

Full Length Research Paper

Combining ability for resistance to rice yellow mottle virus disease in interspecific and intraspecific rice Genotypes

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Estimates of combining ability for resistance to rice yellow mottle virus disease (RYMV) were obtained in the green house of National Crops Resources Research Institute, Uganda. The study involved forty-one F₂'s diallel crosses and their five intraspecific and four interspecific parental lines. Effects of both general combining ability (GCA) and specific combining ability (SCA) for RYMV reactions were significant, with a preponderance of GCA effects. Of parental lines used in this study, Naric 1, Nerica 6 and WAC 116 were good combiners for transferring resistance genes to their progenies. In this study, most genotypes with good resistance were good general combiners expect Gigante, that despite its good mean performance, combined badly for resistance to RYMV. The crosses with the best resistance were Naric 1x Gigante, Naric 1x Nerica 6, Nerica 6 x Nerica 4 and Nerica 1 x Nerica 6. From this study, we suggest that selection of parents as donors for resistance should consider both GCA and parental performance. On the basis of predominant additive genetic effects observed in the present study, we believe that combinations of interspecific parents may be used to produce rice varieties resistant to RYMV, and that selection in early selfing generations should be effective.

Key words: Rice, resistance, yellow mottle virus, combining ability, GCA, SCA.

INTRODUCTION

Rice yellow mottle virus (RYMV) is a major biotic constraint for rice production in Sub-Saharan Africa (Paul et al., 2003). The virus is confined to the African continent and has not been reported in other continents of the world (Banwo et al., 2004). RYMV was first reported in 1966 in Kenya (Bakker, 1974). Since then the virus has spread to most rice growing countries in the sub-continent particularly in West Africa where the virus induces severe crop damage and significant yield losses of up to 100% (Fargette and Konaté, 2004, Taylor et al., 1990). In Uganda, the disease was first reported in 2000 in a subsistence rice field northeast of Lake Victoria, close to the Nile River (Pinel-Galzi et al., 2006). The virus

has since then spread fast and is now found in almost all the major rice growing regions and in all rice ecosystems (Musiiime et al., 2005). The high yield losses have been observed in fields located near the lake Victoria basin (Ochola and Tusiime, 2011). The locally preferred rice varieties with good culinary properties, including 'Supa', have been reported to be highly affected by the virus (Ochola and Tusiime 2011).

The control of RYMV disease was mainly directed to reduce disease pressure by planting resistant varieties (Yuanhong et al., 2006). However, development of RYMV disease resistant genotypes requires on exploitation of natural resistance sources (Zouzou et al., 2008). In Uganda, the rice breeding program has recently introduced new materials that include intraspecific and interspecific rice genotypes to broaden their germplasm. Among these new materials, some have been identified as potential sources of resistance to RYMV (Ochola and

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Tusiime, 2011). These should be used to improve RYMV susceptible rice varieties and thus increase productivity in Uganda. However, introgressing resistance into susceptible cultivars requires an understanding of the nature of inheritance and of the gene action controlling resistance (Kornegay *et al.*, 1980). The information of two types of combining ability; general (GCA) and specific (SCA), from diallel analysis is recognized as important in designing plant breeding programmes (Sarkar *et al.*, 2002). Thus, choice of appropriate parental materials and combinations should help to plan appropriate breeding methodologies to develop resistant lines to RYMV. However, the information of the combining ability in Ugandan local and introduced rice genotypes is not yet adequately understood. The objective of this study was to characterize the inheritance of resistance in locally relevant material, and to identify good combiners for resistance to RYMV to use as parents to develop resistant varieties with high yield.

MATERIAL AND METHODS

Experimental Location

The experiment was conducted in a greenhouse of National Crops Resources Research Institute (NaCRRI)-Namulonge, Uganda (located at 0° 32" N of the Equator and 32° 37" E) during March 2011 to June 2012.

Development of Breeding Population

Nine parents (Table 1) including five *O. sativa* genotypes and 4 *O. sativa* x *O. glaberrima* genotypes were crossed in a full-diallel pattern to produce F1 families. Planting of parents was staggered at weekly intervals in order to synchronize flowering dates. At flowering, the emasculation of the anthers was done without affecting the female reproductive organ a day before opening to prevent self pollination. The vacuum emasculation method was used (Lamo, 2010). This was normally done in the morning between 6h: 00 AM and 9h: 00 AM during the period of anthesis. The flowers were bagged immediately after artificial pollination. The mature seeds from hand crossing were harvested 25 to 30 days after pollination. Seeds from each female panicle were harvested and bagged separately. Rice crossing has a high level of sterility, causing failure to obtain some crosses, with 41 successful crosses which were selfed to generate F2 populations.

Evaluation for RYMV Resistance

The forty one F2's generated and the nine parents were planted in a greenhouse in 4 wooden boxes (each

measuring 7 x 1.5 x 0.6m) using a 25 x 2 alpha lattice design with two replications. Fourteen days after planting, the plants were inoculated with isolate from Iganga (Eastern Uganda). To obtain inoculum, one gram of infected leaf tissue was first crushed in a drop of doubly-distilled water using sterile mortar and pestle until 80% of the leaf tissue material was macerated.

The resultant leaf extract was diluted by addition of 10 ml of doubly-distilled water. The plant infection was achieved through mechanical inoculation. The viral leaf extract was finger-rubbed onto two upper leaves from the base to the top using pieces of cotton wool. Carborandum powder was used as an abrasive material to injure the epidermal tissues of the plant and aid the infection (Konaté *et al.*, 1997). The inoculation was repeated one week later to ensure that adequate levels of inoculum pressure were attained.

Data Collection and Analysis

Severity of RYMV disease symptoms was scored using the IRRI standard scale of 1-9 (IRRI, 2002). In this scale 1: no symptoms observed; 3: leaves green but with sparse dots or streaks and less than 5% reduction of height; 5: leaves green or pale green with mottling and 6%-25% reduction of height, flowering slightly delayed; 7: leaves pale yellow or yellow and 26-75% reduction of height, flowering delayed; and 9: leaves yellow or orange with more than 75% reduction of height, no flowering or some plants dead. The plants were scored at one, two, three and four weeks after inoculation when the disease severity was high and symptoms were clearly expressed and distinguishable.

General analysis of variance of data for resistance was performed with GenStat 14th Ed. Parents and crosses were considered as fixed effects, while replications were considered as random effects. The GCA and the SCA were estimated using Method 1 Model 1 as described by Griffing (1956). The statistical model for this analysis was: $Y_{ijk} = \mu + g_i + g_j + s_{ij} + r_{ij} + e_{ijk}$, where μ : the overall mean, g_i : the GCA effect of the i^{th} parent, g_j : the GCA effect of the j^{th} parent, s_{ij} : the SCA effect of the ij^{th} genotype, r_{ij} : the reciprocal effect of the ij^{th} genotype, and e_{ijk} : the environmental effect of the ijk^{th} observation (Griffing, 1956). Estimates of GCA and SCA effects and standard errors were obtained according to directions provided by Gibson (2014, unpublished paper)

RESULTS

Analysis of Variance in F2 Progenies for Resistance to RYMV

The results in table 2 showed highly significant differences ($P \leq 0.001$) among the genotypes. GCA and

Table 1. Parental lines used in hybridization.

Genotype	Pedigree	Origin	Specie	Reaction to RYMV
Gigante	Local-Mozambique	Africa Rice	O.sativa	R
K 85	Unknown	Local-Uganda	O.sativa	S
K 5	Unknown	Local-Uganda	O.sativa	S
WAC 116	IRIS 251- 42131	Africa Rice	O.sativa	R
WAC 117	IRIS 251- 42157	Africa Rice	O.sativa	R
Naric 1	IRAT 257	IITA Nigeria	O.sativa x O. glaberrima	R
Nerica 1	WAB 450-I-B-P-38-HB	Africa Rice	O.sativa x O. glaberrima	MR
Nerica 4	WAB 450-I-B-P-91-HB	Africa Rice	O.sativa x O. glaberrima	MR
Nerica 6	WAB 450-I-B-P-160-HB	Africa Rice	O.sativa x O. glaberrima	MR

R= Resistant, MR= Moderately Resistant, S= Susceptible. O= Oryza

Table 2. Mean squares and variance components from the analysis of variance for RYMV disease scores in rice parental and F2 populations from a 9 x 9 diallel cross.

Source of variation	d.f	m.s	v.c
Genotypes	49	1.64***	
GCA	8	6.51***	0.65
SCA	27	0.83***	0.39
Recip	14	0.41***	0.18
Error	49	0.05	0.05
^a BR $(2\delta^2g)/(2\delta^2g + \delta^2s)$			0.77
^b BSCGD $(2\delta^2g + \delta^2s)/(2\delta^2g + \delta^2s + \delta^2e) \approx H$			0.97
^c NSCGD $(2\delta^2g)/(2\delta^2g + \delta^2s + \delta^2e) \approx h^2$			0.75

*** significant at 0.001 probability level ; ^a BK= Baker's Ratio (Relative importance of GCA and SCA according to Baker (1978)); ^b Broad sense coefficient of genetic determination (analogous to H); ^c Narrow sense coefficient of genetic determination (analogous to h²); δ^2g and δ^2s , are GCA and SCA components respectively; δ^2e is the error component averaged over two replications. The calculation of all MS and Coefficient of Genetic Determination values are based on entry means.

SCA mean squares were both highly significant ($P \leq 0.001$). The proportion of GCA variance $((2\delta^2gca)/(2\delta^2gca + \delta^2sca) = 0.77)$ was much higher than that of the SCA (Baker, 1978), although both were statistically significant. The broad sense and narrow sense coefficient of genetic determination were high with values of 0.97 and 0.75, respective

General Combining Ability (GCA)

Parental means and GCA effects for resistance to RYMV are presented in Table 3. The O. sativa parents K5 and K85 had significant positive GCA effects ($P \leq 0.001$) and

parental means of 5 and 7, respectively. The parents Nerica 6, Naric 1 and WAC 116 had the strongest negative GCA values ($P \leq 0.001$) and their parental means varied between 3 and 5. Parental lines Nerica 1, and Nerica 4 had non-significant positive GCA effects and Gigante had a non-significant negative GCA effect with the parental means varying between 3 and 5.

Specific Combining Ability

The estimates of SCA effect are given in Table 4. The results showed that out of 22 hybrid combinations four of

Table 3. Estimates of general combining ability effects for resistance to RYMV in rice.

Parents	Parental mean	GCA effect
Gigante	3.33	-0.03 ^{ns}
K5	6.83	0.78 ^{***}
K85	7.00	1.38 ^{***}
Nerica 1	4.42	0.06 ^{ns}
Nerica 4	4.92	0.05 ^{ns}
Nerica 6	3.79	-0.80 ^{***}
Naric 1	3.17	- 0.86 ^{***}
WAC 116	3.00	-0.63 ^{***}
WAC 117	3.00	-0.14 [*]

^{*}, ^{**}, ^{***} Significant at 0.05, 0.01, 0.001 probability levels respectively; ^{ns} not significant at 0.05 probability.

Table 4. Specific combining ability effects for resistance to RYMV in F2 rice populations.

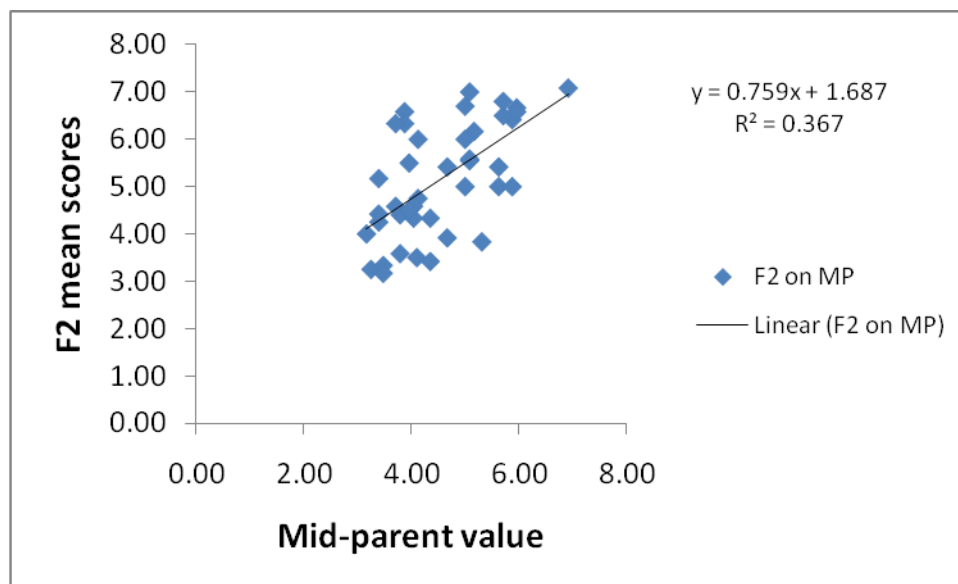
	Male								
	Gig	K5	K85	N1	N4	N6	Naric1	WAC116	WAC117
Female									
Gig	-1.61 ^{***}	0.53 ^{***}	-0.19 ^{ns}	1.42 ^{***}	0.35 ^{**}			-0.13 ^{ns}	-0.34 ^{ns}
K5		0.27 ^{ns}	-0.08 ^{ns}	-0.64 ^{***}	-0.12 ^{ns}		0.58 ^{***}		
K85			-0.76 ^{***}	0.20 ^{ns}	0.19 ^{ns}				
N 1				-0.71 ^{***}	-0.45 ^{**}	-0.77 ^{***}	-0.22 ^{ns}	0.14 ^{ns}	1.41 ^{***}
N 4					-0.19 ^{ns}	-0.38 ^{**}	0.26 ^{ns}	0.08 ^{ns}	
N 6						0.38 ^{**}	-0.10 ^{ns}		0.73 ^{***}
Naric 1							-0.12 ^{ns}		
WAC 116								-0.75 ^{***}	
WAC 117									-1.72 ^{***}

^{**}, ^{***} significant at 0.01, 0.001 probability levels respectively; ^{ns} not significant at 0.05 probability

Table 5. Analysis of variance for regression of F_2 scores on the mid-parental-values.

Source	d.f	m.s.
Regression	1	20.63***
Residual	39	0.91
Total	40	1.40

b= a regression coefficient which is a direct estimate of narrow sense heritability 0.76

**Figure 1.** Regression of RYMV severity scores of F_2 progeny means on that of the mid-parent.

them (viz. K5 x Nerica 1 (-0.64), Nerica 1 x Nerica 6 (-0.45), Nerica 1 x Nerica 4 (-0.77) and Nerica 4 x Nerica 6 (-0.38)) produced significant and negative SCA effect for resistance to RYMV. Five combinations (viz. Gigante x K5 (0.53), Gigante x Nerica 1 (0.35), K5 x Naric 1 (0.58), Nerica 1 x WAC 117 (1.41) and Nerica 6 x WAC 117 (0.73)) possessed significant and positive SCA effect for resistance to RYMV. Note that Gigante, despite its own resistance, did not interact favorably with other parents.

Heritability Estimates of Resistance to RYMV

The regression of the F_2 offspring means against the mid-parent values gave a significant regression coefficient “b” of 0.76 ($P < 0.01$; Table 5), with an r^2 value of 0.37, indicating that 37% (R^2) of the total variation in the progeny mean scores was accounted for by the mid-parental scores (Figure 1). The narrow sense coefficient of genetic determination ($[2\delta^2g / (2\delta^2g + \delta^2s + \delta^2e)] = 0.75$, Table 2) was in almost perfect agreement with the

“b” value. The broad sense coefficient of genetic determination ($[(2\delta^2g + \delta^2s) / (2\delta^2g + \delta^2s + \delta^2e)] = 0.97$) (Table 2) was noticeably larger than “b” respectively, reflecting the significant contribution of SCA effects.

DISCUSSION

Evaluation of RYMV conducted with nine parental lines and their forty one generated F_2 's revealed major and highly significant differences for resistance to RYMV among the genotypes. These differences were due to the considerable genetic diversity among the parents and their respective crosses. GCA and SCA mean squares were both highly significant ($P \leq 0.001$). The effect of the parent on the phenotypic mean of its crosses was evaluated by estimating GCA effects. Desirable parents are those with significant GCA effects in the right direction for the trait of interest (Dabholkar, 1992; Singh and Chaudary, 2004). In the present study, negative GCA effects were desirable, since they indicate a contribution

towards resistance; while positive values indicate a contribution towards susceptibility (Lokko et al. 2004). Naric 1 had the lowest negative GCA value, followed by Nerica 6, WAC 116 and WAC 117, reflecting their ranking in general combining ability. Our results suggest that the use of these parents in breeding programmes for resistance to RYMV would be expected to produce progenies with increased resistance. In the majority of the cases, good general combiners showed low mean scores themselves expect Gigante that despite its own low mean combined badly for resistance to RYMV. It is notable that Gigante, an often used source of resistance to RYMV, did not transmit its resistance effectively to crosses with the other parents in this study. K85 was the worst combiner, as shown by its highly positive GCA value, followed by K5. If these two parents are used in breeding programmes, they should be used cautiously, because their progenies are expected to show increased susceptibility to RYMV.

Specific combining ability (SCA) of a cross reflects the effect of non-additive gene action for a trait. The improvement of crop breeding based on the selection of hybrid combinations use non-additive gene action of a trait as an indicator. Thus, a highly significant SCA effect is desirable for a successful hybrid breeding program. In the present study, estimates of SCA were highly significant; suggesting that resistance levels of certain parent combinations in the progeny were significantly higher or lower than the predictions based on the parents' GCA values. The SCA values may also reflect additive x additive epistasis that can be captured in a pure line, so should be considered along with the mean in order to anticipate which crosses may generate the best pure line progeny.

According to Falconer and Mackay (1996) the regression coefficient "b" from mid-parent offspring regression is a direct estimate of narrow sense heritability (h^2). In this study, narrow sense heritability (h^2) was high, estimated from both variance ratios and regression coefficients. Thus, the additive gene action was considered to be the most important genetic influence on resistance to RYMV. This, along with the relative importance of GCA compared to SCA, indicates that the performance of crosses can be reasonably predicted by the parental GCA values (Baker, 1978). Since the main type of gene action is additive, selection in early generations should be effective and the superiority of selection in one generation will be largely passed from one generation to the next. Similar results were reported in earlier investigations for the inheritance of resistance to RYMV. For instance, Mogga et al. (2010) reported high estimates of narrow sense heritability from both variance ratio (79%) and regression coefficients (82%). Furthermore, Paul et al. (2003), using generation mean analysis, he found that the inheritance of resistance to RYMV depended primarily on additive gene effects with some dominance.

CONCLUSION

In this study it is concluded that the selection of appropriate parent in breeding programmes should be based on both GCA effect and parental mean performance. Based on the predominance of additive gene effects observed in our results, we believe that resistance from interspecific parental lines may be used consistently to produce hybrid rice resistant to RYMV. Moreover, resistance could be improved through a pedigree and/or backcross selection program and the early generation selection for RYMV resistance should be effective.

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