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Assessment of Comparative Virulence and Resistance in Soybean Using Field Isolates of Soybean Rust

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Abstract

A major impediment to breeding for resistance to Asian soybean rust (*Phakopsora pachyrhizi*) is the lack of stable sources of resistance, due to high variability in the pathogen. The objectives of this study were to assess comparative virulence of five diverse field isolates from major soybean producing areas in Uganda, and identify lines with resistance to isolates of soybean rust in seedling and adult plants under screen house and field conditions respectively. When inoculated with the five field isolates, all twelve lines evaluated showed diverse and mixed reactions, suggesting each location differed in soybean rust races and/or virulence. Experimental sites growing many diverse soybean lines yearly had the greatest diversity of soybean rust. The effectiveness of specific resistance genes was restricted to certain locations and gene R*pp2* previously resistant was ineffective producing a susceptible tan reaction at the seedling stage. A positive correlation between mean lesion density at the seedling stage and adult plant severity indicated that using field isolates to screen for seedling resistance can be a useful breeding approach to extrapolate resistance in adult plants. Overall, these results emphasise the relevance of using field isolates from the target areas to evaluate lines for soybean rust resistance.

Keywords: *Phakopsora pachyrhizi*, comparative virulence, stable resistance

1. Introduction

Asian soybean rust (*Phakopsora pachyrhizi* Sydow) is a major threat to soybean production worldwide. The pathogen is an obligate parasite that causes multi-cyclic infections during the growing season. The uredinal stage produces urediniospores responsible for disease development and subsequent yield losses are due to premature defoliation and reduction in photosynthesis that adversely affects grain filling (Kumudini et al., 2010; Miles, Frederick, & Hartman, 2003). Since the disease was first detected in Japan, researchers have focused their efforts on several ways to manage it. Eradication or elimination efforts of the source of soybean rust inoculum are unlikely to succeed due to the wide host range including legumes which are an integral part of most cropping systems (Goellner et al., 2010; Miles et al., 2003; Miles, Pastor-Corrales, Hartman, & Frederick, 2007; Slaminko, Miles, Frederick, Bonde, & Hartman, 2008). Chemical control, though effective, poses a greater challenge since its effectiveness depends on frequent symptom monitoring and timely routine fungicide application (Yorinori et al., 2005). Genetic resistance is currently the most economic and strategically important means of managing rust soybean being pursued by several soybean breeding programmes (Arias et al., 2008). Resistance to rust is conferred mainly by R genes (major genes) and is dependent on specific prevalent soybean rust isolates. Major resistance genes R*pp*1, R*pp2*, R*pp3*, R*pp4*, R*pp5* and R*pp?* (Hyuuga), have been identified to show resistance to specific races of soybean rust (Bromfield & Hartwig, 1980; Garcia et al., 2008; Hartwig, 1986, McLean & Byth, 1980; Monteros, Missaoui, Phillips, Walker, & Boerma, 2007).

Several studies investigating the effectiveness of single soybean rust resistance genes have used single spore isolates with evaluations done under controlled conditions at seedling stage (Li, 2009; Paul & Hartman, 2009; Twizeyimana et al., 2009). However, in nature the soybean rust pathogens often exist as mixtures with either homogeneous or heterogeneous virulence among sub-populations with varying aggressiveness. Single spore isolation does not always capture the variability present within a field isolate (Freire et al., 2008; Paul & Hartman, 2009). Given the high virulence diversity within and among *P. pachyrhizi* isolates, use of single pure isolates in determining soybean rust resistance will not necessarily allow extrapolation to the field level. Moreover, differences have been observed in the capacity of soybean to express resistance depending on the stage of growth

and environment. Seedling evaluation for soybean rust resistance does not always guarantee adult plant resistance (Miles et al., 2008; Ribeiro et al., 2007). Seedling resistance is, however, still important given that soybean rust attacks soybean at any phenological stage of development. Having both seedling and adult plant resistance guarantees complete protection of soybean irrespective of the time when the disease manifests.

In Uganda, previous race characterisation studies using race differentials and molecular analysis showed low racial diversity with three races found in most soybean growing areas (Lamo, 2004). However, the scenario is likely to have changed since two cultivars with specific resistance deployed in 2005 have shown increased susceptibility to soybean rust (Tukamuhabwa et al., 2009). In addition, no cultivar in Uganda utilises any of the available characterised classical resistance genes. The effective use of these exotic gene sources depends on knowledge of how they respond to local soybean rust populations. Differential response of the host genotypes to local rust populations can further help understand the virulence patterns of Asian soybean rust in Uganda. This information would help facilitate utilisation and development of the stable host resistance. Therefore the objectives of this research were to i) assess comparative soybean rust virulence patterns of natural pathogen populations in the different major soybean growing areas and ii) identify soybean lines with stable seedling and adult stage resistance when challenged by geographically diverse field isolates.

2. Materials and Methods

2.1 Soybean Lines

Twelve soybean lines including two susceptible checks (Wondersoya and Nam 2) were used in this study. Their pedigree, origin and source are presented in Table 1. Sources of resistance PI 230970 (R*pp2*), Ankur (R*pp3*), PI 459025 (R*pp4*), G000138-29, Maksoy 1N were previously characterised for rust resistance for three seasons at Namulonge (Oloka, Tukamuhabwa, Sengooba, & Shanmugasundram, 2008). In addition, two high potential materials with resistance genes PI 459024, UG 5; three released cultivars Maksoy 2N, Namsoy 4M and Maksoy 3N. This set of soybean lines was also selected on the basis of a relatively similar growth cycle under the local field conditions to reduce the effect of crop phenology on disease severity during evaluation.

Table 1. A set of 12 putative differential soybean lines used to assess resistance to five field isolates using seedling and adult plants

No	Genotype	Pedigree	Reason for selection	Origin	Source
1	PI230970	G8586	Resistance gene (R*pp2*)	Japan	AVRDC, Taiwan
2	Ankur	G7955	Resistance gene (*Rpp3*)	India	AVRDC, Taiwan
3	PI459025	G10428	Resistance gene (*Rpp4*)	China	AVRDC, Taiwan
4	PI 459024	G10427	Resistant	China	AVRDC, Taiwan
5	G000138-29	(CH#1 x Anoka) x (Clarke 63 x 64.4)	Resistant	China	AVRDC, Taiwan
6	UG 5	-	Resistant	Uganda	MAK, Uganda
7	Maksoy 1N	TGx1835-10E	Resistant	Nigeria	IITA, Nigeria
8	Maksoy 2N	Duiker x GC000138-29	Resistant	Uganda	MAK, Uganda
9	Maksoy 3N	Duiker x TGx 1835-10E	Resistant	Uganda	MAK, Uganda
10	Namsoy 4M	Nam 2 x GC000138-29	Resistant	Uganda	NARO, Uganda
11	Nam 2	87D-668	Susceptible	Nigeria	NARO, Uganda
12	Wondersoya	-	Susceptible	Nigeria	IITA, Nigeria

NARO - National Agricultural Research Organisation - Uganda.

MAK - Makerere University.

IITA -International Institute of Tropical Agriculture.

AVRDC - World Vegetable Centre.

2.2 Field Procedures for Assessment of Adult Plant Resistance

The layout for the field experiment was a randomised complete block design with three replications at the five sites of Makerere University Agricultural Research Institute-Kabanyolo (MUARIK), National Crops Resources Research Institute (NaCRRI), Iki-Iki (IKI), Nakabango (NAK) and Kasese (KAS) in 2010 and 2011 leading to 10 season-location environments. These sites represent areas of high soybean production in Uganda with endemic seasonal soybean rust epidemics. At each site each entry was sown at the same time with 25-30 seeds in 2 metre rows replicated three times in a randomised complete block design. Entries were randomised across sites within a given year with a 60 cm x 5 cm inter- and intra-row spacing. Spreader rows of a susceptible cultivar Nam 2 were planted at the same time every after five rows around each replicate.

2.3 Field Data Collection and Analyses

Reaction types and sporulation were evaluated at the R6 stage (Fehr et al., 1971) when symptoms were clearly seen on susceptible checks using three trifoliate leaves of the mid-canopy. Disease reaction types were recorded as Red Brown (RB), Tan (T) and Mixed (MX). Red brown lesion colour was considered a resistant reaction whereas tan lesions expressed susceptibility (Miles et al., 2003). Mixed reactions had both red brown and tan lesion of the same leaf or different plants of the same soybean line. Field sporulation levels were rated on a 1 to 5 scale (Pham et al. 2009) using the susceptible cultivar Wondersoya as a control. Severity scale was based on 1 to 9 scale where 1- no lesions; 2 = 1-30; 3 = 31-75; 4 = 76-150; 5 = 151-300; 6 = 301-750; 7 = 751-1500; 8 = 1501-3000 and 9 = >3000 lesions (Miles et al., 2008). Prior to analysis severity and sporulation scores were subjected to transformation by square root and arcsine methods respectively to normalise them. Back transformed data was presented as the final results. All analyses were done using GENSTAT software 13th Edition and means compared using standard error (Payne et al., 2010). Interactions between lines and isolates for sporulation and disease severity were analysed using ANOVA.

2.4 Screen House Procedures for Assessment of Seedling Resistance

Two sets of three plants for each of the twelve genotypes were grown in wide trays in the screen house for inoculation in a split plot design. The main plot factor was the five isolates and subplot factor genotype. Using a handheld Liliput® vacuum composite soybean rust field isolates were harvested in June 2011 from random soybean leaves at the R6 stage at each of the five sites (used for the field trial). Isolates from NAK, MUARIK, IKI were inoculated on the same day and those from KAS and NaCRRI a week later. For each isolate, freshly harvested field spores were mixed with distilled deionised water containing the surfactant Tween-20 at 0.5 ml/l. Urediniospore suspensions were diluted to a concentration of 50 000 spores per millimetre using a Neubauer haemocytometer. Prior to inoculating each set of entries, germination ability of the spores was tested on water agar to ensure infectivity. In each set, three trifoliates one from each plant were artificially inoculated with 1.5 ml of spore suspension on the abaxial leaf surface using a Canyon® (Model 5A, England) hand sprayer. After inoculation plants were covered with polythene bags for 24 hours at 22°C-24°C to maintain high relative humidity necessary for infection. Trays containing plants with each isolates were spatially separated within the screen house to avoid any possibility of cross contamination. After 24 hours polythene bags were removed for the duration of the experiment.

2.5 Screen House Data Collection and Analysis

Using 20X magnification lenses, the soybean lines were monitored for lesion colour, incubation period and latent period. Days to appearance of symptoms from inoculation day were recorded as incubation period and days to urediniospore production as latent period. Lesion density (cm^{-2}) and the frequency of sporulating lesions were recorded after 16 days from the middle leaflet and analysed using analysis of variance, means were compared using standard error. Starting at seven days after inoculation, data on lesion density were collected four times at three day intervals (up to 16 days) to plot the areas under disease progress curve (AUDPC) using the following formula:

$$\text{AUDPC} = \sum_{i=1}^{k} 1/2\,[(s_i + s_{i+1})(t_{i+1} - t_i)] \quad (1)$$

Where s_i is the rust severity at time i, t_i is the number of days after the first observation on assessment date i and k is the number of successive observations (Campbell & Madden, 1990).

3. Results

3.1 Reaction Type

Based on reaction types, soybean lines responded differentially to the five field isolates during screen house inoculation (Table 2). Soybean lines PI 230970 (R*pp2*), Nam 2 and Wondersoya produced consistently tan lesions

typical of a susceptible reaction in all locations. However, the two classical resistance gene sources Ankur (R*pp3*) and PI 459025 (R*pp4*), local genotypes UG 5 and Maksoy 3N responded with a red brown phenotype at all locations indicating presence of resistance genes. Mixed reaction responses were observed between lines PI 459024, GC000138-29, Maksoy 1N and Maksoy 2N in all the five isolates tested. All field isolates showed at least one mixed reaction in the twelve soybean lines evaluated. Immune response was not observed in any genotype.

Table 2. Reaction types shown by 12 soybean lines against Ugandan field rust pathogen populations

Source of Isolate	PI 230970	Ankur	PI 459025	PI 459024	UG 5	GC00138-29	Namsoy 4M	Maksoy 1N	Maksoy 2N	Maksoy 3N	Nam 2	Wondersoya
MUAIRK	T	RB	RB	MX	RB	MX	MX	MX	T	RB	T	T
NAK	T	RB	RB	RB	RB	RB	MX	T	T	RB	T	T
IKI	T	RB	RB	RB	RB	RB	MX	MX	T	RB	T	T
NaCRRI	T	RB	RB	RB	RB	MX	MX	MX	MX	RB	T	T
KAS	T	RB	RB	RB	RB	RB	MX	T	MX	RB	T	T

T-Tan, RB red brown, MX-mixed.

3.2 Severity and Sporulation

Table 3 Analysis of variance of soybean rust severity and sporulation of 12 soybean lines against five field isolates at the seedling and adult plant stage

Source of variance	df	Mean Squares		Mean Squares	
		Severity	Probability	Sporulation frequency	Probabilit
Seedling resistance					
Sets	1	1.37	0.130	0.04	0.351
Isolate	4	14.44	<.001	0.16	0.015
Isolate x Sets	4	0.54	0.456	0.03	0.685
Genotype	11	2.44	<.001	2.86	<.001
Isolate x Genotype	44	1.19	0.006	0.13	<.001
Error	59	0.58		0.05	
Adult plant resistance					
Year (season)	1	1.43	<.001	0.63	0.001
Isolate[a]	4	0.86	<.001	1.90	<.001
Isolate x Year (season)	4	2.18	<.001	1.11	<.001
Rep [Isolate x Year (season)]	20	0.02	0.569	0.05	0.930
Genotype	11	2.00	<.001	2.52	<.001
Isolate x Genotype	44	0.20	<.001	0.32	<.001
Genotype x Year (season)	11	0.35	<.001	0.32	<.001
Isolate x Year (season) x Genotype	44	0.19	<.001	0.15	<.001
Pooled Error	160	0.03		0.06	

[a] For adult plant resistance use of the term 'isolate' refers to the location where the field evaluation was done.

In the screen house experiment, analysis of variance indicated that there were significant isolate, genotype and isolate x genotype differences for severity and sporulation for seedling resistance. However, differences in the isolates had the strongest effects for disease severity whereas genotype had the strongest effects on sporulation rate (Table 3). In the field evaluations for adult plant resistance, all sources of variation were significant with isolate, genotype and isolate x year (season) contributing largely to the differences in severity and sporulation. The year (season) and isolate x year (season) effects were, however, more pronounced for severity than sporulation.

In the field, the overall mean severity score was greater in 2010 with more soybean lines producing mixed reaction across test locations than in 2011. However, in 2011 IKI had the lowest mean severity score which was 3.2 less than previous year (Table 4). Susceptible lines Nam 2 and Wondersoya had severity scores consistently greater than the location means with predominantly tan and mixed reaction types. Conversely, Ankur (R*pp3*), PI 459025 (R*pp4*) and Maksoy 3N had red brown lesions across lesions with mean severity generally lower than the mean averages of the locations. There was no relationship between lesion colour and severity during the two seasons of evaluation in the five test locations.

Table 4. Soybean lines used for adult plants resistance assessment in the field, their severity score (1-9), reaction type (in parentheses) and location of isolate origin

Location	Field, 2010					Field, 2011				
	IKI	KAS	MUARIK	NaCRRI	NAK	IKI	KAS	MUARIK	NaCRRI	NAK
Entry										
PI 230970 (R*pp2*)	6.0(RB)	5.3(RB)	3.3(RB)	3.3(RB)	3.3(RB)	2.0(RB)	3.6(RB)	3.3(MX)	3.6(T)	4.0(T)
Ankur (R*pp3*)	6.0(RB)	4.0(RB)	2.3(RB)	2.0(RB)	3.0(RB)	2.0(RB)	2.3(RB)	2.0(RB)	2.6(RB)	3.0(RB)
PI 459025 (R*pp4*)	5.6(RB)	4.0(RB)	2.3(RB)	1.0(RB)	2.0(RB)	3.0(RB)	4.0(RB)	3.3(RB)	2.0(RB)	2.0(RB)
PI 459024	4.0(RB)	5.3(MX)	3.0(RB)	3.3(RB)	3.3(RB)	4.0(MX)	5.3(RB)	2.0(RB)	2.0(RB)	4.0(MX)
GC000138-29	4.0(RB)	3.0(MX)	2.3(RB)	2.0(RB)	2.3(RB)	2.3(RB)	4.0(RB)	3.3(RB)	2.0(RB)	4.0(MX)
UG 5	5.0(RB)	2.6(MX)	2.0(RB)	1.0(RB)	2.3(RB)	2.0(RB)	2.6(RB)	2.6(RB)	4.0(RB)	2.0(RB)
Maksoy 1N	6.6(RB)	5.0(T)	6.0(RB)	5.6(MX)	5.0(T)	2.0(RB)	4.0(RB)	7.3(RB)	3.3(RB)	2.6(RB)
Maksoy 2N	5.6(RB)	6.6(MX)	3.3(RB)	3.6(MX)	4.6(RB)	2.6(MX)	2.0(RB)	6.6(RB)	4.3(T)	4.0(T)
Maksoy 3N	5.0(RB)	3.6(RB)	2.6(RB)	3.0(RB)	3.3(RB)	2.0(RB)	2.0(RB)	2.6(RB)	2.0(RB)	2.0(RB)
Namsoy4M	6.3(RB)	5.3(MX)	4.6(MX)	4.6(MX)	4.3(RB)	3.0(RB)	4.0(MX)	5.3(MX)	4.6(RB)	3.3(RB)
Nam 2	7.6(RB)	4.6(MX)	6.3(MX)	6.6(MX)	6.0(MX)	2.0(RB)	5.6(MX)	6.6(T)	6.6(T)	4.3(T)
Wondersoya	6.6(MX)	8.3(T)	6.3(MX)	5.6(MX)	6.0(MX)	5.0(MX)	7.3(T)	6.6(T)	4.6(T)	5.3(T)
Mean	5.7	4.8	3.7	3.5	3.8	2.7	3.9	4.3	3.5	3.4
SE ±	0.29	0.45	0.48	0.53	0.40	0.28	0.46	0.58	0.41	0.29

RB-red brown; MX-mixed; T-tan.

In the screen house experiments, evaluating the frequency of sporulation per square centimetre, KAS isolate had the greatest mean sporulation frequency (Table 5), 11.1% more than the least sporulating isolate from NAK. Isolates from KAS and NaCRRI resulted in sporulation in all soybean lines whereas NAK did not show sporulation in three lines (Table 5).

Table 5. Sporulation frequency averaged of 12 soybean lines in the screen house for seedling resistance experiment using Ugandan field isolates from five different sites

Genotype	Percentage sporulation frequency of isolate				
	IKI	KAS	MUARIK	NaCRRI	NAK
PI230970 (R*pp2*)	100	89.99	100	85.06	95.66
Ankur (R*pp3*)	73.58	38.46	0	1.31	13.39
PI 459025(R*pp4*)	31.50	30.76	10.87	39.53	10.19
PI 459024	83.97	47.86	80.38	11.31	35.47
GC000138-29	0	45.96	0	16.92	0
UG 5	0	13.21	42.48	18.45	0
Maksoy 1N	100	93.98	99.00	83.75	100
Maksoy 2N	100	94.49	100	98.98	100
Maksoy 3N	2.86	18.45	8.71	11.69	0
Namsoy4M	37.01	80.54	100	58.91	67.20
Nam 2	85.34	96.25	100	99.49	94.31
Wondersoya	100	99.49	100	97.94	100
Mean	59.52	62.45	61.78	51.94	51.35
SE±	12.18	9.55	13.04	11.38	13.04

SE± standard error of the mean.

Table 6. Summary of means of soybean rust infection parameters from inoculation averaged across five Ugandan field isolates

Genotype	Incubation period (IP)	Latent Period (LP)	Number of lesions/square centimetre (LS)	Frequency of sporulating lesions (FS)[1]	AUDPC
PI 230970 (R*pp2*)	5.2	7.2	22.8	94.14	57.7
Ankur (R*pp3*)	4.8	7.6	21.1	25.35	52.2
PI 459025 (R*pp4*)	4.6	7.5	21.9	24.57	55.7
PI 459024	5.1	9.1	19.8	51.80	44.3
GC000138-29	5.7	7.8	21.2	12.58	49.0
UG 5	5.2	8.2	21.7	14.83	56.3
Maksoy 1N	4.8	9.8	29.7	95.34	74.9
Maksoy 2N	5.1	10.2	37.0	98.98	85.8
Maksoy 3N	5.2	7.2	19.5	8.34	46.8
Namsoy4M	4.9	10.4	31.7	68.73	70.0
Nam 2	4.9	10.2	30.6	95.08	78.5
Wondersoya	5.1	9.9	33.7	99.49	81.4
Mean	4.98	8.75	25.9	57.43	62.7
SE±	0.08	0.37	1.78	11.09	4.21

SE± standard error of the mean;

[1] Expressed as a percentage.

Analysis of variance indicated that there were significant ($P \leq 0.01$; ANOVA not shown) differences in incubation and latent period, lesion density, percentage frequency of sporulating lesions and AUDPC for lesion density of lines under screen house conditions. In general, most local cultivars showed longer latent periods that the exotic lines in response to the five isolates tested (Table 6). Namsoy 4M had typically the longest latent period compared to PI 230970 (R*pp2*) and Maksoy 3N with the least. Despite the long latent period of Namsoy 4M, lesions density

was the third highest in all the test lines. Contrary, Maksoy 3N had the shortest latent period and lowest number lesions per square centimetre, 6.4 lower than the overall mean. PI 230970 (R*pp2*) with a classical resistance gene and Maksoy 2N were equally highly sporulating and comparable to the susceptible check lines. Maksoy 3N, GC000138-29 and UG 5 had light sporulation with less than 15% of the uredinia sporulating per square centimetre. Maksoy 2N had the highest AUDPC followed by Wondersoya which differed significantly from all the exotic sources of resistance and cultivar Maksoy 3N (Table 6).

3.3 Correlations Among Soybean Rust Infection Parameters

Using genotype averages across isolates a significant ($P \leq 0.001$) positive correlation was observed between percentage frequency of sporulating lesions and AUDPC (Table 7). Similarly, latent period and number of lesions density were significantly positively correlated with AUDPC. It was noted that incubation period had a negative non-significant correlation with other soybean rust resistance parameters evaluated. Mean disease severity of the adult soybean lines for the two years and seedling lesion density were positively correlated ($r = 0.813$, $P < 0.001$).

Table 7. Correlations among infection parameters evaluated during the seedling stage using five diverse field isolates

	IP	LP	LS	FS
LP	-0.224ns			
LS	-0.209ns	0.857***		
FS	-0.232ns	0.702**	0.799**	
AUDPC	-0.279ns	0.801**	0.972***	0.819***

***$P \leq 0.001$; **$P \leq 0.01$; ns-not significant;

IP-Incubation Period; LP-Latent period; LS-Number of lesions per square centimetre; FS-F frequency of sporulation lesions; AUDPC-Area under disease progress curve.

4. Discussion

The knowledge of rust virulence in soybean rust populations and how soybean lines react to field isolates in different regions is important for successful breeding and deployment of resistance genes (Miles et al., 2011). The reaction types obtained from the five isolates may indicate that each isolate is distinct which is suggestive of the existence of different race populations or virulence patterns (Yamanaka et al., 2010). It was, however, surprising that PI 230970 (R*pp2*) with a classical resistance produced tan lesions. This genotype was recommended for inclusion in the local germplasm after evaluations between 2005 and 2006 at NaCRRI in Uganda (Oloka et al., 2008). This could suggest resistance breakdown and underscores the importance of evaluating for resistance in the target geographic locations due to the differences in diversity and virulence of the rust pathogen.

Mixed lesions were observed on at least one genotype in all locations, which is indicative of a mixture of races with heterogeneous virulence (Miles et al., 2008). This was, however, more pronounced in MUARIK and NaCRRI which have the largest area of different experimental soybean lines every year. Increased virulence diversity could be an evolutionary consequence prompted by deployment of a wide assortment of lines in these two locations.

The significance of isolate-by-genotype interaction for severity and sporulation frequency implies that ranking of lines changes markedly with isolates. This presents a great challenge when breeding for resistance using specific gene resistance to manage soybean rust due to great differences exhibited by the lines. Furthermore, this underscores the importance of evaluating candidate lines using rust populations present in the target areas. In the field, the isolate x year (season) had substantial impact to disease severity greater than sporulation (Table 3). Though the two resistance indices are not completely independent of each other this could suggest greater effect of environment on severity.

This study purposefully used field isolates to understand comparative virulence and identify stable sources of soybean rust resistance. The greater preponderance of mixed reactions in the field attributed to heterogeneous race composition coupled with environmental factors that influenced the amount of inoculum and disease progress during the seasons (Miles et al., 2007, 2008). However, the significance of isolate, genotype and isolate x genotype interaction factors using severity and sporulation rate indices (Table 3) and strong positive correlation of disease severity and lesion density both during seedling and adult plant stages suggests that these are related. Similarly, isolates from KAS, MUARIK and IKI were the most aggressive in both mean seedling and adult plant assessments

compared to those from NaCRRI and NAK. The seedling resistance tests using field isolates can therefore be used to extrapolate resistance under field conditions with better accuracy. On the contrary, seedling and adult plant were observed not to be necessarily correlated during resistance evaluations in Brazil (Ribeiro et al., 2007). Differences obtained in seedling and adult plant resistance done in the US and Paraguay respectively were attributed to differences in virulence of the isolates used and longer, multiple cycles of exposure in the field (Miles et al., 2008). Our study, however, used the same field isolate of similar composition and virulence for both seedling and adult plant resistance hence the comparable results.

The positive correlation between AUDPC and sporulation relate to rapid advance of the disease caused by increased sporulation which results in more secondary infections. It was also observed that AUDPC was directly related to lesion number which is an important disease resistance index. A positive correlation between latent period and AUPDC suggested that lines that expressed disease urediniospores early have slower disease progress. This could imply that the resistance mechanism present in these lines responds rapidly once the rust pathogen is established in the host cells compared to those with a longer latent period.

Overall, the results indicate that soybean rust breeding programmes utilising specific resistance are challenged due to the restricted locations for which such resistance applies. The longevity of R*pp2* was about 5 years, which is relatively short and further limits specific resistance. Great soybean diversity was observed in sites which grown several varieties every season. Latent period, lesion density and proportion of sporulating lesions are important disease resistance parameters that can be used to extrapolate disease progress. Prospecting and exploration for other sources of resistance and strategies such as partial resistance and tolerance is highly recommended.

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