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Segregation of Resistance to Rice blast disease in the Ugandan Local Commercial Variety Namche2 with SSR Markers

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ABSTRACT

Rice blast caused by *Magnaporthe oryzae* is one of the rice diseases with high economic importance. Several resistant genes (R-genes) have been discovered and they occur on all chromosomes in the rice genome. In addition, molecular markers have been discovered to tag specific R-genes. This is important in shortening the breeding cycle for resistance to this disease as well as discovering resistance in varieties whose resistance genes have not been well understood. In the current study, A population of 100 individuals from a cross between NamChe2, a resistant parent, and Supa Soroti, a susceptible parent, was used. This population was evaluated using a rice blast isolate from Namulonge in central Uganda and scores were taken using the Standard Evaluation System (SES) of 0-9, where 0 indicates no symptoms and 9 indicates 75% of the leaf area with lesions. The individuals were then graded with those that scored 0-3 considered resistant (R) while those with scores 4-9 were considered susceptible. Eleven SSR markers were tested for polymorphism between the two parents. Nine markers were polymorphic. The nine markers were run on the population and the chi-square goodness of fit test indicated that all nine markers segregated in a normal Mendelian fashion with a 1:2:1 ratio. Both the regression and chi-square test of independence were significant for SSR marker RM21 (R^2= 0.127, P<0.001 and χ^2 = 8.30, P<0.05). This shows that 12.7% of the resistance observed in the population can be explained by the marker scores. The marker also showed an accuracy of 68% with a false negative rate of 55% Therefore, marker RM21 could be utilized by breeding programs for marker-assisted selection and introgression of resistance genes from NamChe2 at later generations in combination with thorough phenotyping to improve resistance durability among these varieties.

Keywords: *Magnaporthe oryzae*, R-genes, rice blast fungus, SSR markers

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RÉSUMÉ

La pyriculariose du riz, causée par *Magnaporthe oryzae*, est l'une des maladies du riz les plus importantes sur le plan économique. Plusieurs gènes de résistance (gènes R) ont été découverts et sont présents sur tous les chromosomes du génome du riz. De plus, des marqueurs moléculaires ont été identifiés pour marquer des gènes R spécifiques. Cela est crucial pour réduire le cycle de sélection pour la résistance à cette maladie ainsi que pour identifier la résistance dans des variétés dont les gènes de résistance ne sont pas bien compris. Dans cette étude, une population de 100 individus issue d'un croisement entre NamChe2, un parent résistant, et Supa Soroti, un parent sensible, a été utilisée. Cette population a été évaluée avec un isolat de pyriculariose provenant de Namulonge, dans le centre de l'Ouganda, et les scores ont été enregistrés selon le système d'évaluation standard (SES) de 0 à 9, où 0 indique l'absence de symptômes et 9 correspond à 75 % de la surface foliaire présentant des lésions. Les individus ont ensuite été classés : ceux ayant obtenu des scores de 0 à 3 étaient considérés comme résistants (R), tandis que ceux ayant des scores de 4 à 9 étaient considérés comme sensibles. Onze marqueurs SSR ont été testés pour leur polymorphisme entre les deux parents, dont neuf se sont révélés polymorphes. Ces neuf marqueurs ont été appliqués à la population, et le test du chi-carré d'ajustement a indiqué que tous les neuf marqueurs présentaient une ségrégation conforme à la loi mendélienne normale avec un ratio 1:2:1. À la fois les tests de régression et de chi-carré d'indépendance étaient significatifs pour le marqueur SSR RM21 (R^2 = 0,127, P < 0,001 et χ^2 = 8,30, P < 0,05). Cela indique que 12,7 % de la résistance observée dans la population peuvent être expliqués par les scores des marqueurs. Le marqueur a également montré une précision de 68 % avec un taux de faux négatifs de 55 %. Par conséquent, le marqueur RM21 pourrait être utilisé par les programmes de sélection pour la sélection assistée par marqueur et l'introgression du gène de résistance provenant de NamChe2 dans des générations ultérieures, en combinaison avec un phénotypage approfondi, afin d'améliorer la durabilité de la résistance parmi ces variétés.

Mots-clés : *Magnaporthe oryzae*, gènes R, champignon de la pyriculariose, marqueurs SSR

INTRODUCTION

Rice blast is one the most devastating rice diseases affecting rice production in Uganda. The disease causes yield losses of up to 100%, especially under favorable conditions (Onaga and Asea, 2016). Host plant resistance has a competitive advantage over other control methods because it is cost-effective and environmentally friendly. However, many studies have indicated that resistance to rice blast among the already developed resistant varieties breaks down within 2-3 growing seasons, which is caused by the high evolutionary rate of the fungus leading to the formation of new virulent strains of the fungus.

According to Tanksley and Mccouch (1997) and Imam *et al.*, (2014) landraces, local commercial varieties and wild rice relatives are a source of R-genes, and these have been characterized using differential races of *Magnaporthe oryzae*. These sources of resistance have been known for being able to co-evolve with the fungus population, thereby reducing the selection pressure on the pathogen population. This, in turn, reduces the chances of the pathogen evolving into new virulent forms that break down the existing resistance. To improve the selection efficiency for resistance, marker-assisted selection is the best breeding method to employ by the breeding programmes. Over 100 major blast resistance genes have been discovered in the rice genome (Sharma *et al.*, 2012; A. Singh *et al.*, 2012). Also, several DNA-based markers associated with these R genes were found and effectively deployed in MAS to select genotypes resistant to *M. oryzae* (Roychowdhury, 2011). In addition, these DNA-based markers have been utilized to identify the presence of R genes in elite genotypes. This is important to efficiently deploy these R genes in elite susceptible genotypes (Singh *et al.*, 2012). The current study aimed to determine the segregation

patterns of existing SSR markers in the population created from two local varieties. In addition, the study aimed to test the association of the resistance gene in the local resistant variety NamChe2 and the already published SSR markers to obtain SSR markers that can always be used in the rice breeding programme for MAS for rice blast resistance. This, in turn, is useful in shortening the breeding cycle and improving efficiency in the selection for rice blast resistance, especially when using local varieties as sources of resistance. This particular experiment was carried out to determine the association between selected SSR markers and resistance in the population of 100 randomly selected rice plants from a cross of Supa Soroti (susceptible parent) x NamChe2 (Resistant parent). Phenotypically the plants were tested with the Namulonge isolate due to its high virulence compared to all the other isolates in rice growing areas (Onaga *et al*., 2020). This will give baseline information for MAS even when using other isolates.

MATERIALS AND METHODS

This experiment was carried out at the biotechnology laboratory located at Makerere University Agricultural Research Institute Kabanyolo (MUARIK).

Plant materials. Seeds of the 100 F2 individuals from a population derived from a cross between Supa Soroti (Susceptible parent) and NamChe2 (Resistant parent) were pre-germinated by soaking in water for 24 hours. These were later incubated at a temperature of 32^{o}C for 24 hours to break dormancy. These were seeded in buckets filled with wet forest soil. These germinated in 7 days after sowing and within 21 days after sowing they formed seedlings from which leaf samples were taken for DNA extraction before inoculation.

DNA extraction. Young leaves from the two parents and 100 F2 plants were sampled at two weeks old. The Total DNA was extracted using the CTAB (Cetyl Tri Methyl Ammonium Bromide) method (Thompson and Murray, 1989). Fresh young rice leaf tips of about 2cm were cut, placed in paper bags, labeled and then put on ice. These were then placed in a mortar where 700 µl of extraction buffer (CTAB), and were ground using a pestle. The mixture was poured into a sterile 1.5µl Eppendorf tube. The samples were warmed at 65^{o}C for 30 minutes with occasional gentle mixing. 700 µl of chloroform were added and inverted for 10 minutes and the mixture was separated by centrifuging at 13000 rpm for 10 minutes. 600 µl of the upper aqueous layer was removed off and placed in a new Eppendorf tube, 200µl of ammonium acetate was added, mixed gently to make 2.5 Molar of the solution and left at room temperature for 30 minutes. The samples were then centrifuged at 13000 rpm for 10 minutes. 1000µl of the upper layer was then transferred to the new tube and 0.6 Volume of isopropanol was added and mixed gently, after which the solution was centrifuged again at 13000rpm for 10 minutes. The supernatant was discarded and the white pellet observed at the bottom was washed with 500µl of 70% of ethanol twice, after which the tubes were centrifuged for 2-3 minutes. The ethanol was poured off and the pellet air dried. 30µl of sterile distilled water was added to dissolve the DNA molecule and stored at -20^{o}C.

DNA quantification and dilution. Quantification of DNA was done using a NanoDrop ND-1000 spectrophotometer (ND-1000; NanoDrop, Thermo Scientific, Wilmington, DE, USA). The quality of DNA was assessed by running the samples on a 1.2% agarose gel electrophoresis and viewed under UV light. The highly concentrated DNA was diluted in sterile distilled water to a working concentration of 50 ng/µl and stored at 4^{o}C for further PCR-based marker analysis.

PCR procedures. Eleven pairs of primers were selected from published markers (Singh *et al*., 2015; Manoj *et al*., 2017) (Table 1) These primers were selected from different chromosomes on which different R-genes are located and are anticipated to occur in the local resistant genotype. Polymerase chain reaction (PCR) amplification was first performed on each of the two parents (Supa Soroti and NamChe2) to test for polymorphism to distinguish the parents that is, to test if the markers can distinguish the different forms of the gene (alleles). These were later performed on a population of 100 individuals. The PCR master mix contained

0.2 mM of dNTPs, 2 mM of $MgCl_2$, 1XPCR buffer and 0.5 µl of each primer. The 10 µl reaction PCR volume was subjected to 35 amplification cycles in a thermocycler (ARKTIK Thermocycler). The amplification consists of one cycle of heat denaturation at 94°C for 2 minutes. This is followed by 35 cycles of denaturation at 94°C for 1 minute, 55°C of annealing for 1 minute and 72°C of elongation for 2 minutes. The PCR products were run on a 2% agarose gel and stained with GR green at 130V for one hour. Agarose gel was viewed under ultraviolet light using Gel Doc 1000 single wavelength mini trans-illuminator.

Inoculum and inoculation. The inoculum containing the Namulonge isolate was used in this study. This inoculum was stored at 4°C from the previous study as isolated by Nakiyaga *et al*., 2020. The isolate was rejuvenated and multiplied on potato dextrose broth where the fungus grows a layer of conidia on top which turns black after 2-3 weeks. The suspension was made by blending the isolate and diluting with distilled water at a concentration of $1.5x10^5$ conidia/ml using a Neubauer haemacytometer under a compound microscope (Akagi *et al*., 2015). Two drops of 0.05% tween 20 were added to the inoculum to facilitate the adhesion of the pathogen to the 21-day-old rice leaves (Nakiyaga *et al*., 2020). The inoculum was sprayed on the 21-day-old seedlings of all the 100 F2 plants. These were covered with a white polyethylene sheet for 24 hours to allow high humidity conditions for spore growth and attachment onto the leaves. The polyethylene sheet was removed the plants were constantly watered and data was collected 28 days after inoculation to attain the maximum disease score for severity using the standard evaluation system of (IRRI, 2013).

Table 1. List of randomly selected SSR markers that were used in the study

Major gene/QTL	Chromosome location	Linked marker	SSR	L.D (cM)	Expected Band size(bp)	Primer sequences	Reference
Pi7(t)	11	RM229			116	Forward: cactcacacgaacgactgac Reverse: cgcaggttcttgtgaaatgt	(Manoj *et al*., 2017)
Pi9	6	RM541		0.6	158	Forward: tataaccgacctcagtgccc Reverse: ccttactcccatgccatgag	(Cho *et al.*, 2001)
Pi54	11	RM206		0.6	147	Forward: cccatgcgtttaactattct Reverse: cgttccatcgatccgtatgg	(Singh *et al.*, 2015)
Pi-1	11	RM224		0.0	157	Forward: atcgatcgatcttcacgagg Reverse: tgctataaaaggcattcggg	(Fuentes, 2000)
Pi5-(t)	11	RM21		0.0	157	Forward: acagtattccg-taggcacgg Reverse: gctccatgagggtgg-tagag	(Singh *et al.*, 2015)
Piz-5	6	RM527		0.3	233	Forward: ggctcgatctagaaaatccg Reverse: ttgcacaggttgcgatagag	(Fjellstrom *et al*., 2006)
Pi-b	2	RM208		1.2	173	Forward: tctgcaagccttgtctgatg Reverse: taagtcgatcattgtgtg-gacc	(Hayashi *et al*., 2010)
Pi-ta	12	RM247		5.0	131	Forward: tag-tgccgatcgatgtaacg Reverse: catatggttttgacaaagcg	(Eizenga *et al* 2006)
Pi33	8	RM72		11.5	166	Forward: ccggcgataaaacaatgag Reverse: gcatcggtcctaactaaggg	(Singh *et al.*, 2015)
Pitp(t)	1	RM246		0.0	116	Forward: gagctccatcagccattcag Reverse: ctgagtgctgctgcgact	(Singh *et al*. 2015)
Pi27(t)	1	RM259		0.6	162	Forward: tggagtttgagaggaggg Reverse: cttgttgcatggtgccatgt	(Zhu *et al.*, 2004)

Statistical analysis. Phenotypic data was collected on the 100 F2 individuals from a cross between NamChe2 (resistant) x Supa Soroti (susceptible). Scores of 0-3 were graded as resistant (R) and scores of 4-9 were graded as susceptible (S), while accessions with genotypes RR and Rr were all considered resistant and those with rr were considered susceptible. Marker data was tested for segregation where individuals that scored 2 were considered heterozygotes, 1 were considered homozygotes for resistance and 0 were considered homozygotes recessive. The data were analyzed for segregation using the chi-square goodness of fit test.

The genotypic and phenotypic data was analyzed using Genstat 18th edition using a single factor ANOVA for regression to test for association between the marker scores and the phenotypic scores of rice blast resistance. The genotypic data was the explanatory variate and the phenotypic data was the response variate. A Chi-square test of independence was also calculated to test for association between the resistance gene in the population and the markers,

The chi-square test of independence for each marker was calculated according to the formula;

$$\text{Chi} - \text{Square test of Independence} = \chi^2 = \sum_{i=1}^{r}\sum_{j=1}^{c}\frac{(O_{ij} - E_{ij})^2}{E_{ij}}$$

O_{ij} = Observed value of either resistance or susceptible individuals
Eij = Expected value of either resistance or susceptible individuals

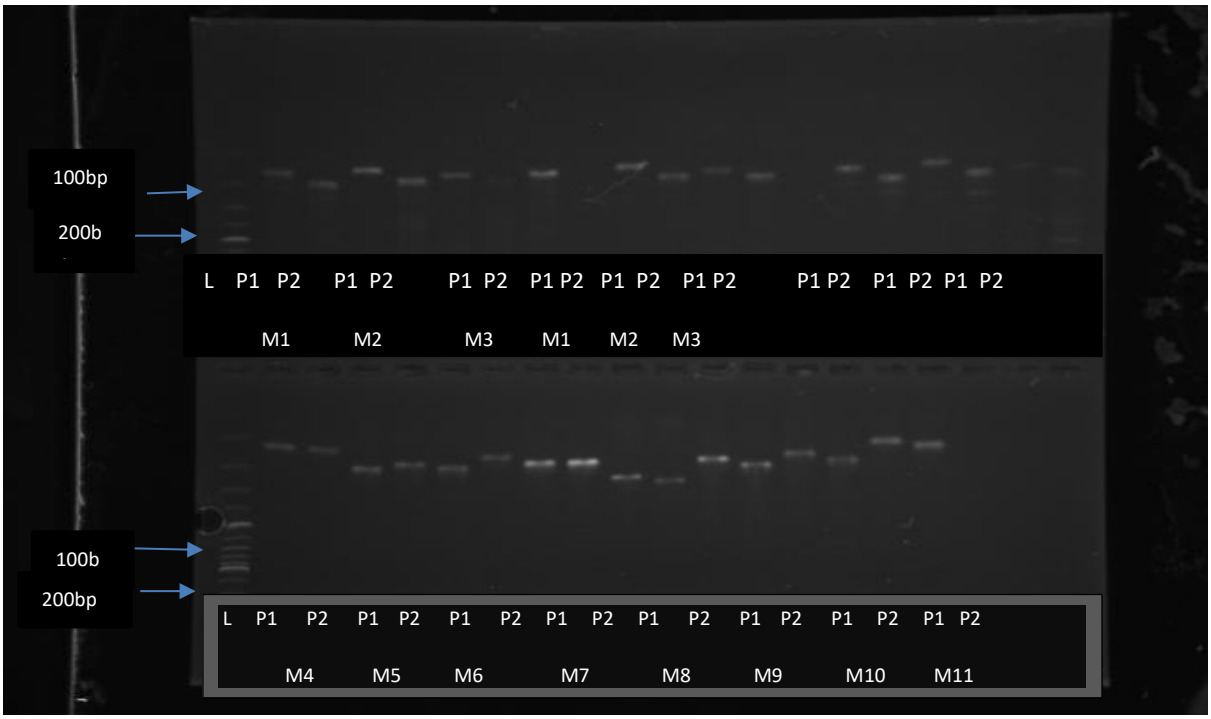
This was used to evaluate whether the markers co-segregate with the resistance gene in the NamChe2 or they don't.

RESULTS

Genetic analysis of resistance to rice blast in the population. Under screen house conditions, the susceptible parent Supa Soroti was highly susceptible to the Namulonge isolate, showing a lesion score of 7.0 while NamChe 2 was highly resistant, having a low lesion score of 1.0. Also, the 100 randomly selected F2 individuals segregated differently, where 71 plants showed resistance while 29 were susceptible. These observed frequencies were non-significant when tested under the chi-square goodness of fit. Therefore, these frequencies fit the single gene model with P = 0.97 compared to the expected ratio of 3:1 (Table 2).

Table 2. Segregation of the F2 population for rice blast resistance

Cross	Total number of F2 plants	Resistant F2 plants	Susceptible F2 plants	χ^2-value	P-value
Supa Soroti x NamChe2	100	71	29	0.97	P = 0.32



P1-Supa Soroti, P2-NamChe2, M1-RM21, M2-RM72, M3-RM259, M4-RM229, M5-RM541, M6-RM206, M7-RM224, M8-RM527, M9-RM208, M10-RM247 and M11-RM246

Figure 1. Gel picture showing polymorphism of markers between the susceptible parent (Supa Soroti) and a resistant parent NamChe2

Polymorphism of the markers between NamChe2 (resistant) and Supa Soroti (susceptible). Nine SSR markers (RM72, RM21, RM259, RM 541, RM206, RM224, RM208, RM247 and RM246) of the eleven tested showed polymorphism between the two parents with different band lengths (Figure 1). All the polymorphic markers showed band lengths between 100bp-200bp.

Segregation of the SSR markers in the population. Nine markers out of the eleven showed polymorphism between the two parents (Supa Soroti and NamChe2) (Figure 1).These nine markers also showed non-significance for the Mendelian ratio of 1:2:1 for co-dominant markers implying that they can be used for MAS (Table 3).

Association of linked markers to the resistance gene among the tested F2 population. Results from the simple linear regression analysis show that among the nine polymorphic markers between Supa Soroti (susceptible) and NamChe2 (resistant), only one SSR marker RM 21 was significant at ($P<0.001$). This marker showed the band for resistance at ~140bp and the susceptible band was recorded at ~180bp, indicating a strong association between RM 21 and the resistance gene in the population. In addition, the Chi-square test of independence showed that eight SSR markers out of nine were non-significant (Tables 4 and 5). However, there was a significant ($P<0.05$) association between SSR marker RM21 and the resistance gene in the Supa Soroti x NamChe2 population. This association indicates that Marker RM21 is associated with the resistance gene to the isolate *Magnaporthe oryzae* from Namulonge in the population. The results of the independence test were consistent with the regression analysis; both showing a significant association between marker RM21 and resistance in the population.

Table 3. Chi-square goodness of fit for the segregation of SSR markers in the 100 F2 rice lines created from NamChe2 and Supa Soroti

MARKER	Total number of individuals scored	Genotypes observed			χ^2 (1:2:1)	P-value (α=0.05, DF=2)
		AA	Aa	Aa		
RM206	100	23	43	22	1.5^{ns}	0.47
RM541	100	32	40	28	4.2^{ns}	0.11
RM208	100	24	60	16	5.3^{ns}	0.07
RM246	100	24	42	34	1.0^{ns}	0.60
RM247	100	20	59	21	3.3^{ns}	0.19
RM259	100	24	42	34	4.6^{ns}	0.10
RM21	100	22	49	29	1.0^{ns}	0.60
RM72	100	22	48	30	1.4^{ns}	0.49
RM547	100	31	45	24	1.98^{ns}	0.37

AA- homozygous dominant individuals for resistance, Aa- heterozygotes, aa-susceptible individuals, P-probability value, ns-non significant, $\chi 2$ is the chi-square, R- resistant, H- Heterozygote, S- Susceptible and DF is the degree of freedom

Table 4. Simple linear regression analysis of the nine polymorphic SSR markers with phenotypic data of blast severity score of the 100 individuals from a population of Supa Soroti x NamChe2

		SSR MARKERS								
Source of variation	DF	RM541	RM206	RM 527	RM208	RM247	RM246	RM21	RM72	RM259
Regression	1	0.85^{ns}	0.24^{ns}	0.85^{ns}	2.4^{ns}	0.01^{ns}	2.4^{ns}	20.9^{***}	3.9^{ns}	0.25^{ns}
Residual	98	1.675	1.682	1.675	1.66	1.684	1.66	1.471	1.644	1.682
Total	99	1.667	1.667	1.667	1.667	1.667	1.667	1.667	1.667	1.667
R^2		0.005^{ns}	0.001^{ns}	0.005^{ns}	0.015^{ns}	0.000^{ns}	0.014^{ns}	0.127^{***}	0.024^{ns}	0.001^{ns}

SOV-source of variance, DF- degree of freedom, ^{ns}-non-significance, R^2- regression coefficient

Table 5. Chi-square test of independence of the nine polymorphic SSR markers from M. *oryzae* resistance in F2 population from a cross between Supa Soroti (S) x NamChe2 (R)

	OBSERVED						EXPECTED							P-VALUE
MARKERS	R(2)	R(1)	R(0)	S(2)	S(1)	S(0)	R(2)	R(1)	R(0)	S(2)	S(1)	S(0)	$\chi 2$	
RM541	20	25	15	9	14	17	17.4	23.4	19.2	11.6	15.6	12.8	3.54^{ns}	0.17
RM206	20	25	15	16	18	6	21.6	25.8	12.6	14.4	17.2	8.4	1.50^{ns}	0.47
RM527	20	26	14	10	19	11	18.0	27.0	15.0	12.0	18.0	10.0	0.81^{ns}	0.67
RM208	16	36	8	7	25	8	13.8	36.6	9.6	9.2	24.4	6.4	1.57^{ns}	0.46
RM247	11	35	14	10	24	6	12.6	35.4	12	8.4	23.6	8	1.35^{ns}	0.51
RM246	16	35	9	12	16	12	16.8	30.6	12.6	11.2	20.4	8.4	4.25^{ns}	0.12
RM21	15	34	11	7	15	18	13.2	29.4	17.4	8.8	19.6	11.6	8.30*	0.02
RM259	16	24	20	8	18	14	14.4	25.2	20.4	9.6	16.8	13.6	0.61^{ns}	0.74
RM72	15	29	16	7	19	14	13.2	28.8	18	8.8	19.2	12	1.17^{ns}	0.56

χ^2 critical = 5.99, Df = 2

χ^2- chi square, Df- degree of freedom, R- resistant (scores 0-3), S- susceptible (scores 4-9), Df- degree of freedom, P-value- probability value. Marker scores 2- Homozygous resistant, 1-heterozygotes and 0-Homozygous susceptible, ns- non-significant (marker and resistance are independent), *(significant at P<0.05, marker and the resistance gene in the population are associated)

From the contusion matrix mode, accuracy of the marker is moderate at 67%. The False Negative rate is 18% while the false positive rate is high at 55%. This means that 66%, of the predictions by the marker were correct, encompassing both true positives (resistant plants) and true negatives (susceptible plants). The false positive rate of 55% is quite high because the model misclassified actual susceptible plants as resistant. which could lead to taking up of more susceptible plants as breeding lines in the pedigree. The false negative rate is relatively low at 18% which means that the model rarely misclassifies positive (resistant) as negative (susceptible).

Table 6 Performance of SSR marker R21 using the confusion matrix

Categories	Number of F2 plants
TP	49
TN	18
FP	22
FN	11
Accuracy (TP+TN)/(TP+TN+FP+FN)	0.67
FNR(FP/(FN+TP))	0.18
FPR(FP/(FP+TN))	0.55

FN- False Negatives, FP- False Positives, TP- True Positives, TN- True Negatives, FPR- False Positive Rate and FNR- False Negative Rate

DISCUSSION

Several studies have uncovered the genetics of blast resistance in rice in the last five years. Several dominant R-genes coffering complete resistance have been discovered in the rice genome. Over 100 R-genes and 350 QTL have been mapped on different chromosomes of the rice genome, characterized and extensively studied. Most of the R genes occur as gene clusters on the same chromosome of which some are either linked or allelic.

In the present study, eight SSR markers lacked association with the resistance in the population (Table 5). This could have been caused by epistasis which reduces the power of locus detection as explained by (Verhoeven *et al.*, 2010). This makes them ineffective for introgression of resistance into the susceptible rice genotypes. SSR marker RM21 was associated with blast resistance in the population created between local commercial varieties (Supa Soroti x NamChe2). This makes the marker effective for the introgression of resistance through MAS. According to Singh *et al.* (2015), SSR marker RM21 is linked to R-gene Pi5-(t) along chromosome 11. This indicates that the resistance gene Pi5-(t) could be present in this population. Results from screening Monogenic lines indicate that the gene Pi5-(t) is resistant to the isolate of *M. oryzae* from Namulonge. Gowda *et al.* (2006) were also able to map a new resistance gene Pi38 on chromosome 11 in the population between Tadukan (resistant) x C039 (susceptible) and it was linked to SSR marker RM21 at a linkage distance of 16cM and 4cM from SSR marker RM206. The association studies they carried out also showed an association between Pi38 and SSR marker RM21.

The high FPR of 55% makes the marker less reliable for early selection. This means that there will be misclassification of individuals which directly translates into many individuals incorrectly identified as possessing the resistance gene. This directly can lead to errors in marker-assisted selection. In addition, the selection of inferior plants can decline the genetic quality of the population. Therefore, SSR marker RM21 is suitable for late stages selection because other selection criteria like phenotypic evaluation could have narrowed down the population, hence reducing the effect of false positives (Yu *et al.*, 2006). The low FNR ensures that susceptible lines are rarely classified as resistant, making it a more reliable marker when narrowing down resistant genotypes in advanced generations. However, to utilize this marker, there is a need to complement it with other markers in addition to phenotypic assessments to mitigate the high FNR and

enhance the utility and reliability of the marker RM21 (Collard *et al*., 2005). Therefore, SSR marker RM21 is insufficient when used alone. However, it holds promise as part of a marker-assisted selection (MAS) strategy for later stages of breeding.

CONCLUSION AND RECOMMENDATIONS

This study aimed to test for the association between resistances in the population created from crossing two local commercial varieties (Supa Soroti (susceptible) and NamChe2 (resistant)). Eleven SSR markers were tested on the parents for polymorphism and nine of them were found polymorphic between Supa Soroti and NamChe2. Nine markers were run on the population of 100 individuals and each genotype was scored. The chi-square goodness of fit was used to test for segregation and all the nine SSR markers (RM259, RM72, RM541, RM247, RM246, RM547, RM208, RM21 and RM206) fit the segregation ratio of 1:2:1 for codominant markers. Therefore, all the nine markers segregated normally in a Mendelian fashion. However, both the chi-square test of independence (χ^2=8.34, P<0.05) and simple linear regression (R^2=0.127, P<0.001) showed that RM21 was closely associated with resistance in the population, explaining 12.7% of the phenotypic variation in the population. However, the marker had a false negative rate of 55% which suggests that the marker can be used in late-generation selection in combination with thorough phenotypic disease assessments. In addition, more markers that complement SSR marker RM21 should be identified to increase its efficiency for marker-assisted selection for resistance to rice blast disease in populations derived from local varieties.

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STATEMENT OF NO-CONFLICT OF INTEREST

The Authors declare no conflict of interest in the paper.

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