45	TGx 1990 78F	IITA	*Rpp1*, *Rpp3*
46	TGx 1990 80F	IITA	*Rpp1*, *Rpp3*
47	TGx 1990 95F	IITA	*Rpp1*, *Rpp3*
48	TGx 1990 97F	IITA	*Rpp1*, *Rpp3*
49	TGx 1990 110FN	IITA	*Rpp1*, *Rpp3*
50	TGx 1991 10F	IITA	*Rpp1*, *Rpp3*
51	TGx 1993 4FN	IITA	*Rpp1b*
52	TGx 1995 5FN	IITA	*Rpp1b*

[a]TGx: Tropical *Glycine max* crosses; Maksoy 1N was used as susceptible check.

[b]AVRDC: Asian Vegetable Research Development Centre (currently known as the World Vegetable Centre), Arusha, Tanzania; USDA: United States Department of Agriculture, USA; SeedCo, Zimbabwe; ARI: Agricultural Research Institute, Tanzania; IITA: International Institute of Tropical Agriculture, Nigeria.

[c]*Rpp*: Resistance to *P. pachyrhizi*.

TABLE 3 Disease severity assessment scale used to evaluate soybean genotypes for resistance to *Phakopsora pachyrhizi* in field trials

	Leaflet surface covered by lesions (%)	
Soybean rust rating	Range	Midpoint[a]
1	0	0.00
2	0–2.5	1.25
3	2.5–5	3.75
4	5–10	7.50
5	10–15	12.50
6	15–25	20.00
7	25–35	30.00
8	35–67.5	51.25
9	67.5–100	83.75

[a]The midpoint value is used for all statistical analyses.

test of homogeneity of variances across locations was performed to assess whether the variances were equal for all locations. As significant differences ($p < .001$) were observed between the locations, the analysis was conducted for individual locations. Mean separations were performed using Tukey–Kramer grouping of least significant difference at $\alpha = 0.05$. Genotypes with disease severities of less than 10%, a sporulation level of 0 or 1, and an RB infection type, relative to the susceptible checks, were categorized as resistant to *P. pachyrhizi*.

3 | RESULTS

We screened a collection of 71 soybean genotypes at five different locations in Tanzania and Uganda in the 2016 and 2017 cropping seasons. In both years, poor seed germination occurred due to low seed viability, and therefore the number of genotypes evaluated differed across all sites. In 2016, 20 genotypes were tested at three different sites, while 11 genotypes were tested at two different sites (Table 4). In 2017, 15, 1, and 8 genotypes were tested at five, four, and three different sites, respectively, while 28 genotypes were tested at two different sites (Table 5). Data were collected for the observed infection types, disease severity, and sporulation levels.

Overall, infection types and sporulation levels did not differ significantly between locations for the majority of the genotypes that developed TAN infection types and moderate to abundant sporulation in comparison with the susceptible checks (Tables 4 and 5). For instance, genotypes such as SC Saga, SC Squire, and SC Sequel, which are known to possess partial resistance only (Tichagwa, 2004), showed TAN infection types at Mubuku, Ngetta, and Iringa (Tables 4 and 5). This finding confirms that soybean rust resistance is scarce and that resistance-breaking pathotypes of the fungus are common and widespread. Some of the genotypes containing single resistance genes, including PI 459025B (*Rpp4*), PI 200526 (*Rpp5*), and PI 567102B (*Rpp6*) developed RB infection types with little sporulation at the Mikumi site (Table 4). Although this could be interpreted as an absence of resistance-breaking pathotypes in that region, we noted that Mikumi was the only site where the majority of the genotypes did not have a TAN reaction and the RB infection type was the most common.

Accession PI 594538A, which carries *Rpp1b*, developed RB infections at all three locations in 2016 (Table 4) and at Mikumi and Iringa in 2017 (Table 5). Accession Hyuuga, which carries *Rpp3* and *Rpp5*, also had an RB reaction in all three locations in 2016 (Table 4). This finding suggests that *P. pachyrhizi* pathotypes that are able to overcome *Rpp1b*, or *Rpp3* in combination with *Rpp5*, are not generally present in the *P. pachyrhizi* population, in contrast to pathotypes that have broken the other resistance genes.

Although the majority of the genotypes developed similar infection types, a few developed different infection types when compared between locations. For instance, whereas accession SC Squire had an RB infection type at Mubuku and at Mikumi, the same accession developed a TAN infection type at Ngetta (Table 4). Similarly, accession TGx 1990 55F had a TAN infection type at Mubuku, while the same accession developed an RB infection type at both Ngetta and Mikumi (Table 4). IM infection types were observed on two genotypes, namely TGx 1993 4FN and TGx 1995 5FN, at the Mubuku site, while the same genotypes had RB infection types in the other locations (Tables 4 and 5). These findings demonstrate that the rust populations differ between the various locations within the same cropping season. Moreover, infection types for some of the genotypes also differed between the two years. For example, whereas accession TGx 1990 114FN and TGx 1987 34F developed RB infection types in 2016 (Table 4), both genotypes developed a TAN infection type in 2017 (Table 5). In both years, the susceptible checks had TAN infection types. This may similarly be attributed to differences in the rust populations between the two years.

To assess disease development with a higher resolution, disease severities (the percentage of leaf area affected by soybean

TABLE 4 Soybean rust disease severity ratings, infection types, and sporulation levels for selected genotypes in Uganda (Mubuku and Ngetta) and Tanzania (Mikumi) in the 2016 growing season

	Mubuku			Ngetta			Mikumi		
Genotype	Severity (mean ± *SE*)[a]	IT[b]	SL[c]	Severity (mean ± *SE*)	IT	SL	Severity (mean ± *SE*)	IT	SL
AGS 3829	29 ± 0.9	TAN	1	56.1 ± 2.6	TAN	3	4.6 ± 0.6	RB	1
AGS 339	22.5 ± 2.6	TAN	3	33.2 ± 1.2	TAN	2	1.9 ± 0.5	RB	1
Hyuuga	2.4 ± 2.0	RB	1	3.2 ± 1.0	RB	1	8.6 ± 0.8	RB	1
PI 594538A	4.6 ± 2.2	RB	1	2.3 ± 2.1	RB	1	8.7 ± 1.1	RB	1
SC Saga	46.8 ± 3.2	TAN	3	23.4 ± 2.3	TAN	2	3.5 ± 0.7	RB	1
SC Sequel	15.0 ± 0.7	TAN	2	20.3 ± 1.5	TAN	1	4.2 ± 0.7	RB	1
SC Squire	16.2 ± 0.6	RB	1	34 ± 0.9	TAN	3	4.8 ± 3.3	RB	1
TGx 1989 42F	29.6 ± 2.5	TAN	2	6.6 ± 1.2	TAN	1	11.3 ± 1.4	TAN	2
TGx 1989 19F	43.2 ± 3.8	TAN	3	24.6 ± 2.3	TAN	3	3.9 ± 0.8	RB	2
TGx 1987 14F	47.8 ± 3.2	TAN	3	6.8 ± 0.8	TAN	2	5.6 ± 0.7	RB	1
TGx 1987 34F	16.8 ± 1.3	RB	1	6.4 ± 0.7	RB	1	2.2 ± 0.5	RB	1
TGx 1990 110FN	37.0 ± 2.6	TAN	2	24.6 ± 2.3	TAN	2	6.0 ± 0.7	RB	1
TGx 1993 4FN	1.0 ± 0.7	IM	0	5.6 ± 0.7	RB	2	2.2 ± 1.1	RB	1
TGx 1990 114FN	11.6 ± 1.7	RB	2	4.3 ± 0.9	RB	1	6.5 ± 1.0	RB	1
TGx 1990 55F	33.9 ± 2.6	TAN	3	4.2 ± 0.7	RB	1	4.5 ± 0.7	RB	1
TGx 1990 2F	25.7 ± 4.1	TAN	3	15.3 ± 1.4	TAN	2	1.9 ± 0.5	RB	1
TGx 1987 62F	14.6 ± 1.5	TAN	3	6.8 ± 0.6	TAN	1	5.5 ± 1.5	RB	1
TGx 1990 21F	11.1 ± 1.0	RB	2	6.4 ± 0.7	RB	1	3.4 ± 0.5	RB	1
TGx 1990 5F	10.8 ± 0.8	RB	1	18.0 ± 2.4	TAN	2	2.2 ± 0.5	RB	1
TGx 1995 5FN	1.0	IM	0	2.1 ± 1.2	RB	1	4.6 ± 0.8	RB	1
TGx 1989 45F	22.9 ± 2.5	TAN	1	6.4 ± 0.7	RB	2	nd[d]		
TGx 1990 48FN	18.6 ± 2.2	TAN	2	6.9 ± 0.9	TAN	2	nd		
TGx 1990 57F	5.2 ± 0.3	RB	1	13.4 ± 1.3	TAN	2	nd		
TGx 1990 78F	18.5 ± 2.6	TAN	2	3.5 ± 0.7	RB	1	nd		
TGx 1990 80F	15.6 ± 1.4	TAN	2	3.6 ± 0.9	RB	1	nd		
TGx 1990 95F	40.5 ± 2.9	TAN	3	13.4 ± 1.3	RB	2	nd		
TGx 1990 15F	15.3 ± 2.0	TAN	2	nd			nd		
TGx 1990 97F	18.8 ± 1.6	TAN	2	nd			nd		
TGx 1990 3F	11.2 ± 0.7	TAN	1	nd			nd		
TGx 1987 10F	14.6 ± 0.7	RB	1	nd			nd		
TGx 1989 41F	nd			14.1 ± 1.4	TAN	2	2.6 ± 0.7	RB	1
TGx 1989 68FN	nd			15.1 ± 1.1	TAN	3	5.2 ± 0.6	RB	1
TGx 1989 11F	nd			20.9 ± 2.2	TAN	3	nd		
TGx 1989 40F	nd			7.8 ± 1.0	TAN	2	nd		
TGx 1989 49FN	nd			6.8 ± 0.6	TAN	2	nd		
TGx 1989 53FN	nd			6.8 ± 0.3	RB	1	nd		
TGx 1990 40F	nd			8.2 ± 1.0	TAN	2	5.2 ± 0.6	RB	1
TGx 1989 5F	nd			9.5 ± 0.8	RB	2	nd		
TGx 1990 52F	nd			25.3 ± 2.0	TAN	3	nd		
TGx 1990 67F	nd			19.2 ± 2.1	TAN	2	nd		
AGS 423	nd			nd			10.0 ± 0.8	RB	2
AGS 459	nd			nd			12.4 ± 1.5	RB	1

(Continues)

TABLE 4 (Continued)

Genotype	Mubuku			Ngetta			Mikumi		
	Severity (mean ± *SE*)[a]	IT[b]	SL[c]	Severity (mean ± *SE*)	IT	SL	Severity (mean ± *SE*)	IT	SL
PI 200492	nd			nd			37.4 ± 3.8	TAN	3
PI 200526	nd			nd			11.9 ± 1.1	RB	1
PI 459025B	nd			nd			9.4 ± 1.3	RB	1
PI 567102B	nd			nd			10.7 ± 1.0	RB	1
TGx 1987 31F	nd			nd			4.5 ± 0.7	RB	1
TGx 1987 32F	nd			nd			2.9 ± 0.7	RB	1
TGx 1987 8F	nd			nd			9.5 ± 0.8	RB	1
TGx 1988 5F	nd			nd			6.1 ± 1.0	RB	1
TGx 1989 21F	nd			nd			4.6 ± 0.8	RB	1
TGx 1990 110FN	nd			nd			12.0 ± 1.6	RB	3
TGx 1990 46F	nd			nd			2.5 ± 0.5	RB	1
TGx 1990 52F	nd			nd			4.5 ± 0.7	RB	1
TZA 448	nd			nd			10.2 ± 1.3	RB	1
TGx 1987 64F	nd			nd			9.1 ± 0.9	RB	1
Line 8	nd			nd			29.5 ± 1.5	TAN	2
Maksoy 3N	47.2 ± 1.1	TAN	3	53.6 ± 4.7	TAN	3	nd		

[a]Severity (mean ± *SE*) was rated on a scale of 1–9 (Table 3).

[b]IT: infection type; TAN: tan-coloured lesions; RB: reddish-brown; IM: immune.

[c]SL: sporulation level; 0, no sporulation; 1, little sporulation; 2, moderate sporulation; 3, abundant sporulation.

[d]nd: not determined.

rust) were assessed based on a modified nine-point disease severity scale (Walker et al., 2011; Table 3). This is particularly relevant for infections that were classified as TAN, as considerable differences in disease severity were observed once disease occurred. Intriguingly, we observed significant differences in disease severities for the majority of the genotypes between the various locations (Tables 4 and 5). For instance, accession TGx 1987 14F, which showed the highest disease severity (47.8%) at Mubuku, showed a significantly lower disease severity at both Ngetta (6.8%) and Mikumi (5.6%) in 2016 (Table 4). Similarly, accession AGS 3829, which showed a high disease severity (56%) at Ngetta, showed a lower disease severity at Mubuku (29%) and a much lower one at Mikumi (4.6%; Table 4). In contrast, in all years, susceptible checks consistently had high disease severities. These data provide further support for the notion that significant variation in *P. pachyrhizi* populations exists between the various locations.

Besides differences in disease severities for the same genotypes between locations, disease severities for some of the genotypes also differed between the two years. For example, accession TGx 1990 57F showed a low disease severity (5.2%) at Mubuku in 2016 (Table 4), while the same accession showed a high disease severity (32%) at that location in 2017 (Table 5). Similarly, accession AGS 3829 showed a high disease severity (56.1%) at Ngetta in 2016, which was slightly higher than the local check, but a significantly lower disease severity at that same location (20.2%) in 2017. These data suggest that not only does significant variation exist in the *P. pachyrhizi* populations between the various locations, but also between the two years at the same location. However, at some of the locations the disease severities of the same genotypes did not differ significantly between the two years. For instance, accession TGx 1990 48FN at Mubuku had disease severities of 18.6% and 17.2% in 2016 and in 2017 (Table 5), respectively. Other genotypes that showed similar disease severities at the same location between the years include TGx 1990 21F and TGx 1990 114FN at Mubuku and Mikumi (Tables 4 and 5). These findings suggest that the same pathotype is present at these locations in both years, although this may also be the consequence of similar susceptibilities of these genotypes to different isolates.

Overall, our data point towards a significant variation in the local *P. pachyrhizi* populations between the different sites and between the two years and shows that resistance-breaking pathotypes within those populations are common. Thus, most soybean genotypes are susceptible and provide little basis for promising soybean disease resistance management. Nevertheless, genotypes Hyuuga, PI 594538A, TGx 1987 34F, TGx 1990 21F, TGx 1990 114FN, TGx 1993 4FN, and TGx 1995 5FN developed an RB infection type with little sporulation and relatively low disease severities in comparison to the susceptible checks across all three locations in 2016 (Table 4). Of these seven genotypes, TGx 1993 4FN and TGx 1995 5FN also developed an RB infection type, with little to no sporulation and very low disease severities across the five tested locations in 2017 (Table 5). Thus, these two genotypes appear to be resistant against

TABLE 5 Soybean rust (*Phakopsora pachyrhizi*) disease severity ratings, infection types, and sporulation levels for selected soybean genotypes in Uganda (Mubuku and Ngetta) and Tanzania (Mikumi, Iringa, and Suluti) in the 2017 growing season

	Uganda						Tanzania								
	Mubuku			Ngetta			Mikumi			Iringa			Suluti		
Genotype	Severity (mean ± *SE*)[a]	IT	SL	Severity (mean ± *SE*)	IT	SL	Severity (mean ± *SE*)	IT	SL	Severity (mean ± *SE*)	IT	SL	Severity (mean ± SE)	IT	SL
AGS 339	37.4 ± 3.8	TAN	3	23.1 ± 1.6	TAN	2	6.0 ± 1.4	TAN	1	34.3 ± 5.4	TAN	1	24.7 ± 5.2	TAN	2
TGx 1990 114FN	11.3 ± 3.1	TAN	1	12.5 ± 1.6	TAN	1	4.6 ± 0.8	TAN	1	16.4 ± 3.3	TAN	1	11.5 ± 3.8	TAN	1
TGx 1993 4FN	1.0 ± 0.2	IM	0	2.2 ± 0.5	RB	1	1.6 ± 0.4	RB	1	0.9 ± 0.1	RB	0	9.2 ± 2.6	RB	1
TGx 1995 5FN	1.0 ± 0.1	IM	0	4.5 ± 1.3	RB	1	1.3 ± 0.5	RB	1	5.7 ± 0.8	RB	0	4.0 ± 1.0	RB	1
TGx 1987 34F	11.4 ± 2.6	TAN	2	19.0 ± 6.9	TAN	2	2.6 ± 0.7	TAN	1	16.3 ± 3.2	TAN	1	19.3 ± 2.0	TAN	2
TGx 1987 62F	16.1 ± 2.5	TAN	3	13.3 ± 2.6	TAN	2	2.1 ± 0.6	TAN	1	10.7 ± 3.2	TAN	1	7.3 ± 2.0	TAN	1
TGx 1987 14F	14.3 ± 2.0	RB	1	18.2 ± 1.3	TAN	2	3.1 ± 0.5	TAN	1	12.2 ± 1.8	TAN	1	12.7 ± 1.8	TAN	2
TGx 1989 45F	27.3 ± 3.3	TAN	3	24.3 ± 2.0	TAN	3	7.1 ± 1.3	TAN	1	41.9 ± 6.9	TAN	3	19.0 ± 2.3	TAN	1
TGx 1990 15F	24.3 ± 2.0	TAN	2	15.2 ± 2.1	TAN	2	4.1 ± 2.4	TAN	1	43.9 ± 6.3	TAN	3	30.9 ± 6.1	TAN	2
TGx 1990 21F	12.7 ± 1.0	TAN	2	41.6 ± 5.5	TAN	3	4.3 ± 1.0	RB	1	16.4 ± 3.3	TAN	2	16.2 ± 3.0	TAN	2
TGx 1990 3F	10.4 ± 1.6	TAN	2	14.2 ± 2.3	TAN	1	5.1 ± 0.4	RB	1	28.8 ± 4.3	TAN	2	14.1 ± 2.4	TAN	1
TGx 1987 10F	16.3 ± 2.0	TAN	2	19.4 ± 4.0	TAN	1	3.1 ± 0.5	TAN	1	28.3 ± 1.2	TAN	1	21.2 ± 2.6	TAN	1
TGx 1990 48FN	17.2 ± 1.1	TAN	2	22.6 ± 6.6	TAN	2	4.3 ± 0.3	RB	1	44.3 ± 4.2	TAN	3	14.9 ± 2.4	TAN	2
TGx 1990 57F	32.0 ± 5.4	TAN	3	12.0 ± 1.6	TAN	1	8.5 ± 1.3	TAN	1	24.6 ± 3.5	TAN	2	14.4 ± 1.8	TAN	1
TGx 1990 5F	12.0 ± 1.6	TAN	2	23.0 ± 2.4	TAN	2	5.0 ± 1.5	TAN	1	34.8 ± 6.6	TAN	2	19.9 ± 4.2	TAN	2
TGx 1990 78F	28.1 ± 6.7	TAN	2	21.1 ± 4.1	TAN	2	nd			33.3 ± 4.1	TAN	2	nd		
TGx 1990 80F	23.3 ± 1.9	TAN	1	16.1 ± 2.3	TAN	2	nd			48.4 ± 6.1	TAN	2	25.8 ± 4.5	TAN	2
TGx 1990 97F	28.4 ± 3.2	TAN	1	nd			nd			42.9 ± 5.4	TAN	2	17.8 ± 2.7	TAN	2
Maksoy 2N	16.0 ± 1.4	TAN	3	14.8 ± 6.1	TAN	2	nd			nd			nd		
Maksoy 3N	33.1 ± 1.4	TAN	3	50.2 ± 4.0	TAN	3	nd			nd			nd		
Maksoy 4N	21.2 ± 1.3	TAN	2	14.6 ± 2.2	TAN	2	nd			nd			nd		
AGS 3,829	nd			20.2 ± 2.3	TAN	2	nd			nd			nd		
AGS 461	nd			nd			nd			33.3 ± 4.1	TAN	2	18.4 ± 4.2	TAN	2
GC 4,051,321	nd			nd			nd			36.8 ± 6.3	TAN	2	27.1 ± 4.6	TAN	3
PI 594538A	nd			nd			3.2 ± 1.9	RB	1	4.8 ± 1.2	RB	1	nd		
PI 230,970	nd			nd			6.7 ± 1.3	RB	1	49.8 ± 4.9	TAN	3	nd		
SC Saga	nd			nd			3.6 ± 0.9	TAN	1	19.4 ± 2.2	TAN	2	nd		

(Continues)

TABLE 5 (Continued)

Genotype	Uganda						Tanzania								
	Mubuku			Ngetta			Mikumi			Iringa			Suluti		
	Severity (mean ± *SE*)[a]	IT	SL	Severity (mean ± *SE*)	IT	SL	Severity (mean ± *SE*)	IT	SL	Severity (mean ± *SE*)	IT	SL	Severity (mean ± SE)	IT	SL
SC Sequel	nd			nd			3.5 ± 0.7	TAN	1	50.2 ± 4.0	TAN	3	nd		
SC Squire	nd			nd			5.6 ± 1.2	TAN	1	32.2 ± 1.3	TAN	3	nd		
TGx 1987 32F	nd			nd			7.4 ± 6.5	TAN	3				nd		
TGx 1987 64F	nd			nd			10.3 ± 2.0	TAN	2	49.4 ± 5.7	TAN	3	nd		
TGx 1987 8F	nd			nd			3.2 ± 0.7	TAN	2	9.1 ± 2.2	RB	1	nd		
TGx 1988 5F	nd			nd			7.1 ± 1.2	TAN	1	29.5 ± 4	TAN	2	16.6 ± 2.8	TAN	2
TGx 1989 19F	nd			nd			9.9 ± 2.1	TAN	1	33.0 ± 9.6	TAN	2	28.4 ± 3.2	TAN	3
TGx 1989 42F	nd			nd			6.4 ± 1.4	TAN	2	36.4 ± 5.6	TAN	3	nd		
TGx 1990 2F	nd			nd			3.9 ± 0.8	TAN	1	46.4 ± 3.3	TAN	2	34.0 ± 6.5	TAN	3
TGx 1990 95F	nd			nd			4.6 ± 0.8	TAN	1	41.2 ± 4.8	TAN	3	7.1 ± 1.8	TAN	1
TGx 1991 10F	nd			nd			5.3 ± 0.8	TAN	1	21.6 ± 2.0	TAN	2	10.6 ± 1.8	TAN	1
TGx 1987 129F	nd			nd			nd			29.2 ± 6.6	TAN	3	6.8 ± 1.4	TAN	1
TGx 1987 31F	nd			nd			nd			36.1 ± 6.7	TAN	2	nd		
TGx 1987 65F	nd			nd			nd			41.9 ± 5.8	TAN	2	12.7 ± 1.8		
TGx 1987 88F	nd			nd			nd			7.8 ± 1.7	RB	2	28.3 ± 5.8	TAN	3
TGx 1988 3F	nd			nd			nd			48.8 ± 6.1	TAN	2	nd		
TGx 1989 11F	nd			nd			nd			35.4 ± 4.4	TAN	2	20.9 ± 2.2	TAN	2
TGx 1989 20F	nd			nd			nd			41.2 ± 6.1	TAN	3	34.0 ± 6.3	TAN	3
TGx1989 48FN	nd			nd			nd			30.5 ± 3.9	TAN	2	14.0 ± 2.7	TAN	1
TGx 1989 53FN	nd			nd			nd			28.1 ± 3.0	TAN	2	15.2 ± 2.6	TAN	2
TGx 1989 68FN	nd			nd			nd			24.5 ± 5.4	TAN	2	nd		
TGx 1989 75FN	nd			nd			nd			34.3 ± 6.6	TAN	2	20.1 ± 3.9	TAN	2
TGx 1990 40F	nd			nd			nd			40.7 ± 7.4	TAN	3	12.5 ± 1.6	TAN	2
TGx 1990 52F	nd			nd			nd			20.6 ± 1.8	TAN	2	10.5 ± 1.7	TAN	1
TGx 1990 55F	nd			nd			nd			27.8 ± 4.4	TAN	2	32.1 ± 6.0	TAN	2
TGx 1990 67F	nd			nd			nd			24.9 ± 4.9	TAN	2	25.1 ± 4.6	TAN	3
Maksoy 1N	33.1 ± 1.1	TAN	2	38.8 ± 1.9	TAN	2	nd			nd			nd		
Soya 2	nd			nd			18.9 ± 5.1	TAN	2	59.1 ± 4.1	TAN	3	37.0 ± 4.6	TAN	3

[a] Severity (mean ± *SE*) was rated on a scale of 1–9 (Table 3).

various rust populations that occurred in different locations in the two years and may provide a basis for improved soybean rust resistance management in the future.

4 | DISCUSSION

Deployment of host resistance is the best approach to manage soybean rust caused by *P. pachyrhizi* (Hartman et al., 2005). High virulence diversity exists among *P. pachyrhizi* pathotypes and populations with different virulence spectra occur across soybean-growing regions worldwide (Akamatsu et al., 2017; Godoy et al., 2016; Murithi et al., 2016). Furthermore, several pathotypes have been identified among field isolates of *P. pachyrhizi* in Africa (Murithi et al., 2017), Japan (Yamaoka et al., 2014), South America (Akamatsu et al., 2013), and the USA (Twizeyimana & Hartman, 2012). Considering the geographical variability of the pathogen, it is important to identify sources of resistance that can be deployed to effectively control *P. pachyrhizi* populations at different locations. Therefore, continuous screening of soybean genotypes at different locations is important and may aid in the identification of useful resistance sources that can be introduced into local breeding programmes. However, field screening for resistance of soybean to rust is challenging due to variable weather conditions during the cropping seasons, which may affect seed germination as well as the occurrence of natural soybean rust infections. Sometimes the growth stages from flowering to seed filling for the early maturing soybean varieties may not coincide with favourable conditions for rust infection (Twizeyimana et al., 2011). Such variable environmental factors could have contributed to the low disease pressure that led to the low disease severities observed at the Mikumi site in both 2016 and 2017. Previous studies have shown that soybean rust establishment is negatively affected by temperatures above 28 °C and low rainfall (Bonde et al., 2012; Del Ponte et al., 2006).

In our current study, the majority of the genotypes that were tested were found to be susceptible to soybean rust, although disease severities differed between locations and years. This finding confirms the existence of rust populations at various locations that are able to overcome known resistance genes. However, of the 71 soybean genotypes tested at the different locations over the two years of this study, two genotypes were found to be able to resist the rust populations that occurred in all locations, namely TGx 1993 4FN and TGx 1995 5FN. The source of rust resistance in these lines is thought to be the USDA accession PI 594538A, which carries *Rpp1b*. This particular accession was also tested in our study and indeed also showed a low disease severity and RB infection type at the three locations that were assessed in 2016, namely Mubuku, Ngetta, and Mikumi, as well as at the Mikumi and Iringa sites in 2017. No data were obtained for this accession at the remaining three locations in 2017 due to poor seed germination. Our findings are consistent with previous studies in which PI 594538A developed RB or IM infection types upon challenge with rust isolates from African countries (Murithi et al., 2017; Twizeyimana et al., 2009), and also with isolates from South America and the USA (Akamatsu et al., 2013; Paul et al., 2015; Twizeyimana et al., 2009, 2011).

In addition to USDA accession PI 594538A, three other USDA genotypes that carry single previously characterized resistance genes were found to be resistant to soybean rust, namely PI 459025B (*Rpp4*), PI 200526 (*Rpp5*), and PI 567102B (*Rpp6*), although it should be noted that these were tested in only one location. Moreover, cultivar Hyuuga (carrying both *Rpp3* and *Rpp5*), which originates from southern Japan, was also found to be resistant to soybean rust in the three locations that were tested in 2016. Unfortunately, seeds of accession PI 462312 (*Rpp3*) did not germinate at any of the locations tested in this study. USDA genotypes such as PI 200492 (*Rpp1*), PI 594538A (*Rpp1b*), PI 230970 (*Rpp2*), PI 462312 (*Rpp3*), PI 459025B (*Rpp4*), PI 200526 (*Rpp5*), and PI 567102B (*Rpp6*) have been reported earlier to be resistant to rust (Oloka et al., 2008; Pham et al., 2009; Twizeyimana et al., 2008; Walker et al., 2011) and have been successfully used in soybean breeding programmes (Childs et al., 2018). However, our study revealed high disease severities and TAN infection types on genotypes PI 200492 (*Rpp1*) and PI 230970 (*Rpp2*) at the Mikumi and Iringa locations in Tanzania. High disease severity and sporulation levels on PI 230970 (*Rpp2*) were also reported in Nigeria in 2005 (Twizeyimana et al., 2008). The high disease severity on PI 200492 (*Rpp1*) is not surprising, as it was previously shown that the *Rpp1* gene is ineffective against rust isolates from East Africa (Murithi et al., 2017). Therefore, our findings imply the occurrence of novel *P. pachyrhizi* pathotypes that overcome resistance conferred by the *Rpp1* and *Rpp2* genes. In contrast, the *Rpp4*, *Rpp5*, and *Rpp6* genes were still able to prevent rust infection in the single location where they were tested. Although accession PI 462312 (*Rpp3*) did not germinate at any of the locations, recent studies found this accession to be resistant to rust isolates collected in Uganda and Tanzania (Murithi et al., 2017).

Besides PI 594538A (*Rpp1b*), the other known source of rust resistance for the majority of the IITA breeding lines that were tested in this study is soybean cultivar UG 5 (Hartman et al., 2011), which contains two resistance genes, *Rpp1* and *Rpp3* (Paul et al., 2015). Cultivar UG 5 has been reported as highly resistant against *P. pachyrhizi* isolates in Nigeria, Uganda, and the USA (Oloka et al., 2008; Paul et al., 2015; Twizeyimana et al., 2008). This cultivar was not included in our current study due to lack of seeds at the time of testing. However, considering that we found that the majority of the IITA soybean genotypes were susceptible to soybean rust in this study, we anticipate that pathotypes with a more complex virulence spectrum have evolved that have overcome resistance conferred by the *Rpp1* and *Rpp3* resistance genes in East Africa. This could also suggest that some of the IITA lines could contain *Rpp1*, *Rpp3*, or their combination due to independent segregation of the resistance genes.

The rapid evolution of *P. pachyrhizi* continues to threaten the available resistance genes, as soybean rust populations are able to quickly overcome resistance once it is deployed. Thus, efforts should

be directed towards enhancing the durability of the resistance genes that are still effective (Johnson, 2000). Resistance gene pyramiding that involves combining (stacking) of multiple resistance genes in a single cultivar can contribute to the durability of resistance, provided that these genes mediate recognition of different matching effector proteins of the rust fungus (Mundt, 2014). In this manner, the different resistance genes present in a stack confer recognition of multiple effectors simultaneously, which is more difficult for the pathogen to overcome, as this will require mutations in multiple effector genes to occur simultaneously (McDonald & Linde, 2002). In our study, genotypes TGx 1993 4FN, TGx 1995 5FN, and PI 594538A (all presumably carrying *Rpp1b*), and cultivar Hyuuga (carrying *Rpp3* and *Rpp5*) were all resistant against soybean rust at different locations in both years. Potentially, genotypes PI 459025B (*Rpp4*), PI 200526 (*Rpp5*), and PI 567102B (*Rpp6*) can also be used, but these should first be tested in other locations to confirm their effectiveness in these regions as well. Previous studies have demonstrated the effectiveness of pyramided lines carrying multiple *Rpp* genes against isolates from Brazil and Japan (Yamanaka et al., 2013, 2015). Similarly, in a study in Bangladesh involving 13 different rust isolates, soybean lines carrying two or three resistance genes had higher levels of resistance compared to lines carrying only a single gene (Yamanaka & Hossain, 2019). Thus, making combinations of these genes, including those identified in previous studies (Murithi et al., 2017), may aid in the development of durable resistant soybean cultivars for use in Tanzania and Uganda.

Finally, more efforts should be put into screening for novel sources of resistance, especially among wild relatives of soybean and nonhost legumes. This could aid in identification of novel resistance genes to combine in gene stacks to prevent the breakdown of stacks that are based on resistance genes that have been deployed as singule genes and have been overcome already. Overall, the data that we obtained revealed a significant variation in the *P. pachyrhizi* populations at the different sites and over the two years that our experiment took place. Resistance-breaking pathotypes were found to be common and only two genotypes showed an RB infection type, with very low disease severities across the various locations in 2017. Therefore, these genotypes may provide a basis for improved soybean rust resistance management in the future.

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Conflict of interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Akamatsu, H., Yamanaka, N., Soares, R.M., Ivancovich, J.G., Lavilla, M.A., Bogado, A.N. et al. (2017) Pathogenic variation of South American *Phakopsora pachyrhizi* populations isolated from soybeans from 2010 to 2015. *Japan Agricultural Research Quarterly*, 51, 221–232.

Akamatsu, H., Yamanaka, N., Yamaoka, Y., Soares, R.M., Morel, W., Ivancovich, J.G. et al. (2013) Pathogenic diversity of soybean rust in Argentina, Brazil, and Paraguay. *Journal of General Plant Pathology*, 79, 28–40.

Ali, N. (2010). Soybean processing and utilization. In: Singh, G. (Ed.) *The Soybean. Botany, Production and Uses*. Wallingford, UK: CAB International, pp. 345–374.

Bandyopadhyay, R., Ojiambo, P.S., Twizeyimana, M., Asafo-Adjei, B., Frederick, R.D., Pedley, K.F. et al. (2007) First report of soybean rust caused by *Phakopsora pachyrhizi* in Ghana. *Plant Disease*, 91, 1057.

Bonde, M.R., Nester, S.E. & Berner, D.K. (2012) Effects of daily temperature highs on development of *Phakopsora pachyrhizi* on soybean. *Phytopathology*, 102, 761–768.

Bromfield, K.R. & Hartwig, E.E. (1980) Resistance to soybean rust and mode of inheritance. *Crop Science*, 20, 254–255.

Bromfield, K.R. & Melching, J.S. (1982) Sources of specific resistance to soybean rust. *Phytopathology*, 72, 706.

Bromfield, K.R., Melching, J.S. & Kingsolver, C.H. (1980) Virulence and aggressiveness of *Phakopsora pachyrhizi* isolates causing soybean rust. *Phytopathology*, 70, 17–21.

Childs, S.P., Buck, J.W. & Li, Z. (2018) Breeding soybeans with resistance to soybean rust (*Phakopsora pachyrhizi*). *Plant Breeding*, 137, 250–261.

Del Ponte, E.M., Godoy, C.V., Li, X. & Yang, X.B. (2006) Predicting severity of Asian soybean rust epidemics with empirical rainfall models. *Phytopathology*, 96, 797–803.

FAOSTAT (2018) *Food and Agricultural Organization of United Nations (FAO) database*. Available at: http://www.fao.org/faostat/en/#data/QC [Accessed 10 January 2021].

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

Godoy, C.V., Seixas, C.D.S., Soares, R.M., Marcelino-Guimaraes, F.C., Meyer, M.C. & Costamilan, L.M. (2016) Asian soybean rust in Brazil: past, present, and future. *Pesquisa Agropecuária Brasileira*, 51, 407–421.

Goellner, K., Loehrer, M., Langenbach, C., Conrath, U., Koch, E. & Schaffrath, U. (2010) *Phakopsora pachyrhizi*, the causal agent of Asian soybean rust: pathogen profile. *Molecular Plant Pathology*, 11, 169–177.

Hartman, G.L., Miles, M.R. & Frederick, R.D. (2005) Breeding for resistance to soybean rust. *Plant Disease*, 89, 664–666.

Hartman, G.L., West, D.E. & Herman, T.K. (2011) Crops that feed the world 2. Soybean – worldwide production, use, and constraints caused by pathogens and pests. *Food Security*, 3, 5–17.

Hartwig, E.E. (1986) Identification of a fourth major gene conferring resistance to soybean rust. *Crop Science*, 26, 1135–1136.

Johnson, R. (2000) Classical plant breeding for durable resistance to diseases. *Journal of Plant Pathology*, 82, 3–7.

Kumudini, S., Godoy, C.V., Board, J.E., Omielan, J. & Tollenaar, M. (2008) Mechanisms involved in soybean rust-induced yield reduction. *Crop Science*, 48, 2334–2342.

Levy, C. (2005) Epidemiology and chemical control of soybean rust in Southern Africa. *Plant Disease*, 89, 669–674.

Li, S., Smith, J.R., Ray, J.R. & Frederick, R.D. (2012) Identification of a new soybean rust resistance gene in PI567102B. *Theoretical and Applied Genetics*, 125, 133–142.

Marchetti, M.A., Uecker, F.A. & Bromfield, K.R. (1979) Uredial development of *Phakopsora pachyrhizi* in soybeans. *Phytopathology*, 65, 822–823.

McDonald, B.A. & Linde, C. (2002) Pathogen population genetics, evolutionary potential and durable resistance. *Annual Review of Phytopathology*, 40, 349–379.

McLaren, N.W. (2008) Reaction of South African soybean cultivars to rust caused by *Phakopsora pachyrhizi*. *South African Journal of Plant and Soil*, 25, 49–54.

McLean, R.J. & Byth, D.E. (1980) Inheritance of resistance to rust (*Phakopsora pachyrhizi*) in soybeans. *Australian Journal of Agricultural Research*, 31, 951–956.

Melching, J.S., Dowler, W.M., Koogle, D.L. & Royer, M.H. (1989) Effects of duration, frequency, and temperature of leaf wetness periods on soybean rust. *Plant Disease*, 73, 117–122.

Miles, M.R., Frederick, R.D. & Hartman, G.L. (2006) Evaluation of soybean germplasm for resistance to *Phakopsora pachyrhizi*. *Plant Health Progress*, 7. doi: 10.1094/PHP-2006-0104-01-RS

Miles, M.R., Morel, W., Ray, J.D., Smith, J.R., Frederick, R.D. & Hartman, G.L. (2008) Adult plant evaluation of soybean accessions for resistance to *Phakopsora pachyrhizi* in the field and greenhouse in Paraguay. *Plant Disease*, 92, 96–105.

Mundt, C.C. (2014) Durable resistance: a key to sustainable management of pathogens and pests. *Infection, Genetics and Evolution*, 27, 446–455.

Murithi, H.M., Beed, F., Madata, C., Haudenshield, J. & Hartman, G.L. (2014) First report of *Phakopsora pachyrhizi* on soybean causing rust in Tanzania. *Plant Disease*, 98, 1586.

Murithi, H.M., Beed, F., Soko, M., Haudenshield, J. & Hartman, G.L. (2015) First report of *Phakopsora pachyrhizi* causing rust on soybean in Malawi. *Plant Disease*, 99, 420.

Murithi, H.M., Beed, F., Tukamuhabwa, P., Thomma, B.P.H. & 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 Pathology*, 65, 176–188.

Murithi, H.M., Haudenshield, J., Beed, F., Mahuku, G., Joosten, M.H.A.J. & Hartman, G.L. (2017) Virulence diversity of *Phakopsora pachyrhizi* isolates from East Africa compared to a geographically diverse collection. *Plant Disease*, 101, 1194–1200.

Ojiambo, P.S., Bandyopadhyay, R., Twizeyimana, M., Lema, A., Frederick, R.D., Pedley, K.F. et al. (2007) First report of rust caused by *Phakopsora pachyrhizi* on soybean in Democratic Republic of Congo. *Plant Disease*, 91, 1204.

Oloka, H.K., Tukamuhabwa, P., Sengooba, T. & Shanmugasundram, S. (2008) Reaction of exotic soybean germplasm to *Phakopsora pachyrhizi* in Uganda. *Plant Disease*, 92, 1493–1496.

Paul, C., Frederick, R.D., Hill, C.B., Hartman, G.L. & 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 Disease*, 99, 1059–1069.

Pham, T.A., Miles, M.R., Frederick, R.D., Hill, C.B. & Hartman, G.L. (2009) Differential responses of resistant soybean entries to isolates of *Phakopsora pachyrhizi*. *Plant Disease*, 93, 224–228.

Pivonia, S. & Yang, X.B. (2004) Assessment of the potential year-round establishment of soybean rust throughout the world. *Plant Disease*, 88, 523–529.

Pretorius, Z.A., Kloppers, F.J. & Frederick, R.D. (2001) First report of soybean rust in South Africa. *Plant Disease*, 85, 1288.

Sanginga, N. (2003) Role of biological nitrogen fixation in legume-based cropping systems; a case study of West Africa farming systems. *Plant and Soil*, 252, 25–39.

Schneider, R.W., Hollier, C.A., Whitam, H.K., Palm, M.E., McKemy, J.M., Hernández, J.R. et al. (2005) First report of soybean rust caused by *Phakopsora pachyrhizi* in the continental United States. *Plant Disease*, 89, 774.

Tesfaye, A., Kifle, B., Haudenshield, J.S. & Hartman, G.L. (2017) First report of *Phakopsora pachyrhizi* causing rust on soybean in Ethiopia. *Plant Disease*, 101, 1049.

Tichagwa, J.S.. 2004. Breeding for resistance to soybean rust in Zimbabwe. In: Moscardi, F., Hoffmann- Campo, C.B., Saraiva, O.F., Galerani, P.R., Krzyzanowski, F.C. & Carraõ-Panizzi, M.C. (Eds.) *Proceedings of VII World Soybean Research Conference, IV International Soybean Processing and Utilization Conference, III Congresso Mundial de Soja (Brazilian Soybean Conference)*. Londrina, Brazil: Embrapa Soybean, pp. 349–353.

Twizeyimana, M. & Hartman, G.L. (2012) Pathogenic variation of *Phakopsora pachyrhizi* isolates on soybean in the United States from 2006 to 2009. *Plant Disease*, 96, 75–81.

Twizeyimana, M., Ojiambo, P.S., Hartman, G.L. & Bandyopadhyay, R. (2011) Dynamics of soybean rust epidemics on an early and late maturing soybean cultivar. *Plant Disease*, 95, 43–50.

Twizeyimana, M., Ojiambo, P.S., Ikotun, T., Ladipo, J.L., Hartman, G.L. & Bandyopadhyay, R. (2008) Evaluation of soybean germplasm for resistance to soybean rust (*Phakopsora pachyrhizi*) in Nigeria. *Plant Disease*, 92, 947–952.

Twizeyimana, M., Ojiambo, P.S., Sonder, K., Ikotun, T., Hartman, G.L. & Bandyopadhyay, R. (2009) Pathogenic variation of *Phakopsora pachyrhizi* infecting soybean in Nigeria. *Phytopathology*, 99, 353–361.

Walker, D.R., Boerma, H.R., Harris, D.K., Schneider, R.W., Buckley, J.B., Shipe, E.R. et al. (2011) Evaluation of USDA soybean germplasm accessions for resistance to soybean rust in the southern United States. *Crop Science*, 51, 678–693.

Wrather, J.A., Anderson, T.R., Arsyad, D.M., Gai, J., Ploper, L.D., Porta-Puglia, A. et al. (1997) Soybean disease loss estimates for the top 10 soybean producing countries in 1994. *Plant Disease*, 81, 107–110.

Yamanaka, N. & Hossain, M.M. (2019) Pyramiding three rust-resistance genes confers a high level of resistance in soybean (*Glycine max*). *Plant Breeding*, 138, 686–695.

Yamanaka, N., Lemos, N.G., Uno, M., Akamatsu, H., Yamaoka, Y., Abdelnoor, R.V. et al. (2013) Resistance to Asian soybean rust in soybean lines with the pyramided three *Rpp* genes. *Crop Breeding and Applied Biotechnology*, 13, 75–82.

Yamanaka, N., Morishita, M., Mori, T., Lemos, N.G., Hossain, M.M., Akamatsu, H. et al. (2015) Multiple *Rpp*-gene pyramiding confers resistance to Asian soybean rust isolates that are virulent on each of the pyramided genes. *Tropical Plant Pathology*, 40, 283–290.

Yamanaka, N., Yamaoka, Y., Kato, M., Lemos, N.G., Passianotto, A.L.L., dos Santos, J.V.M. et al. (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.

Yamaoka, Y., Yamanaka, N., Akamatsu, H. & Suenaga, K. (2014) Pathogenic races of soybean rust *Phakopsora pachyrhizi* collected in Tsukuba and vicinity in Ibaraki, Japan. *Journal of General Plant Pathology*, 80, 184–188.

Yorinori, J.T., Paiva, W.M., Frederick, R.D., Costamilan, L.M., Bertagnolli, P.F., Hartman, G.E. et al. (2005) Epidemics of soybean rust (*Phakopsora pachyrhizi*) in Brazil and Paraguay from 2001 to 2003. *Plant Disease*, 89, 675–677.

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