Agricultural Journal 10 (3-6): 17-23, 2015
ISSN: 1816-9155

Soybean Genetic Diversity and Resistance to Soybean Rust Disease in Uganda

[1]G. Ssendege, [1]T. Obua, [2]R. Kawuki, [3]M. Maphosa and [1]T. Tukamuhabwa
[1]Makerere University, School of Agricultural Sciences, P.O. Box 7063, Kampala, Uganda, Zimbabwe
[2]National Crops Resources Research Institute, P.O. Box 7084, Kampala, Uganda, Zimbabwe
[3]Lupane State University, Crop and Soil Sciences, P.O. Box AC255, Bulawayo, Zimbabwe

Abstract: This study was conducted to enhance soybean diversity and breeding for resistance to soybean rust (caused by *Phakopsora pachyrhizi*), the greatest obstacle to soybean production in Uganda. Exotic and local soybean genotypes from USDA Germplasm collection, Zimbabwe and the national breeding programme were evaluated for soybean resistance at Makerere University Agricultural Research Institute Kabanyolo (MUARIK) during the 2011 and 2012 seasons. Similarity matrices were calculated from the exotic and local soybean genotype Simple Sequence Repeat (SSR) data fingerprint using NTSYSpc Version 2.2. Significant genetic variation was present among exotic soybean accessions indicating high divergence among them. However, three local soybean varieties Maksoy 1N, Maksoy 2N and Maksoy 3N had low genetic diversity as revealed by the dendrogram which clustered them into one group. Soybean accessions PI 462312 with the resistance gene *Rpp3*, *PI 416826A*, *PI 417126*, *PI 615437* and *PI 628935* showed resistance to soybean rust at MUARIK and thus are recommended for use in improving genetic diversity and resistance in released varieties in Uganda. However, there is a need to evaluate all the resistant genotypes in other regions of Uganda for broad spectrum soybean rust resistance.

Key words: USDA, SSR, MUARIK, soynean, maksoy

INTRODUCTION

Soybean [*Glycine max* (L.) Merr] plays an important nutritive role in the food and feed industry in many countries (Hartman *et al*., 2011). However, the crop is currently threatened by soybean rust, caused by *Phakopsora pachyrhizi* which has become a worldwide threat to soybean production. Under heavy infestation, losses of up to 75% have been experienced in unprotected soybean crops (Yorinori *et al*., 2005). In Uganda, the deployed resistance genes in varieties Maksoy 1N (TGx 1835-10E) from Nigeria and Namsoy 4m (Nam II×GC00138-29) developed by Makerere University have broken down due to the emergence of more virulent and aggressive strains of soybean rust. In addition, repeated use of closely related parents in the breeding programme has narrowed the genetic base of the available soybean germplasm, consequently increasing its genetic vulnerability to new races of soybean rust. Therefore, there is need to identify new diverse sources of soybean rust resistance from exotic genotypes to cope with the highly variable rust pathogen. The introduction of exotic soybean germplasm enables broadening of the current genetic base by availing new favorable alleles and or allele combinations that can be subjected to selection (Chung and Singh, 2008; Guzman *et al*., 2007).

Despite the importance of maintaining genetic diversity in crop improvement, most exotic soybean genotypes have a narrow adaptation hence there is need to evaluate their performance under local conditions before their use. Therefore, local characterisation of exotic soybean genotypes will enable the selection of adapted, diverse genotypes for broadening the genetic base for soybean rust resistance breeding. One option for establishing relatedness and or similarity of parental materials is through their phenotypic characterisation. However, the commonly used phenotypic descriptors for genetic diversity enhancement in soybean are less informative and limited given the common ancestry of most commercial varieties (Priolli *et al*., 2002). It is on this basis that DNA-based markers are an ideal tool used for genetic analysis in soybean (Kuroda *et al*., 2009; Li *et al*., 2009). The soybean breeding programme at Makerere University obtained 89 soybean genotypes from the USDA Soybean Germplasm Collection and Seed Co. Private Limited a leading soybean breeding company in Zimbabwe in an effort to broaden the genetic base for various traits with an emphasis on resistance to soybean rust. In this study, the level of resistance to soybean rust and genetic diversity among the introduced soybean germplasm was quantified.

Corresponding Author: G. Ssendege, Makerere University, School of Agricultural Sciences, P.O. Box 7063, Kampala, Uganda, Zimbabwe

MATERIALS AND METHODS

SSR genetic fingerprinting: Ninety-two soybean accessions of which 76 were obtained from the USDA Germplasm Collection Centre (selected on the basis of being possessing soybean resistance traits) originating from Brazil, China, India, Indonesia, Japan and Vietnam, 13 commercial varieties from Zimbabwe [Buffalo, Dinamri, Duiker, Edamame 1, Roan, Safari, Santa, SCSI, Serenade, Siesta, Solitaire, Soparo and Gazelle] and three local genotypes [Maksoy 1N (TGx 1835-10E), Maksoy 2N (TGx 1835-10E×Duiker) and Maksoy 3N (Duiker×GC00138-29)] were used. Maksoy 1N and Maksoy 3N were used as a susceptible and resistant check, respectively.

The 20 days after germination, genomic DNA was extracted from young fresh leaves of seedlings using the CTAB Method, described by Doyle and Doyle (1990). DNA quality and concentration was determined using the Nanodrop (Thermo Scientific, USA) and concentrations adjusted to 10 ηg μ L^{-1}. The DNA was assayed with ten polymorphic SSR primer-pairs (Table 1). These markers are distributed on ten linkage groups and are highly informative according to previous studies (Song *et al*., 2004). PCR was performed in a Gene Amp 9700 (Bio-Rad, USA) thermocycler in a 20 μL reaction volume containing 40 ng of template DNA, 0.5 μm of each primer, 0.2 mm dNTPs, 1 U of Taq polymerase, 2.5 mm $MgCl_2$ and 1×PCR buffer. The cycling profile involved an initial denaturing cycle of 94°C for 2 min, followed by 35 cycles of 94°C for 35 sec; annealing at 46°C for 55 sec, extension at 72°C for 45 sec and a final extension cycle at 72°C for 5 min. The PCR amplicons were fractionated by electrophoresis on 3% (w/v) Metaphor (Lonza Bioscience, Singapore) agarose horizontal gel stained with Gel Red™ Nucleic Acid Stain (Biotium, USA), at 110 volts for 2 h along with a 100 bp ladder as a size-standard. Gel images were taken using a Bio Doc-It™ Imaging System (Bio-Rad, USA).

Data collection and analysis: Distinct amplified fragments were scored for presence or absence (0). The Polymorphism Information Content (PIC) a measure of allelic diversity at a given locus was calculated for the 10 loci, according to the formula referred by Wang *et al*. (2008):

$$PIC = 1-\sum_{j=1}^{n} P^2ij$$

where, Pij is the frequency of the jth allele for the ith marker. The similarity matrices were calculated from the generated data and clustered using NTSYSpc software, Version 2.2 (Rohlf, 1998) employing the Unweighted Pair Group Method of the Arithmetic average (UPGMA) to generate a dendrogram. The generated information was used to infer genetic diversity of the soybean genotypes.

Field evaluation for soybean rust resistance: The field experiment comprised the 92 soybean genotypes which were evaluated at Makerere University Agricultural Research Institute Kabanyolo (MUARIK) for the two consecutive growing seasons of 2011B and 2012A (A and B) refer to the first and second seasons, respectively. Severe seasonal soybean rust epidemics commonly occur at the MUARIK location. The soybean genotypes were each planted with a spacing of 60×5 cm in two rows measuring 2 m which were replicated twice in a randomized complete block design. The entire field was surrounded by the susceptible variety Nam I to ensure early and intense inoculum build up. Soybean rust severity was assessed at reproductive stages: R2 (full bloom), R4 (full pod formation) and R6 (full seed formation) using a scale of 1-9, where 1 = no disease and 9 = 90% and above disease severity (Kawuki *et al*., 2004). Soybean rust sporulation was scored among the soybean genotypes using a scale of 1-5 at the R6 stage where 1 = no infection and 5 = high sporulation and reaction types based on leaf lesion colour. Evaluation for sporulation was done in the field with the aid of a×10 handheld lens. Soybean lines without any visible rust symptoms were considered Immune (I), those with Red-Brown (RB) lesions were considerd resistant and lines with Tan-coloured infection type (TAN) were susceptible. For all the assessments three mid-canopy leaves from the inner most 20 plants were assessed for the resistance indices.

Table 1: Polymorphic SSR loci and primers sequence used for genotyping soybean genotypes

Primer	Linkage group	Forward	Reverse	Motif	Position
Satt308	M	GCGTTAAGGTTGGCAGGGTGGAAGTG (26)	GCGCAGCTTTATACAAAAATCAACAA (26)	(ATT)21	130.76
Satt009	N	CCAACTTGAAATTACTAGAGAAA (23)	CTTACTAGCGTATTAACCCTT (21)	(ATT)14	28.52
Satt173	O	TGCGCCATTTATTCTTCA (18)	AAGCGAAATCACCTCCTCT (19)	(ATT)18	58.40
Sct_189	I	CTTTTCCTGGCAATGAT (17)	AAAATCGCAAAACCTTAGT (19)	(CT)17	113.77
Satt263	E	CACCCAATCATGATAGCATTTTAT (24)	CTCATGGAATTGTCTTTCAGTTTC (24)	(ATT)19	45.40
Satt406	J	GCGTGAGCATTTTTGTTT (18)	TGACGGGTTTAATAGCAT (18)	(ATT)31	38.19
Sat_074	F	GGGTGAGAAATACATGCAACTTACA (25)	GGGCATCAAAATTGATATTAAATGTCTAA (29)	(AT)30	142.35
Satt197	B1	CACTGCTTTTTCCCCTCTCT (20)	AAGATACCCCCAACATTATTTGTAA (25)	(ATT)20	46.39
Satt286	C2	GCGGCGTTAATTTATGCCGGAAA (23)	GCGTTTGGTCTAGAATAGTTCTCA (24)	(ATT)17	101.75
Sat005	D1b+W	TATCCTAGAGAAGAACTAAAAAA (23)	GTCGATTAGGCTTGAAATA (19)	(ATT)19	75.29

Data analysis: The data on soybean rust severity and sporulation were subjected to analysis using Genstat 13th edition and mean values were compared among the soybean lines at 5% least significant difference. Rust severity and sporulation data were normalized by angular transformation prior to the analysis of variance. Rust severity and reaction types were compared among the soybean germplasm. Soybean genotypes with low rust scores were considered to have a greater resistance potential against soybean rust.

RESULTS

SSR genetic fingerprinting: The analysis of the 92 soybean genotypes using ten SSR primer pairs showed polymorphism among the soybean genotypes with a total of 53 different alleles amplified (Fig. 1). The number of alleles per locus ranged from three for Sct-189, Satt 263 and Satt 286 to 10 alleles for locus Satt 173 with an average of 5.3 alleles per locus (Table 2). The average PIC of the ten markers was 0.769. Marker Satt 263 had the highest PIC (0.834) while marker Satt 197 had the lowest PIC (0.698). Marker Satt 173 produced the highest number of amplified bands (457) while marker Satt 263 produced the lowest number of amplified bands (116) in all the 92 accessions. Number of alleles produced per genotype ranged from 1-46 with an average of 26 amplicons per genotype.

A dendogram based on the amplified polymorphisms resolved the soybean genotypes into six distinct groups at a similarity coefficient level of 0.57 (Fig. 2). A total of 31 genotypes, including 25 from the USDA Germplasm Collection and six from Zimbabwe were included in Group 1 which was further divided into two subgroups at the similarity coefficient level of 0.65. The first subgroup comprised 23 genotypes including 19 from USDA Germplasm collection and four genotypes (Duiker, Roan, Dinamri and Siesta) from Zimbabwe. The second subgroup comprised eight genotypes including six from USDA Germplasm Collection and two (Safari and Santa) from Zimbabwe. Group 2 comprised 12 genotypes including 10 from USDA Germplasm Collection and two (SCSI and Soprano) from Zimbabwe. Three genotypes PI 567053, PI 567102B and Serenade were clustered in group 3. Group 4 comprised eight genotypes including seven from USDA Germplasm Collection and one genotype, Edamame from Zimbabwe. Group 5 comprised 37 soybean genotypes including 31 from USDA Germplasm Collection, three from Uganda and three genotypes from Zimbabwe. The group was sub divided into three sub groups at similarity coefficient of 0.67. Sub Group 1 comprised 10 genotypes including seven from USDA Germplasm Collection and three local Ugandan varieties (Maksoy 1N, Maksoy 2N and Maksoy 3N). Sub Group 2 comprised 24 genotypes including 21 from USDA Germplasm Collection and three genotypes (Gazelle,

Table 2: SSR loci and allele statistics of soybean genotypes at ten different loci

Primer	Allele per locus	Total fragments	PIC
Satt308	9.0	438.0	0.738
Satt009	4.0	161.0	0.821
Satt173	10.0	457.0	0.769
Sct-189	3.0	142.0	0.752
Satt263	3.0	116.0	0.834
Satt406	8.0	328.0	0.814
Sat-074	4.0	185.0	0.763
Satt197	4.0	209.0	0.698
Satt286	3.0	147.0	0.734
Satt005	5.0	230.0	0.766
Mean	5.3	241.3	0.769

PIC = Polymorphic Information Content

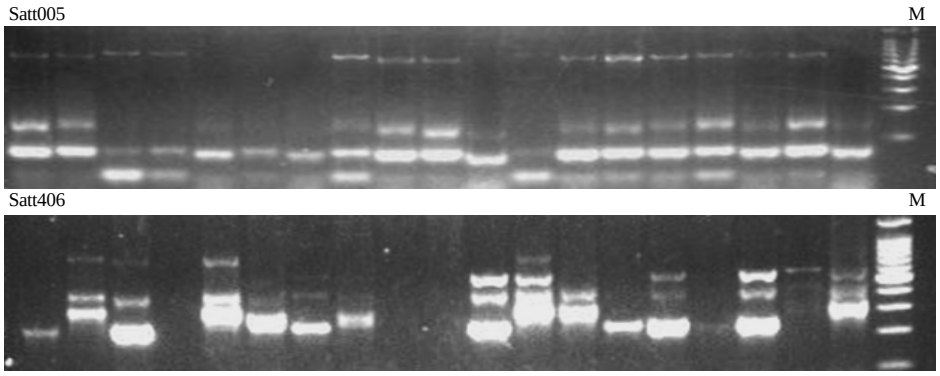

Fig. 1: The electrophoretic patterns of DNA bands amplified by SSR markers from selected soybean genotypes. From left to right are the soybean genotypes Soprano, PI 644103, Safari, PI 416810, PI 605865B, PI 567102B, PI 417120, PI 567053, PI 605885B, PI 567059, PI 471904, PI 628850, Duiker, SCSI, PI 628903, Gazelle, PI 417132, PI 606405, PI 628863 and the M-100bp ladder

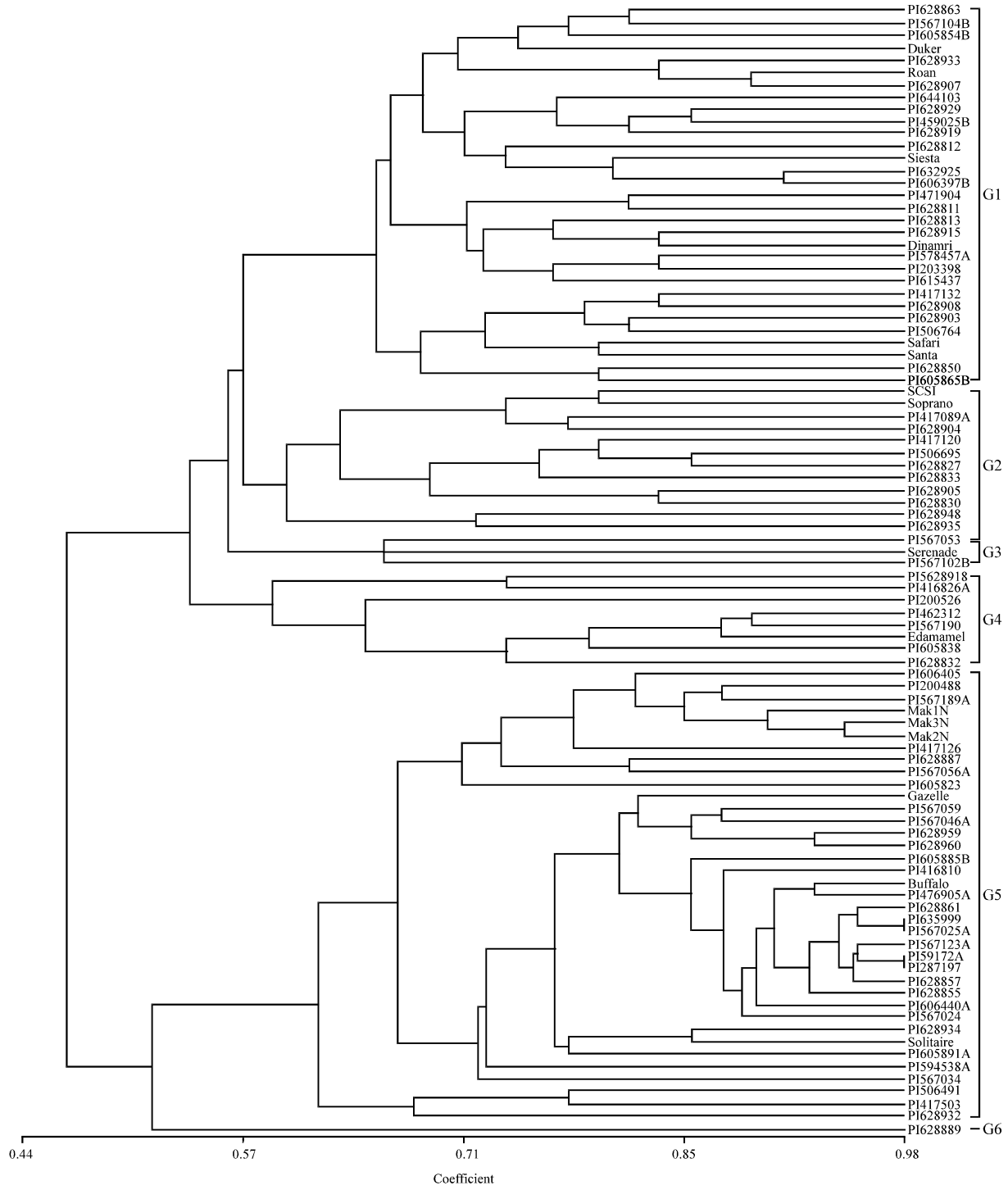

Fig. 2: A dendrogram of genetic relationship among 91 soybean genotypes based on 10 SSR loci. Six groupings (G1 to G6) are apparent at 0.57 coefficient

Buffalo and Solitaire) from Zimbabwe. Sub Group 3 comprised only three genotypes (PI506491, PI417503 and PI628932) from USDA Germplasm Collection. Group 6 had only one genotype (PI628889) from USDA Germplasm Collection. Soybean genotypes (PI685999 and PI567025A, PI59172A and PI287197) had the highest

Table 3: Response of soybean genotypes to soybean rust at MUARIK in 2011B and 2012A

Plot No	Genotype	RR2(1-9)	RR4(1-9)	RR6(1-9)	RS(1-5)	RRT
1	Buffalo	1.8	4.0	5.5	4.0	T
2	Dinamri	1.8	3.8	6.3	5.0	T
3	Duiker	1.8	3.0	4.0	2.8	T
4	Edamame 1	1.5	3.0	4.0	2.5	T
5	Gazelle	1.8	3.3	5.0	3.3	T
6	Maksoy 1N	1.5	2.8	4.8	3.3	T
7	Maksoy 2N	1.8	3.0	5.0	3.5	T
8	Maksoy 3N	1.5	2.8	4.5	2.8	T
9	PI 200488	1.3	2.5	3.8	2.5	T
10	PI 200526	1.8	2.0	3.5	2.5	RB
11	PI 203398	1.5	3.5	4.5	3.0	T
12	PI 287197	1.8	4.0	6.0	3.8	T
13	PI 416810	1.3	1.8	3.0	2.0	RB
14	PI 416826A	1.0	2.0	2.8	1.0	I
15	PI 417089A	1.5	2.0	3.5	2.3	RB
16	PI 417120	1.3	3.0	4.5	3.0	T
17	PI 417126	1.0	2.0	2.8	1.0	I
18	PI 417132	1.5	2.5	3.5	2.3	RB
19	PI 417503	1.5	2.5	3.0	2.0	RB
20	PI 459025	1.5	2.5	3.5	2.3	RB
21	PI 462312	1.3	1.8	3.0	2.0	RB
22	PI 471904	1.0	2.0	3.3	2.3	RB
23	PI 476905A	1.5	3.0	4.5	2.8	T
24	PI 506491	1.0	2.3	3.8	2.5	RB
25	PI 506695	1.5	2.5	3.8	2.5	T
26	PI 506764	1.3	2.3	3.0	2.0	RB
27	PI 567024	1.3	2.5	3.5	2.5	T
28	PI 567025A	1.3	1.8	3.3	2.3	RB
29	PI 567034	1.5	2.5	3.0	2.0	RB
30	PI 567046A	1.8	2.5	4.0	2.8	T
31	PI 567053	1.5	3.0	4.8	3.3	T
32	PI 567056A	1.5	2.8	4.5	3.0	T
33	PI 567059	1.3	2.0	3.0	2.0	RB
34	PI 567102B	1.3	2.5	4.0	2.8	T
35	PI 567104B	1.8	3.0	5.0	3.5	T
36	PI 567123A	1.8	3.8	5.8	3.8	T
37	PI 567189A	1.3	2.3	3.3	2.3	RB
38	PI 567190	1.3	2.8	3.5	2.5	T
39	PI 578457A	1.5	2.8	4.5	3.0	T
40	PI 59172A	1.3	2.5	3.3	2.3	RB
41	PI 594538A	1.3	2.3	3.8	2.5	T
42	PI 605823	2.0	4.3	6.5	5.0	T
43	PI 605838	1.3	2.0	3.0	2.0	RB
44	PI 605854B	1.3	2.0	3.3	2.0	RB
45	PI 605865B	1.3	2.5	3.5	2.3	RB
46	PI 605885B	1.3	2.5	3.3	2.3	RB
47	PI 605891A	1.3	2.5	4.3	2.8	T
48	PI 606397B	1.5	2.5	3.5	2.5	T
49	PI 606405	1.3	2.5	3.0	2.0	RB
50	PI 606440A	2.0	4.3	5.3	3.5	T
51	PI 615437	1.3	1.8	2.8	1.0	I
52	PI 628811	2.3	3.5	5.0	3.5	T
53	PI 628812	1.0	2.0	3.0	2.0	RB
54	PI 628813	2.5	4.3	6.5	4.0	T
55	PI 628827	2.0	3.5	5.0	3.5	T
56	PI 628830	1.8	3.0	4.3	2.8	T
57	PI 628832	1.5	3.0	3.5	2.5	T
58	PI 628833	1.5	3.3	5.0	3.3	T
59	PI 628850	1.3	3.3	5.0	3.5	T
60	PI 628855	1.8	4.5	6.0	5.0	T
61	PI 628857	1.8	4.3	5.5	3.5	T
62	PI 628861	2.0	4.3	5.8	5.0	T
63	PI 628863	1.0	2.3	3.8	2.5	T
64	PI 628887	1.8	4.0	5.0	3.3	T
65	PI 628889	1.8	4.3	5.0	3.5	T
66	PI 628903	1.8	3.5	5.3	3.3	T
67	PI628904	1.8	3.8	5.5	3.5	T
68	PI628905	2.3	4.3	5.8	3.8	T
69	PI628907	1.5	3.8	6.0	3.8	T
70	PI628908	2.0	2.8	4.3	3.0	T
71	PI628915	2.3	4.0	6.0	3.8	T
72	PI628918	2.0	3.5	4.8	3.3	T
73	PI628919	2.8	4.5	6.3	5.0	T
74	PI628925	2.0	3.3	5.5	3.8	T
75	PI628929	1.8	3.3	5.0	3.3	T
76	PI628932	1.3	2.3	3.8	2.5	T
77	PI628933	2.0	4.0	6.0	5.0	T
78	PI628934	2.3	4.0	5.3	3.5	T
79	PI628935	1.0	1.8	3.0	2.0	RB
80	PI628948	1.3	3.3	4.5	3.3	T
81	PI628959	1.8	4.3	5.5	3.5	T
82	PI628960	2.3	3.5	5.5	3.8	T
83	PI635999	1.3	2.3	3.8	2.5	T
84	PI644103	1.5	3.5	4.3	2.8	T
85	Roan	2.0	3.3	5.5	3.5	T
86	Safari	1.5	3.5	5.0	3.3	T
87	Santa	1.8	3.5	5.0	3.3	T
88	SCSI	2.0	3.3	5.0	3.5	T
89	Serenade	2.0	3.8	5.8	3.5	T
90	Siesta	1.8	3.8	5.5	3.5	T
91	Solitaire	2.0	3.3	5.3	3.5	T
92	Soprano	1.8	3.8	5.3	3.5	T
	Mean	1.6	3.0	4.4	2.9	
	LSD	1.6	2.2	1.8	1.7	
	CV %	16.6	15.9	10.9	12.4	
	F probability[a]	<0.001	<0.001	<0.001	<0.001	

[a]ANOVA conducted on angular transformed values; RR2, RR4 and RR6 are Rust severity at growth stages R2, R4 and R6, respectively; RS = Rust Sporulation; RRT = Rust Reaction Type; I = Immune; RB = Red Brown and T = Tan

genetic similarity (GS = 98%) suggesting that they were genetically similar. However, genotypes PI200526 and PI628889 were the most genetically dissimilar (GS = 44%).

Reaction of exotic soybean genotypes to soybean rust disease: The mean reaction of 89 exotic soybean and three local soybean genotypes to soybean rust at MUARIK for two consecutive seasons of 2011B and 2012A are presented in Table 3. There was progressive increase in rust severity among genotypes as they matured. The differences in mean rust severity among genotypes at full bloom, full pod and full seed were highly significant ($p<0.001$). The full seed stage was however the most suitable stage for soybean rust reaction and assessment. Evaluations done at full seed had the greatest soybean rust severity in most of the genotypes.

Three soybean genotypes (PI416826A, PI417126 and PI615437) had the lowest rust sporulation score (1) while six genotypes Dinamri, PI 605823, PI 628855, PI 628861, PI 628919 and PI 628933 had the highest rust sporulation score (5). Mean rust sporulation for the 92 soybean genotypes was 2.9. The differences in mean rust sporulation among the genotypes were highly significant ($p<0.001$). A total of 66 soybean genotypes had TAN

reaction thus were highly susceptible to soybean rust, while 22 genotypes had RB. Three soybean genotypes (PI615437, PI416826A and PI417126) had immune reaction and thus were classified as resistant to soybean rust. There was no clear cut relationship between the final severity score at full bloom and reaction type. However, most sporulation scores greater than or equal to 2.5 had TAN reaction phenotypes. All the release varieties had a TAN reaction whereas genotypes PI416826A, PI417126 and PI615437 had an immune reaction to soybean rust.

DISCUSSION

The major objective of SSR genotyping was to infer the genetic diversity available within the assembled exotic germplasm. The study demonstrated that there was high genetic diversity in the exotic germplasm as shown by selected SSR markers in soybean despite considerable inbreeding (Fu *et al*., 2007). Dealing with similar work Chotiyarnwong *et al*. (2007) reported an average of 11.8 alleles per locus and genetic diversity of 0.83 among 149 Thai indigenous and 11 recommended soybean varieties using 18 SSR markers. Using 129 Chinese soybean genotypes and 60 SSR markers, Wang *et al*. (2006) reported an average of 12.2 alleles per locus and mean PIC value of 0.78. Diwan and Cregan (1997) reported an average of 10.1 alleles per locus and genetic diversity of 0.80 among 35 North American soybean genotypes using 20 SSR markers. The 5.3 alleles per locus observed in this study is much lower than that obtained in the previous studies. These differences could be attributed to the number and location of loci in the soybean genome sampled in the study. However, the genetic diversity (PIC) of 0.77 obtained is comparable to the one reported by Wang *et al*. (2006) using 60 SSR markers.

The local check varieties Maksoy 1N, Maksoy 2N and Maksoy 3N were grouped in the same cluster, whilst exotic soybean genotypes from the USDA Germplasm Collection and Zimbabwe were scattered in all clusters of the dendrogram implying that they were genetically different. The high genetic diversity observed among the evaluated genotypes justifies the inclusion of some of them into the national soybean breeding programme for purposes of broadening the germplasm base for rust resistance in Uganda. The test materials included two genotypes (PI462312 and PI459025B) with known specific resistance genes *Rpp3* and *Rpp4*, respectively which had low disease resistance indices. These findings corroborate findings by Maphosa *et al*. (2012) who observed greater resistance to soybean rust disease in these classic genotypes. This therefore implies that these specific resistance genes could be used in improving resistance in released genotypes with desirable agronomic traits but susceptible to soybean rust disease.

Soybean genotypes PI417089A and PI59172A previously found with RB resistance reaction to rust in the United States were also found to be resistant in this study which suggests that there are effective against a broad soybean pathogen range. Reported a TAN reaction type in soybean genotypes PI203398, PI567024, PI567046A, PI567104B, PI567123A and PI594538A which was similarly observed in this study. Genotypes with an immune response and RB reactions are potential sources of resistance since in previous studies, host-pathogen interaction that resulted in RB reactions tended to have longer latent periods, lower rates of increase in pustules over time and smaller lesions (Walker *et al*., 2011).

CONCLUSION

The low genetic diversity among local soybean varieties justifies the need for introducing exotic genotypes to expand the soybean germplasm base. Therefore, inclusion of soybean rust resistant, diverse germplasm in soybean breeding program may provide the genetic variability and new alleles for soybean improvement. Furthermore, genotypes PI628935, PI615437, PI416826A and PI417126 are useful sources of soybean rust resistance genes that can be incorporated into high-yielding and adapted cultivars. The genes responsible for the resistance in these genotypes need to characterised. More studies should be conducted on the evaluated genotypes for resistance to soybean rust across the major soybean producing areas given that *Phakopsora pachyrhizi* populations exhibit high pathogenic diversity.

ACKNOWLEDGEMENTS

Researchers would like to thank RUFORUM and AGRA for funding this research and the Makerere University soybean breeding programme for the germplasm and technical assistance.

REFERENCES

Chotiyarnwong, O. P. Chatwachirawong, S. Chanprame and P. Srinives, 2007. Evaluation of genetic diversity in Thai indigenous and recommended soybean varieties by SSR markers. Thai J. Agric. Sci., 40: 119-126.

Chung, G. and R.J. Singh, 2008. Broadening the genetic base of soybean: A multidisciplinary approach. Crit. Rev. Plant Sci., 27: 295-341.

Diwan, N. and P.B. Cregan, 1997. Automated sizing of fluorescent-labeled Simple Sequence Repeat (SSR) markers to assay genetic variation in soybean. Theor. Appl. Genet., 95: 723-733.

Doyle, J.J. and J.L. Doyle, 1990. Isolation of DNA from fresh tissue. Focus, 12: 13-15.

Fu, Y.B., G.W. Peterson and M.J. Morrison, 2007. Genetic diversity of Canadian soybean cultivars and exotic germplasm revealed by simple sequence repeat markers. Crop Sci., 47: 1947-1954.

Guzman, P.S., B.W. Diers, D.J. Neece, S.K. Martin and A.R. LeRoy *et al*., 2007. QTL associated with yield in three backcross-derived populations of soybean. Crop Sci., 47: 111-122.

Hartman, G.L., E.D. West and T.K. Herman, 2011. Crops that feed the World 2, soybean-worldwide production, use and constraints caused by pathogens and pests. Food Secur., 3: 5-17.

Kawuki, R.S., E. Adipala, J. Lamo and P. Tukamuhabwa, 2004. Responding to the soybean rust epidemic in sub-Saharan Africa: A review. Afr. Crop Sci. J., 11: 301-318.

Kuroda, Y., N. Tomooka, A. Kaga, S.M.S.W. Wanigadeva and D.A. Vaughan, 2009. Genetic diversity of wild soybean (Glycine soja Sieb. et Zucc.) and Japanese cultivated soybeans (G. max (L.) Merr.) based on microsatellite (SSR) analysis and the selection of a core collection. Genet. Resour. Crop Evol., 56: 1045-1055.

Li, X.H., K.J. Wang and J.Z. Jia, 2009. Genetic diversity and differentiation of Chinese wild soybean germplasm (G. soja Sieb. and Zucc.) in geographical scale revealed by SSR markers. Plant Breed., 128: 658-664.

Maphosa, M., H. Talwana and P. Tukamuhabwa, 2012. Enhancing soybean rust resistance through *Rpp2*, *Rpp3* and *Rpp4* pair wise gene pyramiding. Afr. J. Agric. Res., 7: 4271-4277.

Priolli, R.H.G., C.T. Mendes-Junior, N.E. Arantes and E.P.B. Contel, 2002. Characterization of Brazilian soybean cultivars using microsatellite markers. Genet. Mol. Biol., 25: 185-193.

Rohlf, F., 1998. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Version 2.02, Exeter Software, Setauket, New York.

Song, Q.J., L.F. Marek, R.C. Shoemaker, K.G. Lark and J.E. Specht *et al*., 2004. A new integrated genetic linkage map of the soybean. Theor. Appl. Genet., 109: 122-128.

Walker, D.R., H.R. Boerma, D.V. Phillips, R.W. Schneider and J.B. Buckley *et al*., 2011. Evaluation of USDA soybean germplasm accessions for resistance to soybean rust in the southern United States. Crop Sci., 51: 678-693.

Wang, L., Y. Guan, R. Guan, Y. Li and Y. Ma *et al*., 2006. Establishment of Chinese soybean Glycine max core collections with agronomic traits and SSR markers. Euphytica, 151: 215-223.

Wang, L.X., R.X. Guan, Y.H. Li, F.Y. Lin and W.J. Luan *et al*., 2008. Genetic diversity of Chinese spring soybean germplasm revealed by SSR markers. Plant Breed., 127: 56-61.

Yorinori, J.T., W.M. Paiva, R.D. Frederick, L.M. Costamilan and P.F. Bertagnolli *et al*., 2005. Epidemics of soybean rust (*phakopsora pachyrhizi*) in brazil and paraguay from 2001 to 2003. Plant Dis., 89: 675-677.