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Characterization of rice blast resistant genes and combining ability in Ugandan local commercial disease-resistant varieties

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ABSTRACT

Rice blast disease caused by *Magnaporthe oryzae* is one of the most serious diseases that affect rice production in Uganda causing up to 100% yield loss in severe cases. Local commercial varieties of rice like NamChe2 and NERICA 15 have shown broad-spectrum resistance to several isolates of *M. oryzae* in Uganda; however, the resistance genes in these varieties have not been characterized. This study aimed to test the allelic relationship between resistance genes of these local resistant commercial varieties and known broad-spectrum R genes. Seven resistant genotypes, including two monogenic differential lines (WH13-3260(Pi7t) and WH13-3203(Pi54)) and five local commercial resistant varieties (NamChe2, NERICA15, NERICA6, NERICA4, and IR64) were crossed in a half diallel mating design to generate F1 resistant x resistant crosses which were later selfed to generate F2 populations. The 16 F2 populations were planted in a randomized complete block design (RCBD) of two replications with 50 plants per replication per cross. Individual plants from two replications were evaluated 28 days after inoculation and plants with scores of 0-3 were considered resistant while those with higher scores were considered susceptible. Means of the evaluated plants were used to analyze for combing ability. Combining ability results showed that resistance among resistant genotypes is controlled more by non-additive effects with a low narrow sense coefficient of genetic determination (0.19), low Baker's ratio (0.22) and high broad sense coefficient of genetic determination (0.89). Genotypes IR64, WH13-3260 and NamChe2 had significant negative GCA effects indicating that they are good combiners for resistance to blast disease and therefore can be utilized to improve other locally adapted varieties for resistance to rice blast disease. The Chi-square goodness of fit revealed that the cross of NamChe2 x WH13-3203(Pi54) did not segregate giving a ratio of 1:0 (R: S) indicating that NamChe2 carries an allele of the gene Pi54. Crosses between the monogenic lines and the other commercial resistant varieties segregated with different epistatic ratios like NamChe2 x WH13-3260 (9:7), NERICA15x WH13-3203 (3:1, 13:3), NERICA4 x WH13-3203 (9:7), NERICA4 x WH13-3260 (27:37), 6 x WH13-3203 (9:7), NERICA6 x WH13-3260 (9:7 and 27:37) and IR64 x WH13-3260(9:7). This shows that the genes within these varieties and those of monogenic lines are different. This study sets a basis for using the local resistant variety NamChe2 as a source of resistance to rice blast disease. Other local resistant sources could also be characterized using other characterized R genes.

Key words: Broad-spectrum resistance, combining ability, *Magnaporthe oryzaei*, monogenic differential lines, R genes, Rice blast

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RÉSUMÉ

Le brûlure du riz, causé par *Magnaporthe oryzae*, est l'une des maladies les plus graves affectant la production rizicole en Ouganda, pouvant entraîner jusqu'à 100 % de pertes de rendement dans les cas sévères. Des variétés commerciales locales de riz, telles que NamChe2 et NERICA15, ont montré une résistance à large spectre face à plusieurs isolats de *M. oryzae* en Ouganda. Cependant, les gènes de résistance dans ces variétés n'ont pas encore été caractérisés. Cette étude visait à tester la relation allélique entre les gènes de résistance de ces variétés commerciales locales résistantes et les gènes de résistance (genes R) à large spectre connus. Sept génotypes résistants, comprenant deux lignées différentielles monogéniques (WH13-3260(Pi7t) et WH13-3203(Pi54)) et cinq variétés commerciales locales résistantes (NamChe2, NERICA15, NERICA6, NERICA4 et IR64) ont été croisés selon un plan de croisements en diallèle partiel pour générer des croisements résistant x résistant. Les plantes individuelles ont été évaluées 28 jours après inoculation, et les plantes ayant des scores de 0 à 3 ont été considérées comme résistantes, tandis que celles avec des scores plus élevés ont été jugées sensibles. Le test du Chi-carré a révélé que le croisement NamChe2 x WH13-3203(Pi54) ne présentait pas de ségrégation, avec un ratio de 1:0 (R: S), indiquant que NamChe2 porte un allèle du gène Pi54. Les croisements entre les lignées monogéniques et les autres variétés commerciales résistantes ont montré des ratios épistatiques différents, tels que NamChe2 x WH13-3260 (9:7), NERICA15 x WH13-3203 (3:1, 13:3), NERICA4 x WH13-3203 (9:7), NERICA4 x WH13-3260 (27:37), NERICA6 x WH13-3203 (9:7), NERICA6 x WH13-3260 (9:7 et 27:37), et IR64 x WH13-3260 (9:7). Cela montre que les gènes au sein de ces variétés et ceux des lignées monogéniques sont différents. Cette étude établit une base pour l'utilisation efficace de la variété locale résistante NamChe 2 comme source de résistance à la brûlure du riz. D'autres sources locales de résistance pourraient également être caractérisées en utilisant d'autres gènes R déjà identifiés.

Mots-clés: Résistance à large spectre; *Magnaporthe oryzae*; Lignées différentielles monogéniques; Gènes de résistance (genes R); Brûlure du riz

INTRODUCTION

Rice blast caused by *Magnaporthe oryzae*, is one of the most devastating diseases in rice production in Uganda. Deployment of resistance into local commercial varieties is the most effective and environmentally safe control measure compared to chemical application. However, resistant varieties are normally not durable due to the emergence of new virulent strains of *M. oryzae* (Onaga and Asea, 2016).To widen the diversity of sources of resistance to rice blast, novel sources of resistance, especially those with broad-spectrum kinds of resistance are needed by the rice breeding programme in Uganda. Resistant x Resistant crosses can be developed using a diallel mating design. according to Niyongabo (2012), crosses involving upland varieties inherit resistance to rice blast disease non-additively, suggesting the presence of epistatic/dominance effects.

Mulbah *et al.* (2015), who performed a combining ability study for horizontal resistance to rice blast, reported that blast resistance is controlled by additive gene effects with a few partial dominant gene effects present. They recommended selecting genotypes with reduced size of lesions in older leaves and increased size in younger leaves because smaller size lesions are connected to a race-specific type of resistance (Bonman, 1992). They also identified genotypes with a good GCA, like LAC23 and Payleito, for trait lesion size and AUDPC, respectively. In addition, these genotypes were good candidates for lesion size and AUDPC, respectively. In Uganda, a study of GCA and SCA effects using a full diallel mating design was conducted by Niyongabo (2012), revealing that the parents NERICA10, Koshihikari and IR64 had a great combining ability for rice blast resistance because they had negative GCA values. In addition, resistance in these genotypes was found to be mainly non-additively inherited**.**

On the contrary, Zewdu *et al.* (2017) also studied the combining ability of the locally adapted genotypes

with genotypes introduced from Korea. They obtained significant SCA and GCA effects among the genotypes using the Namulonge isolate. Their results also suggested that the inheritance of resistance to rice blast disease is mainly additive. Therefore, the inheritance and combining ability of resistance to rice blast disease depends on the genotypes being assessed (Quenouille *et al.*, 2013).

The previous studies did not include NERICA15 and NamChe 2 in their crossing block, yet these are materials identified to possess blast resistance. Therefore, it is important to study the ability of these genotypes to combine for resistance to the blast isolate from Namulonge. The uniqueness of the strain came from its high virulence characteristics. This particular strain belongs to a dominant lineage in Africa (lineage 3) with signs of positive selection, an indication of rare alleles accumulation in the backbone genome of *M. oryzae*. This is different from other isolates obtained from Doho irrigation scheme, Lira, Budaka and other areas in Uganda (Onaga *et al.*, 2020). This isolate is also virulent to recently released NERICA varieties which were previously resistant in Namulonge (Onaga *et al.*, 2016). This will guide on decision-making of parents for use in improving resistance in the locally preferred but susceptible varieties or landraces in Uganda. The rice genome has cloned over 100 dominant R genes and 350 QTL (Devi *et al.*, 2015). Many of these R genes occur as clusters on particular chromosomes, for example Pi5, Pi3, Pi15, and Pi56(t) on chromosome 9, Pik, Pik-h, Pik-m, Pik-p, Pik-s, Pi1, Pif, Pilm2, Pi7(t), Pi18(t), and Pi43(t) on chromosome 11 and Pi- ta, Pi-ta2, and Ptr(t) on the chromosome 12 (Sharma *et al.*, 2012; Liu *et al.*, 2015; Singh *et al.*, 2015). Therefore, all the resistant genes occur as alleles at a certain complex locus or clusters at a particular chromosome (Kinoshita,1995) as cited by ((Pan *et al.*, (1998).

Allelism studies conducted by Pan *et al.* (1998) indicate that the efficient utilization of resistant local cultivars depends on knowledge of the genetic control of their resistance, which can be achieved by studying the allelic relationship between R genes within those local cultivars and the Near Isogenic lines whose resistance gene has already been characterized. Knowledge of the allelic relationships among the resistance genes is necessary to conduct a gene pyramiding process to strengthen resistance to the disease.

In Uganda, several local varieties have shown resistance to several isolates of *M. oryzae*. Recently a study by Mutiga *et al.* (2017) where *M. oryzae* populations in Uganda were characterized using race differentials, NERICA4 was reported to be resistant to most of the races of the pathogen. In addition, NERICA15 was found to be resistant to >95% of isolates while NERICA4 was found to be resistant to >91% of the isolates from Africa (Mutiga *et al.*, 2017). NamChe2 on the other hand has consistently shown resistance to several blast isolates. However, the genetic basis of resistance among these local resistant varieties is not well understood. Therefore, the major objective of this study was to characterize the resistance genes carried by the local varieties using the allelism test. In this situation, local resistant varieties whose resistance had not been characterized are crossed with monogenic differential lines carrying already characterized resistant genes. This is important for the efficient utilization of the resistance genes in these varieties and the characterization of rice blast resistant genes (R genes) within these local resistance sources.

MATERIALS AND METHODS

Genetic material and mating design. To generate the crosses that were used in this study, seven resistant genotypes, including NERICA15, NamChe2, NERICA4, NERICA6, IR64, and two monogenic lines (WH13-3203(Pi54) and WH13-3260(Pi7t)) whose resistant gene had already been characterized were used as parents (Table 1). The seven parents were crossed in a 7x7 half-diallel mating design. These crosses generated 16 successful hybrids. The 16 F1 hybrids were then selfed to generate about 100 F2 individuals of the 16 hybrids. The 16 F2 populations plus the seven parents, amounting to 23 entries, were planted in an RCBD with two blocks with each entry having 50 plants per block. These were inoculated with the Namulonge isolate of *M. oryzae*. Infected leaves were collected from the old rice field at Namulonge. The leaf samples were first washed with sodium hypochlorite and then in distilled water before being incubated in petri dishes at 32^{o}C to enhance sporulation. After incubation, single spores were picked under a microscope with a pin-loop and placed on V8 media with rice bran. The fungal

growth started within 7 days after spore placement on media (Mishra *et al*., 2015; Kulmitra *et al*., 2017).

The fungus was then sub-cultured to obtain pure cultures that were stored at 4°C for preparation of inoculum. The isolate was 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 was added to the inoculum to facilitate adhesion of the pathogen to the 21-day old rice leaves (Nakiyaga *et al*., 2020). The prepared inoculum was sprayed on the leaves until they soak with the liquid and the plants were covered with a polyethene sheets for 24 hours to create an environment with a high humidity. After 28 days after inoculation each plant was scored for rice blast disease severity on the 0 to 9 scale (IRRI, 2013) where 0 shows no lesions observed and 9 means 75% of the leaf area has lesion and also most of the leaves on the plant are infected.

The 23 entries (16 crosses and 7parents) were also evaluated for combining ability following half diallele analysis method II and model 1 (Griffing, 1956) in Genstat 18th edition. Below is the linear model that was used in the analysis of combining ability of the crosses: $y_{ijk} = \bar{\bar{Y}} + g_i + g_j + SCA_{ij} + R_k + e_{ijk}$, where y_{ijk} is the observations made on an individual cross, $\bar{\bar{Y}}$ is the grand mean, g_i and g_j are the GCA effects of the i^{th} and j^{th} parents, R_k is the replication mean effects, e_{ijk} is the experimental error.

Table 1. Characteristics of selected parental lines

Genotype/parent	Resistant/susceptible	R gene	Pedigree	Source of seed
NERICA4	Resistant	Not known	CG14/WAB56-104	NaCRRI
NERICA15	Resistant	Not known	CG14/WAB56-104	NaCRRI
NERICA6	Resistant	Not known	CG14/WAB56-104	NaCRRI
NamChe2	Resistant	Not known	NM7-8-2-B-P-11-6	NaCRRI
IR64	Resistant	Pi30(t)	Indica cultivar,(IR5657-33-2-1/IR2061-465-1-5-5)	NaCRRI
WH13-3203	Resistant	*Pi54*	IRBLKh-K3/CO39	IRRI
WH13-3260	Resistant	*Pi7(t)*	IRBL7/Moroberekan	IRRI

Table 3. Summary of the GCA effects of the parents that were involved in hybrid formation

Parents	Mean score	SE_{GCA}	GCA effects
NERICA15	2.5	0.013	0.11***
NamChe2	2.0	0.015	-0.25***
NERICA4	3.0	0.014	0.25***
NERICA6	2.5	0.015	0.04***
IR64	1.0	0.018	-0.58***
WH13-3203	3.0	0.014	-0.06***
WH13-3260	3.0	0.013	0.18***

SE_{GCA} =[((p/ni)*((p-1)/p(p+2)))* MS error/r]1/2

*****, ** Significance at probability level<0.001 and 0.01 respectively SE_{GCA}- Standard error of the GCA effect, ni-number of cross combinations, MS error- mean square of error and r is number of replications**

RESULTS

Results from the analysis of variance revealed that the genotypes were highly significant (P<0.001), Table 2). In addition, both the GCA and SCA effects were significant (P<0.001) and high. SCA effects were also significant (P<0.001), indicating a high genetic variability among the parents which reflected on the crosses and an involvement of non-additive effects in the inheritance of resistance to rice blast disease. The Narrow sense coefficient of genetic determination (NSCGD) was 0.19 which translates into 19% of the genetic variation for rice blast resistance being due to additive effects (transferred from parents to the hybrids) and 81% due to non-additive effects. The broad sense coefficient of genetic determination (BSCGD) was 0.89, indicating that 89% of the variation observed was due to genetic factors of the genotypes and only 11% was due to environmental effects and errors. The predictability ratio (Baker's ratio) was 0.22, indicating that 22% of the observed significant variation among the genotypes was due to additive effects. In addition, Baker's ratio indicates poor prediction of the performance of the crosses from their parents, implying that late selection at later generations is recommended.

Table 2. Analysis of variance for resistant parents in a half-diallele mating design

SOV	D.f	MS	F-test
ENTRIES	22	0.547***	7.42
GCA	6	0.531***	7.20
SCA	16	0.553***	7.50
Error	22	0.074	
σ^2 (GCA)	0.067		
σ^2 (SCA)	0.479		
NSCGD (NSH)	0.19		
BSCGD(BSH)	0.89		
BR	0.22		

***** significance at P<0.001, ns-non-significant, σ^2-Variance component, NSCGD- Narrow sense coefficient of genetic determination, BSCGD-Broad sense coefficient of genetic determination, BR-Baker's ratio, D.f- degree of freedom, MS- mean squares, SOV-source of variation.**

Three parents, NamChe2 (-0.25), IR64(-0.58), and WH13-3203(-0.06) had significant negative GCA effects (P<0.001), making them good combiners for rice blast resistance while four parents, NERICA15, NERICA4, NERICA6 and WH13-3260 showed significant positive GCA effects which makes them poor at transmitting rice blast resistance to the hybrids (Table 3).

According to the specific combining ability shown in Table 4, Eight cross combinations NERICA15 x IR64 (-1.02), IR64 X WH13-3206(-1.07), NERICA15 x NamChe2 (-0.56), NERICA15 x WH13-3260 (-0.45), NERICA15 x NERICA6 (-0.48), NamChe2 x NERICA4 (-0.58), WH13-3203 x WH13-3260 (-1.07) and NERICA6 x WH13-3203 (-0.80) had the highest negative significant SCA effects (P<0.001, P<0.01 and P<0.05). SCA effects, makes these crosses the most desirable for resistance to blast because such crosses can have a greater number of resistant plants making selection for resistance easier. However cross combinations of NERICA15 x NERICA4 (-0.37), NERICA 6 x WH3260 (-0.33) were non-significant. All the crosses with positive undesirable SCA effects were non-significant except for cross combination NERICA15 x WH13-3260 (0.45) which was significant at P<0.05. However, looking at its mean performance of 3.0, it cannot be categorized as the worst performing cross. This could be attributed to the non-additive effects (loci

interactions (epistasis)) which could have occurred in a complementary manner to cause resistance among these crosses.

In terms, of the mean performance of the crosses, cross NamChe2 X WH13-3203 (2.6) had the lowest mean score followed by WH13-3203 X WH13-3260 (2.8). However, both crosses NamChe2 x WH13-3203 and WH13-3203 x WH13-3260 had positive non-significant SCA effects. The low mean scores are due to the fact that all the individuals individually scored in these two F2 populations generally had scores between 0-3.

Table 4. SCA effects for the cross combinations from the half diallele mating design for rice blast resistance

	NERICA15	NamChe2	NERICA4	NERICA6	IR64	WH13-3203	WH13-3260
NERICA15		-0.56***	-0.37^{ns}	-0.48**	-1.02***	0.11^{ns}	0.45*
NamChe2			-0.58**	M	M	0.26^{ns}	-0.54**
NERICA4				0.01^{ns}	M	0.28^{ns}	0.20^{ns}
NERICA6					M	-0.80***	-0.33^{ns}
IR64						M	-1.07***
WH13-3203							0.54^{ns}
WH13-3260							

SE_{SCA}=0.19 where; $SE_{SCA}=\sqrt{ems} * c$ and c=(P^2+P+2)/(P+1) (P+2)

*****, **, *significance at P<0.001, 0.01 and 0.05 respectively, ns- non-significance and SE_{SCA}-standard error of the SCA effects, P is the number of parents, ems is the error mean square, r- one-half the average number of crosses per parental combination and c is a coefficient**

The chi-square goodness of fit analysis was conducted to determine the number of genes involved in resistance among the crosses, intra-loci interactions and the similarity between the R genes. Different ratios were observed from **t**his analysis output. Due to poor germination of the seed from the hybrids and F2 hybrid sterility, the number of plants evaluated was reduced to half the total expected number (50 F2 individuals from each of the two replications) in some crosses. (Table 6), despite pre-germination treatments.

A cross between the local variety NamChe2 and a monogenic differential line WH13-3203 that carries Pi54 (Pi-kh) did not segregate, giving a ratio of 1:0 (R: S) (Table 6), implying that NamChe2 could be carrying a resistance locus (Pik-h). However, a cross between NamChe2 and the monogenic differential line WH13-3260 that carries the gene Pi7 (t) is segregated in a 9:7 ratio. In addition, the cross between the two monogenic differential lines also did not segregate, giving a segregation ratio of 1:0. None of the other resistant local varieties showed the allelism ratio of 1:0. The chi-square test however showed the crosses between other local varieties and monogenic lines segregated in different ratios of 3:1 (NERICA15 x WH13-3203, NERICA15 x WH13-3260), 9:7(NERICA4 x WH13-3203, NERICA6 x WH13-3260, NEICA6 x WH13-3203 and IR64 x WH13-3260), 13:3 (NERICA15 x WH13-3260 and NERICA15 x WH13-3203) and 27:37(NERICA6 x WH13-3260 and NERICA4 x WH13-3260), probably resulting from different genes interacting at different loci. Crosses between NERICA15, a highly resistant local genotype, and the two monogenic lines showed non-significance of two ratios that is one for a single dominant gene (3:1), and 13:3 which suggests that the genotype could have two loci with both dominant and recessive epistasis. Crosses of NERICA6 and the monogenic lines WH13-3260 indicated two ratios of 9:7 (duplicate recessive epistasis) and 27:37 (three complementary genes). The crosses among local resistant genotypes (NERICA15 x NamChe2, NERICA15 x NERICA4, NERICA15 x NERICA6, NERICA15 x IR64 and NamChe2 x NERICA4) also segregated with two different epistatic ratios (9:7 and 27:37), indicating that there could be either two or three dominant genes, occurring on different

chromosomes, govern resistance to rice blast among these genotypes.

Table 5. Mean performance of the resistant x resistant crosses

Cross combination	MEAN PERFORMANCE
NERICA6 X WH13-3203	4.0
NERICA 15 X NERICA 4	3.9
IR64 X WH13-3260	3.9
NERICA 15 X NERICA 6	3.8
NERICA15 X IR64	3.8
NERICA4 X WH13-260	3.8
NamChe2 X NERICA4	3.8
NERICA6 X WH13-3260	3.8
NamChe2X WH13-3260	3.7
NERICA4 X WH13-3203	3.7
NERICA 15 X NamChe2	3.6
NERICA4 X NERICA6	3.5
NERICA15 X WH13-3203	3.1
NERICA15 X WH13-3260	3.0
WH13-3203 X WH13-3260	2.8
NamChe2 X WH13-3203	2.6
LSD (5%)	0.8
SED	0.4
CV %	12.0
Minimum	2.6
Maximum	4.0

LSD- least significant difference, CV- coefficient of variation, SED-standard error of difference

Table 6. Allelism test for rice blast resistance genes to *M. oryzae* isolate from Namulonge present in the local commercial genotypes crossed with monogenic differential lines.

Population	#of plants	Reaction	Observed ratio		Expected ratio (R:S)	χ^2	P-value
			R	S			
NERICA15 X WH13-3203	60	R X R	47	13	3:1, 13:3	0.35^{ns}, 0.33^{ns}	0.55, 0.56
NERICA15 X WH13-3260	60	R X R	44	16	3:1, 13:3	0.089^{ns}	0.76, 0.12
NamChe2 X WH13-3203	70	R X R	70	0	1:0	-	-
NamChe2 X WH13-3260	71	R X R	36	35	9:7	0.887^{ns}	0.35
NERICA4 X WH13-3203	57	R X R	29	28	9:7	0.67^{ns}	0.41
NERICA4 X WH13-3260	55	R X R	26	29	27:37	0.58^{ns}	0.45
NERICA6 X WH13-3203	72	R X R	42	30	9:7	0.13^{ns}	0.72
NERICA6 X WH13-3260	51	R X R	28	23	9:7, 27:37	0.04^{ns}, 3.38^{ns}	0.85, 0.07
IR64 X WH13-3260	59	R X R	36	23	9:7	0.55^{ns}	0.46
WH13-3203 X WH13-3260	77	R X R	77	0	1:0	-	-
NERICA15 X NamChe2	50	R X R	29	21	9:7	0.06^{ns}	0.80
NERICA15 X NERICA4	54	R X R	23	31	27:37	0.004^{ns}	0.95
NERICA15 X NERICA6	55	R X R	29	26	9:7, 27:37	0.28^{ns}, 2.5^{ns}	0.598, 0.11
NERICA15 X IR64	63	R X R	36	27	9:7	0.02^{ns}	0.89
NamChe2 X NERICA4	50	R X R	24	26	9:7, 27:37	1.38^{ns}, 0.69^{ns}	0.24, 0.41

R-resistant, S-susceptible, ns-non-significant, χ^2- Chi-square calculates, P-value- Probability value, #- number of plants, R-resistant individuals, S- susceptible individuals

DISCUSSION

Parent and crosses combining ability. Numerous combining ability studies have been conducted on the Ugandan locally grown resistant varieties and different researchers have reported resistance in these varieties being either additive or non-additively inherited. Niyongabo (2012) reported varieties NERICA10, Koshihikari and IR64 as parents with good combining ability while Zewdu *et al.* (2017) reported Korean varieties to have a better combining ability compared to the local varieties. In the current study, parents IR64, NamChe2 and WH13-3260 showed high negative significant GCA effects and therefore are good combiners that can be utilized to improve other locally adapted varieties for resistance to rice blast disease. Both SCA and GCA were highly significant; however, the SCA variance was predominant over the GCA variance, indicating a primarily non-additive control for blast resistance in the studied lines. Consequently, the narrow sense coefficient of genetic determination was very low (0.19), with non-additive (dominance and epistasis) gene effects accounting for over 81%. This high non-additive effect makes parent-offspring transmission of resistance challenging. Niyongabo (2012) reported similar results on upland local varieties in Uganda. This implies that to effectively breed and select for resistance to rice blast among local varieties, several crosses have to be made and evaluations should be made in several locations and seasons to increase the precision in estimating their heritability in terms of resistance to rice blast disease.

Also, as a consequence of the high non-additive effect, Baker's ratio was low (0.22), indicating that the performance of the crosses cannot be easily predicted from the performance of the parents. However, the broad sense coefficient of genetic determination was high (0.89), accounting for 89% of the variation observed among the crosses being due to genetic factors, with a very low influence of the environmental factors (11%). Zewdu *et al.* (2017) reported different results among Korean genotypes that showed a high Bakers ratio and reported that resistance among these varieties was additively inherited. In addition, Mulbah *et al*. (2015), combining ability results for horizontal blast resistance in rice shows that resistance is mainly controlled by additive gene effects with a few partial dominant gene effects present; therefore, selection can be made at early generation. This contradicts the current study because resistant genotypes were crossed with each other, leading to gen interaction (dominance and epistatic effects), consequently lowering the additive effects and increasing dominance effects that are more contingent on the environment. This indicates that inheritance for resistance to rice blast disease depends on the genotypes tested, the race of the fungus used and how crosses are made (Quenouille *et al.*, 2013).

Allelism among the resistant x resistant F2 progenies. The F2 population of the NamChe2 x WH13-3203 (Pik-h) did not carry any susceptible individuals, with a ratio of 1:0 (R: S). Therefore, these two parents could be carrying identical or allelic genes of resistance to the *M. oryzae* isolate from Namulonge, indicating that one of the resistance genes in NamChe2 is allelic to Pik-h. Similar results were obtained by Pan *et al*. (1998) in a population created between a monogenic line JDC and a Chinese rice cultivar GA20. Their results showed no susceptible individuals; hence, an allele of the Pik locus was discovered in cultivar GA20. In addition, Inukai *et al*. (1994) reported similar results of a 1:0 ratio in the F2 populations of Indian variety AUS373 crossed with Japanese differential cultivars (JDCs) and Chinese susceptible cultivar Lijiangxintuanheigu (LTH), indicating the presence of similar allelic genes to the locus Pi-k. In this case, the R gene Pik-h (Pi54) has already been characterized and it occurs in a gene cluster with other genes like Pik, Pik-m, Pik-p, Pik-s, Pi1, and Pi7t located along chromosome 11 (Kumari *et al.*, 2013; Devanna *et al.*, 2014; Ingh *et al*., 2015; Thakur *et al*., 2015; Vasudevan *et al*., 2015). This particular gene is one of the broad spectrum resistance genes reported by Wang *et al*. (2013). This implies that the local variety NamChe2 can be utilized as a source of the broad-spectrum resistance gene Pi54 to improve other locally adapted but important cultivars in Uganda. This, in turn, reduces resistance breakdown caused by the evolution of the fungus. In addition, using NamChe2 as a source of resistance gene Pi54 has a higher advantage since local varieties are reported to co-evolve with the pathogen strains in a particular area, reducing selection pressure on the pathogen population (Bonman, 1992). However, the F2 population of NamChe2 x WH13-3260 segregated with a phenotypic ratio of 9:7, suggesting a duplicate recessive epistasis probably due to the complementary interaction between Pi7 (t) and another different dominant gene in NamChe2. Also, the two monogenic differential lines WH13-3203 and WH13-3260, carrying different R genes (Pik-h and

Pi7(t) respectively), had no susceptible individuals, confirming that Pik-h and Pi7(t) are allelic as proposed by Yang *et al*. (2008).

The F2 populations of NERICA15, with the monogenic differential line carrying Pi54 (Pik-h) and Pi7(t), were fit for phenotypic segregation ratios 3:1 and 13:3, indicating that at least a single dominant gene is present in their genotype. However, the 13:3 epistatic ratio also suggests dominant epistasis, implying that there are both recessive and dominant genes governing the inheritance of resistance to rice blast disease, and these are different and occur on different chromosomes (Fehr, 1987). This leads to the conclusion that understanding the number of genes in the local variety NERICA15 requires molecular tools like genotyping by sequencing to know how many genes are responsible for the resistance observed within this variety. This is important in giving insights on the breeding strategy to use while using this variety as a source of resistance. The F2 population generated from IR64 x WH13-3260 (Pi54) was fit for a 9:7 segregation ratio which makes breeding for resistance to blast complex because it requires understanding the two genes involved. This ratio implies that the gene Pi33 R gene in IR64 was complementary to Pi54. Gene Pi33 was mapped in IR64 on chromosome 8 in a 1.6cM interval between markers RM72 and Y2643L (Bohnert *et al.*, 2004). In their study, the observed resistance was due to a duplicate recessive epistatic effect of rice blast resistance genes Pi33 and Pi54 (Pik-h). In addition, R genes in NERICA4 and NERICA6 did not show allelism with the resistance gene in WH13-3203 (Pi54) and WH13-3260 (Pi7t) because the population fit segregation ratios of 9:7 and 27:37, respectively. For Pi54, this segregation ratio suggests that the gene interacted with another dominant gene in NERICA4 causing a duplicate recessive epistasis. Pi7t has complementary gene action with two other dominant genes in NERICA4 causing a three-gene complementary gene action. This makes breeding and selection for resistance to blast disease challenging. Therefore, marker-assisted section (MAS) and genomic selection could be useful to track both genes during the breeding process while using these genotypes as sources of resistance. Several **s**tudies have reported epistatic effects among R genes especially those that involved pyramiding (Inukai *et al.*, 1994; Prabhu and de Filippi, 1996; Pan *et al*., 1998; Divya *et al.*, 2014). This reduces the efficiency of studying the number of genes possessed by a single genotype which calls for the intervention of molecular tools like high throughput genotyping by sequencing (Next Generation sequencing) to specifically detect the number of genes.

CONCLUSION AND RECOMMENDATION

The study revealed that the mode of gene action among resistant x resistant crosses was mainly non-additive (dominance/epistasis) in nature following the low Narrow sense coefficient of genetic determination (0.19) and low Baker's ratio (0.22). However, the Broad sense coefficient of genetic determination was high (0.89) indicating that most of the variations that were observed among these crosses in terms of rice blast resistance were mainly due to genetic factors. This suggests that selection for resistance to rice blast disease among such crosses can be carried out at later generations and more complex breeding tools like marker-assisted selection can be effective. In addition, parents NamChe2, IR64 and WH13-3260 were good combiners and therefore can easily transmit their resistance to their offspring. Therefore, these parents can be utilized by the breeding programme as sources of resistance to rice blast disease.

The allelism test showed that a cross of NamChe2 with the monogenic line WH13-3203(Pik-h) did not segregate, giving a ratio of 1:0. This suggests that NamChe2 could be carrying a locus that is allelic to R gene Pik-h. NamChe2 x WH13-3260 (Pi7t) segregated in a 9:7 ratio suggesting a duplicate dominant epistasis. Crosses of other local resistant sources with the monogenic lines carrying R genes Pik-h and Pi7t segregated with epistatic ratios 9:7, 13:3 and 27:37 which suggests that the genes within these local varieties are different from those in the local resistant sources and they occur on different chromosomes causing epistatic interactions. Therefore, further allelism studies can be carried out using monogenic lines carrying other R genes to characterize the genes within the rest of the local resistant sources. In addition, molecular tools such as genomic selection and marker-assisted selection could be further explored to characterize the R genes carried by these local resistant commercial varieties so that their resistance can be efficiently utilized by the rice breeding programme to improve elite varieties against rice blast disease.

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STATEMENT OF NO-CONFLICT OF INTEREST

The Authors declare no conflict of interest in the paper.

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