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SCREENING SOYBEAN GENOTYPES FOR RESISTANCE TO RUST DISEASE

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ARTICLE INFO **ABSTRACT** **ORIGINAL RESEARCH ARTICLE**

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Soybean rust, caused by *Phakopsora pachyrhizi* (H. Sydow and Sydow), is one of the most important foliar diseases affecting soybean worldwide. Yield losses due to rust under excessive infestation could be up to 75 % in unprotected fields. The tread of soybean research in Africa is towards developing soybean varieties resistant to rust disease. Molecular screening for rust resistance gene in soybean genotypes was conducted at Council for Scientific and Industrial Research (CSIR) – Crops Research Institute (CRI) Molecular Biology Laboratory, Fumesua in Kumasi. Field screening of soybean genotypes for resistance was conducted at a "hot spot" at Tampola, Navrongo in the Kassena Nankana District of the Upper East Region of Ghana. The study was conducted to determine the presence of rust resistance gene(s) in 34 soybean genotypes and to evaluate the genotypes resistant to *P. pachyrhizi*. Simple Sequence Repeat (SSR) markers revealed genotypes SIT-E TGx1990-3F, SIT-M TGx1987-91F and SIT-M TGx1989-45F have multiple resistance genes (R*pp*1, R*pp*2 and R*pp*3), however, genotype TGx1909-3F was identified not to have resistance gene. Out of the 34 soybean genotypes, SIT-M TGx1989-45F, SIT-M TGx1987-40F, SIT-E TGx1990-3F and SIT-M TGx1987-91F were found to be highly resistant to rust disease during a phenotypic screening at the disease hot spot. Genotypes observed to have resistance gene(s) (R*pp*1, R*pp*2, R*pp*3, R*pp*4, and R*pp*5) to soybean rust could further be exploited and used in the breeding programme.

Introduction

Soybean (*Glycine max (*L.) Merr.) is an important legume crop, with potential for expansion in Africa due to its nutritional benefits and its ability to improve soil fertility by nitrogen fixation. Most traditional foods in Ghana such as gari, banku, kenkey, stew, and sauces are fortified with soybean to increase their nutritional levels. Unfortunately, rust disease reduces the quality and yield of soybean. According to Hartman *et al*. (2005), soybean rust disease (SBR) caused by *Phakopsora pachyrhizi* (H. Sydow and Sydow) is one of the most important foliar diseases affecting soybean worldwide. SBR has been reported throughout the tropics of Asia for many decades (Hartman *et. al*., 1999), Africa

(Levy, 2005) and Ghana (Bandyopadhyay *et al*., 2007). SBR epidemic is caused by the following environmental factors: moisture, temperature, wind, and light. The disease effects yield and its components such as pods per plants, seeds per pods and mean seed weight which is also dependent on the variety/genotypes. Yorinori *et al*. (2005) reported that under excessive infestation; losses up to 75 % can be noticed in unprotected fields. However, the rate of yield losses may vary depending on the existing conditions and such conditions include the genotype, environment and the time during the season when the rust becomes established.

Such a threat influences the net profit of the producers as well as jeopardizing the livelihood and nutritional well-being of millions of people who rely on its oil and protein (Asafo-Adjei *et al*., 2005). An effort to reduce the SBR with fungicides application has led to; high cost of production, environmental pollution and development of *P. pachyrhizi* races tolerant to the fungicides and even with a fungicide application, there may still be yield losses (Calvo *et al*., 2008). Hence, genetic resistance is an economically, environmentally and strategically important means of controlling soybean rust disease.

Five major sources of resistance to SBR have been identified in soybean germplasm making it possible for employing the use of molecular markers for targeting genes of interest. The resistance genes identified in soybean to *P. pachyrhizi* (R*pp*) are R*pp*1 (Mclean and Byth, 1980); R*pp*2 and R*pp*3 (Bromified and Hartwig 1980); R*pp*4 (Hartwig, 1986) and R*pp*5 (Garcia *et al*., 2008). In Ghana, the only research done on soybean rust was to ascertain the presence of the pathogen, *P. pachyrhizi*, in October 2006 by Bandyopadhyay *et al*. (2007) and reported that disease incidence ranges from 50 to 100 % and disease severity ranges from 3 to 40 % of the leaf area on infected plants. Hence, there is the need to screen for resistant soybean genotypes for seed multiplication or breeding against rust. This will make it possible for plant breeders to make progress in developing cultivars resistant to SBR.

Materials and Methods

Experiment 1: Screening For Rust Resistance Gene in Soybean Genotypes Using SSR Molecular Markers

Study Site

This study was conducted at Council for Scientific and Industrial Research (CSIR) – Crops Research Institute (CRI) Molecular Biology Laboratory, Fumesua in Kumasi.

Study Materials

Plant materials used for the study are presented in Table 1.

Table 1: Soybean genotypes/varieties and their sources used for the study

Genotypes/Varieties	Source/Institution*	Country
TGx1909-3F	IITA	Nigeria
SIT-M TGx1990-67F	IITA	Nigeria
SIT-E TGx1987-11F	IITA	Nigeria
SIT-E TGx1988-3F	IITA	Nigeria
TGx1903-7F	IITA	Nigeria
SIT-E TGx1987-86F	IITA	Nigeria
SIT-M TGx1990-45F	IITA	Nigeria
NANGBAAR	CSIR-CRI	Ghana
SIT-E TGx1990-3F	IITA	Nigeria
SIT-E TGx1990-15F	IITA	Nigeria

SIT-E TGx1987-10F	IITA	Nigeria
SIT-E TGx1989-19F	IITA	Nigeria
SIT-M TGX1904-6F	IITA	Nigeria
SIT-E TGx1989-4F	IITA	Nigeria
SIT-M TGx1989-46F	IITA	Nigeria
SIT-E TGx1988-5F	IITA	Nigeria
ANIDASO	CSIR-CRI	Ghana
SIT-M TGx1987-91F	IITA	Nigeria
SIT-M TGx1989-42F	IITA	Nigeria
SIT-M TGx1987-14F	IITA	Nigeria
SIT-E TGx1740-2F	IITA	Nigeria
SIT-E TGx1989-21F	IITA	Nigeria
SIT-E TGx1987-62F	IITA	Nigeria
SIT-E TGx1990-97F	IITA	Nigeria
SIT-M TGx1989-45F	IITA	Nigeria
SIT-E TGx1989-20F	IITA	Nigeria
SIT-E TGx1990-2F	IITA	Nigeria
SIT-M TGx1448-2E	IITA	Nigeria
SIT-E TGX1835-10E	IITA	Nigeria
SIT-M TGx1987-96F	IITA	Nigeria
SIT-M TGx1987-40F	IITA	Nigeria
SIT- E TGx1990-8F	IITA	Nigeria
SIT-E TGx1990-5F	IITA	Nigeria
SIT-M TGx1440-1E	IITA	Nigeria

*****IITA: International Institute of Tropical Agriculture
CSIR-CRI: Council for Scientific and Industrial Research - Crop Research Institute

DNA Isolation

Genomic DNA was isolated from young leaves with DNeasy Plant Mini Kit according to the manufacturer's protocol (Qiagen Sciences), Canada.

DNA Quantity and Quality Estimation

DNA quality was checked on 0.8 % agarose gel in 1X TAE buffer by electrophoresis at 120 volts for 45 mins and stained with ethidium bromide visualized under ultraviolet transilluminator connected to a computer. Serial dilutions were carried out to get the desired quantity (concentration) of DNA for polymerase chain reaction (PCR).

SSR Primers

Five different SBR resistance genes identified and mapped by Song *et al*. (2004) was used to select simple sequence repeat (SSR) molecular markers for the genomic location of the known resistance to *Phakopsora pachyrhizi* (R*pp*) genes. SSR primers (Table 2) were obtained from Soybase (http://www.soybase.org/resources/ssr.php). Nine markers associated with R*pp* genes were used for the molecular analysis to select for resistance genotypes.

Table 2: SSR markers and their primer sequences in relation to five soybean resistance loci on a soybean linkage map

SSR Markers	**Primer sequence**	**Linkage Group**	**Position (cM)**	**Resistance gene**	**References**
Sat_064	Fw: TAG CTT TAT AAT GAG TGT GAT AGA T	G	108.69	R*pp*1	Cregan *et al*. (1999)
	Rv: GTA TGC AAG GGA TTA ATT AAG				
Sat_165	Fw: GCG GAC AGG CAG CCA CAC ATC TTA	J	42.2	R*pp*1	Song *et al*. (2004)
	Rv: GCG GAT TAA ATC AGT TTG TAT CGA				
Satt620	Fw: GCG GGA CCG ATT AAA TCA ATG AAG TCA	J	53.71	R*pp*2	Silva *et al*. (2008)
	Rv: GCG CAT TTA ATA AGG TTT ACA AAT TAG T				
Satt708	Fw: GCG CAA TTT TAA GAG ATT TTC GGG ATA A	C2	115.48	R*pp*2	Song *et al*. (2004)
	Rv: GCG ACT CGG TTG ATT TTT TTT TCA ATT TTT T				
Staga001	Fw: GCG GAG GGG AGT TTG CAG ATT A	C2	119.84	R*pp*3	Song *et al*. (2004)
	Rv: GCG GCA AGG GCA ACT GAA AAA T				
Sat_307	Fw: GCG AAT TGG ACT AAA AGA ATA AGC ATC A	O	123.43	R*pp*3	Song *et al*. (2004)
	Rv: GCG TGT TTG GTA TAG AAA TGA GAA ATA AAA T				
AF162283	Fw: GCG AGT TCT GGA TGT AGG	G	87.94	R*pp*4	Yamanaka *et al*. (2008)
	Rv: GCG AGT TCT GGA TGT AGG				
Sat_166	Fw: GCG CTA ATT TAT CGG GAC CCA ACA TAT	N	38.59	R*pp*4	Song *et al*. (2004)
	Rv: GCG GAA ATA GTG CAT TGA TGA AAA ACA				
Sat_280	Fw: GGC GGT GGA TAT GAA ACT TCA ATA ACT ACA A	N	43.45	R*pp*5	Song *et al*. (2004)
	Rv: GGC GGG CTT CAA ATA ATT ACT ATA AAA CTA CGG				

Polymerase Chain Reaction

Polymerase chain reactions (PCR) were carried out in 10 µl volumes for nine markers. The components of the reaction mixture were PCR water 5.78 µl, 10x buffer 1 µl, $MgCl_2$ (25 mM) 0.9 µl, DNTPs (20 mM) 0.2 µl, forward and reverse primer 0.5 µl each, Taq polymerase 0.12 µl and template DNA 1µl all in 1x PCR buffer. The amplification was carried out in a thermocycler machine (Gene Amp® PCR system 9700 version 3.09, Applied Biosystems, California, USA) with the following conditions: the cycling consisted of 5 mins at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 50 °C, 1 min at 72 °C; followed by 7 mins at 72 °C. Amplification products were left at 4 °C prior to electrophoresis. DNA loading dye (Fermentas) was added to the PCR amplification products and separated by electrophoresis in 2 % agarose gel.

DNA Electrophoresis

The PCR products were run on 2 % agarose gel (7.5 µl ethidium bromide, 200 ml, 1X TBE, 4.0 g agarose) at 120 volts for 45 mins in electrophoretic setup. The DNA was visualized using an ultraviolet transilluminator connected to a computer.

Scoring of Bands

The photographed gels were downloaded onto a computer and weighted bands were scored as presence (1) or absence (0) of the band using DNA ladder as the reference (1 kb Invitrogen and 100 base pair Fermentas).

Experiment 2: Field screening of soybean genotypes for rust resistance to *P. pachyrhizi*

Study Site

The field evaluation was conducted at Tampola, Navorongo in the Kassena Nankana District of the Upper East Region of Ghana located in the Sudan Savannah Agro-ecological Zone. The average annual rainfall, temperature, relative humidity, wind speed, sunshine hours and solar radiation of the area are 885 mm, 28.6 °C, 54 %, 81 km day^{-1}, 7.9 h and 20.4 M J $m^{-2}day^{-1}$, respectively (Ghana Meteorological Agency, 2013). Planting materials are presented in Table 1.

Land Preparation, Layout, Experimental Design, and Planting

The land was not plowed but manually slashed with a cutlass in order to maintain the stability of the pathogen community. It was also not burnt for the same reason. Stumping was done with mattocks and hoes. The debris was also manually collected. Linning and pegging were done at a planting distance of 75 cm between rows and 10 cm within rows. The experimental design used was randomized complete block design (RCBD) with three replications partitioned by two alleys of 1 m each. The two central rows were the test row from which data was taken. Each plot had four rows which were four meters long. Three seeds were planted per hill and thinned to 2 seeds per hill at 2 weeks after planting (WAP).

Fungus Source

The soybean genotypes were screened for rust resistance under natural epiphytotic condition**.** The study site is noted as a hot spot for rust disease. When a hot spot of a disease is known, and natural epidemics are so frequent no artificial inoculations are needed (Tiwari *et al*., 1997). Bromfield (1984) also reported that a single diseased leaf may be enough to initiate a disease epidemic in a field.

Evaluation of Soybean Genotypes For Rust Resistance

Rust severity was recorded using 0 - 9 disease rating scale (Table 3) by Mayee and Datar (1986). The scoring was done after flowering and before pod formation and their averages calculated. Evaluations were made during these reproductive stages of development because spore production and pustule development generally increase after plants begin to flower (Bromfield, 1984) and because variation in disease severity was typically high at these stages, while the most susceptible genotypes were not yet heavily defoliated. Based on disease

rating, soybean test entries were grouped into 6 categories.

Table 3: Disease grade/score

Disease grade/score	% Leaf area affected	Disease reaction
0	Nil	Immune
1	<1	Highly resistant
3	1 - 5	Resistant
5	6 - 25	Moderately resistant
7	26 - 50	Susceptible
9	> 51	Highly susceptible

Source: Mayee and Datar (1986)

Data Analysis

Data collected were subjected to Analysis of Variance (ANOVA) using Statistics statistical package (version 9.0) and means separations were done using Least Significant Difference (LSD) at 5 %.

Results

Experiment 1: Screening For Rust Resistance Gene (S) In Soybean Genotypes Using SSR Molecular Markers

Rust Resistance Alleles Identified By SSR Markers

Out of the nine molecular markers used, Satt620 and Sat_166 were monomorphic. The remaining seven of the markers (Sat_064, Sat_165, Satt708, Staga001, Sat_307, AF162283 and Sat_280) produced polymorphism with significant differences. The screening of soybean genotypes for resistance gene presence was based on these seven markers. Expected alleles showing resistance or susceptibility were scored as present (1) or absence (0) (Table 4).

The banding pattern of primer Staga001 that was linked to rust disease resistance at 251 bp is presented in Plates 4.1.

Table 4. Soybean genotypes and their resistance or susceptible alleles

Soybean Genotypes	**Sat_064**	**Sat_165**	**Satt708**	**Staga001**	**Sat_307**	**AF162283**	**Sat_280**	
	143 bp	**228/277 bp**	**240 bp**	**251 bp**	**212/162/215 bp**	**200 bp**	**224/297 bp**	**Response**
TGx1909-3F	0	0	0	0	0	0	0	S
SIT-M TGx1990-67F	0	0	0	1	0	0	0	R
SIT-M TGx1987-11F	0	0	0	1	0	0	0	R
SIT-E TGx1988-3F	1	0	0	1	0	0	0	R
TGx1903-7F	0	0	0	0	1	0	0	R
SIT-E TGx1987-86F	1	0	0	0	0	0	0	R
SIT-E TGx1990-45F	0	0	0	1	0	0	0	R
NANGBAAR	0	0	0	0	1	0	0	R
SIT-E TGx1990-3F	1	0	1	1	1	0	0	R
SIT-E TGx1990-15F	0	0	0	1	1	0	0	R
SIT-E TGx1987-10F	0	0	0	0	1	0	0	R
SIT-E TGx1989-19F	0	0	1	1	1	0	0	R

Table 4. Soybean genotypes and their resistance or susceptible alleles Cont'd

	Sat_064	**Sat_165**	**Satt708**	**Staga001**	**Sat_307**	**AF162283**	**Sat_280**	
Genotypes	**143 bp**	**228/277 bp**	**240 bp**	**251 bp**	**212/162/215 bp**	**200 bp**	**224/297 bp**	**Response**
SIT-M TGx1904-6F	0	0	1	0	0	0	0	R
SIT-E TGx1989-4F	1	0	1	1	0	0	0	R
SIT-E TGx1989-46F	1	0	0	0	1	0	0	R
SIT-E TGx1988-5F	1	0	1	1	1	0	0	R
ANIDASO	0	0	1	0	1	0	0	R

SIT-M TGx1987-91F	1	0	1	1	1	0	0	R
SIT-M TGx1989-42F	0	1	0	1	0	0	1	R
SIT-M TGx1987-14F	0	0	0	0	0	1	0	R
SIT-E TGx1740-2F	0	0	0	1	0	0	0	R
SIT-E TGx1898-21F	0	0	0	1	0	0	0	R
SIT-E TGx1987-62F	0	1	1	1	0	0	0	R
SIT-E TGx1990-97F	0	0	0	1	0	0	0	R

Table 4. Soybean genotypes and their resistance or susceptible alleles Cont'd

	Sat_064	**Sat_165**	**Satt708**	**Staga001**	**Sat_307**	**AF162283**	**Sat_280**	
Genotypes	**143 bp**	**228/277 bp**	**240 bp**	**251 bp**	**212/162/215 bp**	**200 bp**	**224/297 bp**	**Response**
SIT-M TGx1989-45F	1	0	1	1	1	0	0	R
SIT-E TGx1989-20F	1	0	0	1	0	0	1	R
SIT-E TGx1990-2F	0	0	0	1	0	0	1	R
SIT-M TGx1990-2E	0	0	0	1	0	0	0	R
SIT-E TGx1835-10E	0	1	0	1	0	0	1	R
SIT-M TGx1987-96F	0	1	0	1	1	0	0	R
SIT-M TGx1987-40F	0	1	0	1	0	0	1	R
SIT-E TGx1990-8F	0	0	0	1	1	0	1	R
SIT-E TGx1990-5F	0	0	0	1	1	0	0	R
SIT-M TGx1440-1E	0	0	0	1	1	0	0	R

Allele associated with rust resistant or susceptible gene, 1 = indicates presence of the allele and 0 = indicates absence of the allele
R = Resistant and S = Susceptible

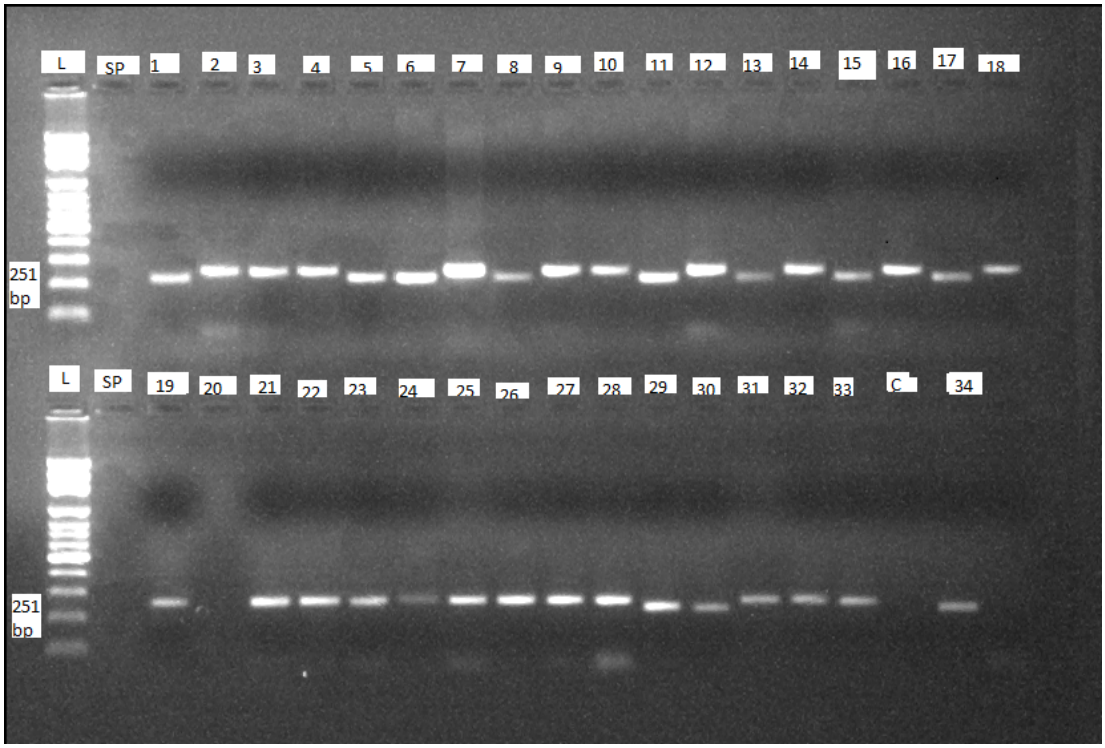

Figure 1: Plate 1. Marker Staga001 detected resistant genotypes at 251 bp
L-100bp DNA ladder, SP-Space, 1- TGx1909-3F, 2- SIT-M TGx1990-67F, 3-SIT-E TGx1987-11F, 4-SIT-E TGx1988-3F, 5- TGx1903-7F, 6- SIT-E TGx1987-86F, 7- SIT-M TGx1990-45F, 8- NANGBAAR, 9- SIT-E TGx1990-3F, 10- SIT-E TGx1990-15F, 11- SIT-E TGx1987-10F, 12- SIT-E TGx1989-19F, 13- SIT-M TGX1904-6F, 14- SIT-E TGx1989-4F, 15- SIT-M TGx1989-46F, 16- SIT-E TGx1988-5F, 17- ANIDASO, 18- SIT-M TGx1987-91F, SP-Space, 19- SIT-M TGx1989-42F, 20- SIT-M TGx1987-14F, 21- SIT-E TGx1740-2F, 22- SIT-E TGx1989-21F, 23- SIT-E TGx1987-62F, 24- SIT-E TGx1990-97F, 25- SIT-M TGx1989-45F, 26- SIT-E TGx1989-20F, 27- SIT-E TGx1990-2F, 28- SIT-M TGx1448-2E, 29- SIT-E TGX1835- 10E, 30- SIT-M TGx1987-96F, 31- SIT-M TGx1987-40F, 32- SIT- E TGx1990-8F, 33- SIT-E TGx1990-5F, C - Control and 34- SIT-M TGx1440-1E.

Experiment 2: Field screening of soybean genotypes for rust resistance to *P. Pachyrhizi*

Results on genotypes to rust severity are shown in Table 5. The Table shows that significant differences ($p < 0.05$) existed among the genotypes in their resistance to rust (*P. pachyrhizi*). Reactions of 34 genotypes to rust revealed that none of the genotypes showed an immune reaction to rust.

Table 5: Rust severity score

Genotypes	% Leaf area affected	Response*
SIT-E TGx1988-3F	4.0	R
TGx1903-7F	23.7	MR
NANGBAAR	23.3	MR
SIT-E TGx1990-3F	0.5	HR

SIT-E TGx1990-15F	20.3	MR
SIT-E TGx1987-10F	4.0	R
SIT-E TGx1989-19F	11.7	MR
SIT-M TGX1904-6F	9.7	MR
SIT-E TGx1989-4F	11.0	MR
SIT-M TGx1989-46F	22.7	MR
SIT-E TGx1988-5F	10.0	MR
ANIDASO	15.7	MR
SIT-M TGx1987-91F	0.7	HR
SIT-M TGx1989-42F	15.7	MR
SIT-M TGx1987-14F	18.0	MR
SIT-E TGx1989-21F	21.3	MR
SIT-E TGx1987-62F	8.3	MR
SIT-M TGx1989-45F	0.4	HR
SIT-E TGx1990-2F	21.3	MR
SIT-E TGx1835-10E (check)	1.3	R
SIT-M TGx1987-40F	0.6	HR
SIT-E TGx1990-8F	18.7	MR
SIT-E TGx1990-5F	20.3	MR
SIT-M TGx1440-1E	3.0	R
TGx1909-3F	69.0	HS
SIT-M TGx1990-67F	68.3	HS
SIT-E TGx1987-11F	50.7	S
SIT-E TGx1987-86F	46.7	HS
SIT-M TGx1990-45F	63.3	HS
SIT-E TGx1740-2F	61.0	HS
SIT-M TGx1990-97F	54.3	HS
SIT-E TGx1989-20F	41.7	S
SIT-E TGx1448-2E	42.7	S
SIT-E TGx1987-96F	27.3	S
Mean	23.9	
LSD ($P < 0.05$)	3.4	
CV (%)	8.9	

*HR = highly resistant, R = Resistant, MR = moderately resistant, S = Susceptible, HS = highly susceptible

Discussion

Screening For Rust Resistance Gene(S) in Soybean Genotypes Using SSR Molecular Markers

The SSR markers used to characterise 34 soybean genotypes showed that molecular diversity existed among the genotypes used for the study. The findings confirmed that most of the genotypes were of different genetic background. Most of the soybean genotypes identified by the markers to have presence of the rust resistance gene(s) were also found to be either highly resistant, resistant or moderately resistant under natural epiphytotic condition. For instance, genotype SIT-E TGx1990-3F and SIT-M TGx1989-45F were discovered by four different SSR markers to have

resistance genes and were also confirmed highly resistant during field screening. This agrees with the assertion that genetic composition of soybean variety/genotype dictates its resistance to disease (Song *et al.*, 2004). Also, all the genotypes detected by SSR marker Satt708 as resistant were also found to have a level of resistance during field screening, making it the best marker identified in selection for resistance to SBR. The SSR markers indicated some potentially useful sources of resistance to SBR that may be valuable to soybean breeders. This corresponds to the findings of the study by Tran *et al*. (2012), who successfully applied molecular markers to detect the presence of resistance (R*pp*5) in HL203, an elite Vietnamese soybean variety to SBR. These results have indicated the significance of marker-assisted selection (MAS) in identifying a targeted gene. From the study, none of the SSR markers used was able to identify all genotypes to be resistant. This could be due to the polygenic nature of the genes controlling the rust resistance. It has been indicated that rust disease resistance is controlled by many recessive genes (Calvo *et al*., 2008). It could also be suggested that genotypes used to identify the markers associated with rust disease resistance are of different genetic background from those used in this study. Besides, the markers might have been identified using genotypes reacting to different strains of the pathogen (Agrios, 2005).

None of the soybean genotypes was identified to carry all the five dominant major resistance genes (R*pp*1, R*pp*2, R*pp*3, R*pp*4, and R*pp*5). This indicated that most of the lines identified as resistant were associated with single gene resistance. This is in conformity with Bonde *et al*. (2006) that, cultivars have single gene resistance. It is also supported by Hartman *et al.* (2005) that, none of the soybean cultivars in present commercial production is resistant to all *P. pachyrhizi* isolates. Long-term utilization of these race-specific genes can prompt the pathogen to mutate and overcome them. This makes the disease devastating and challenges Ghana soybean breeders to develop soybean cultivars that have the multiple resistance genes to provide resistance to different races of *P. pachyrhizi*. To develop suitable varieties, plant breeders should optimize the plant genotype by choosing the most promising resistance genes and combinations to ensure stability/durability of resistance. Marker-assisted backcrossing can be gainfully employed for adding new resistance genes into popular and elite soybean genotypes that have been grown by Ghanaian farmers over the years on account of their unique agronomical characters. Gene pyramiding has also been suggested to be effective to overcome resistance instability conferred by single gene resistance to SBR (Hartman *et al*., 2005).

Field screening of soybean genotypes for rust resistance to *P. pachyrhizi*

The field screening identified 24 soybean genotypes as highly resistant, resistant or moderately resistant to *P. pachyrhizi* and 10 genotypes as either susceptible or highly susceptible. These research results are in consonance with Kim *et al*. (2005) who reported that the soybean reactions to rust depends on the existing genotype, environmental conditions, and the inoculum level. Similar findings were recorded Verma *et al*. (2004) evaluated 242 germplasm lines/cultivars of soybean under natural epiphytotic conditions for resistance to rust and reported only one line (SJ-1) as highly resistant, three lines *viz*., JS-19, RPSP-728, PK-838 as resistant, 16 lines as moderately resistant and rest were either susceptible or highly susceptible. None of the soybean genotypes evaluated on the field showed immune reaction but during the molecular screening, some genotypes were

identified as immune. Also, some genotypes that were known to have resistance gene during molecular screening were found to be susceptible during field evaluation. This was probably due to virulent races of the pathogen and high inoculum build-up due to the yearly planting of soybean and/or alternate host plants at the study site. According to Sweets (2002), the severity of rust infection is influenced by the quantity of inoculum, interaction among hosts, pathogen strains, and existing environmental conditions.

Conclusion

Genotypes SIT-E TGx1990-3F, SIT-M TGx1987-91F and SIT-M TGx1989-45F were known to have resistance genes R*pp*1, R*pp*2 and R*pp*3 to SBR by four different SSR markers (Sat_064, Satt708, Staga001 and Sat_307) and also detected as highly resistant during field screening. All the genotypes (SIT-E TGx1990-3F, SIT-E TGx1989-19F, SIT-M TGx1904-6F, SIT-E TGx1989-4F, SIT-E TGx1988-5F, ANIDASO, SIT-M TGx1987-91F, SIT-E TGx1987-62F and SIT-E TGx1989-45F) detected by SSR marker Satt708 as resistant having R*pp*2 gene was also found to have level of resistance during field screening, making it the best marker identified in selection for resistance to SBR. It could be recommended that soybean genotypes identified to have multiple resistance genes (R*pp*1, R*pp*2, and R*pp*3) during molecular screening and also detected to be highly resistant during field screening should be further be exploited and used in breeding programme against rust disease.

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Appendix 1: Summary ANOVA for soybean rust severity score

Source of Variation	**Degree of freedom (df)**	**Sum of Squares**	**Mean of Square**	**F-value**
Replication	2	25.5	12.76	
Treatment	33	44665.8	1353.51	315.16
Error	66	283.4	4.29	
Total	101	44974.8		
Mean	23.87			
LSD 5(%)	3.38			
CV (%)	8.68			