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Existence of Two Pathotypes of Rice *Yellow mottle virus*, Genus Sobemovirus, in Mali

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Abstract: Screenhouse studies were conducted using 10 RYMV isolates from 6 different localities in Mali against 8 WARDA differential rice genotypes to investigate the possible existence and classification of different pathotypes of RYMV in Mali. The reaction of 8 rice genotypes to the 10 RYMV isolates was different in terms of SPAD and yield reductions. The interaction between isolates and rice cultivar was also significant. AMMI cluster analysis revealed the existence of two pathotypes (HPI and MPI) of RYMV isolates in Mali. Of 8 rice genotypes studied, only Bouake 189 was highly susceptible to the two pathotypes. This information could be useful in the rice breeding programs aiming at deployment of RYMV resistant genotypes to different rice ecologies and localities in Mali.

Key words: RYMV, diversity, pathotypes, genotype, SPAD reduction, yield reduction

INTRODUCTION

Rice *Yellow mottle virus* (RYMV), genus sobemovirus, is the only known virus disease of rice in Africa and it is indigenous to the continent. RYMV was first described in 1966 in Kenya (Bakker, 1970) and has subsequently been reported throughout West Africa, Madagascar, Tanzania, Zanzibar and most recently Mozambique. The spread of the disease has been facilitated by intensive agriculture husbandry practice (Awoderu, 1991) and this disease is limited to rainfed and irrigated lowlands. The virus is highly infectious, environmentally stable and is transmitted both mechanically and by Chrysomelid beetle vectors in the field (Hull, 1988; Abo *et al*., 1998; Nwilene, 1999), but not transmitted by seed (Konaté *et al*., 2001). The estimated yield reduction due to RYMV infection in susceptible lowland cultivars was up to 97% (Reckhaus and Adriamasintseheno, 1995) and as high as 54% in a tolerant upland cultivars (Fomba, 1988). RYMV is known to be one of the most economically damaging diseases of rice in sub-Saharan Africa (Ndjiondjop *et al*., 2001). The virus, depending on the genotype, causes yellowing, mottling and stunting of infected plants with narrowing of emerging leaves and when infection occurs early, the plant normally dies.

Varietal resistance seems the most promising control mechanism. However, two types of resistance had been found so far: a high natural resistance in *Oryza glaberima* land race and a local indica cultivar Gigante (Ndjiondjop *et al*., 2001) and a partial resistance in japonica varities (Albar *et al*., 2003; Ghesquière *et al*., 1997). Irrigated rice farmers generally prefer the higher yielding indica rice which are susceptible to the virus. Therefore, all major rice varieties grown in West African lowlands, such as Bouaké 189, Jaya, BG 90-2 and IR 1529-680-3, are highly susceptible to RYMV (Séré and Sy, 1997).

The existence of different RYMV strains in the field (Konate *et al*., 1997; N'Guessan *et al*., 2000) that are different in their pathogenicity is often a matter of considerable practical importance. Therefore reliable criteria are needed for distinguishing, identifying and pathotyping these strains. Screening for durable resistance need to be done with the most virulent pathotypes as, most often, the breakdown in resistance is attributed to a poor prerelease challenge with appropriate pathogen population (Mekwatanakarn *et al*., 2000).

In this study, our aim was to investigate the existence of a highly virulent pathotype of Rice yellow mottle virus in Mali to be used in screening for durable resistance. Besides, identification of different virulence strains and pathotypes from different localities will be used to identify and characterize rice genotypes for stable resistance to RYMV as such information is useful for developing rice varieties with durable resistant to RYMV in Mali.

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MATERIALS AND METHODS

Rice genotypes: Eight differential rice genotypes (Table 1) used in this study were established by WARDA plant pathology unit to identify difference in virulence among RYMV isolates (WARDA, 2001).

RYMV isolates: Ten RYMV isolates (Table 2) used for this study were collected from rice in 6 different localities in Mali. Before used, each isolate was first propagated in the susceptible rice variety Bouake189, following mechanical inoculation of 21 old plants in the screenhouse. Four weeks after inoculation, leaves from each RYMV isolate bearing typical yellow mottle symptoms were harvested and used for inoculating rice genotypes. By this way, the inoculum of the isolates were standardized.

Inoculation of rice genotypes: The eight young differential rice genotypes were inoculated mechanically (Fauquet and Thouvenel, 1977) with the 10 isolates in the screenhouse 21 days after direct seedling in 3 replicates. Another sets of same eight young differential rice genotypes in 3 replicates not inoculated were used as controls. Infected leaf samples of each RYMV isolate were ground with 0.01 M phosphate buffer pH 7.0 at the ratio of 1:10 (w/v) and the resulting homogenate filtered through cheesecloth. Carborundum powder (600 mesh) was added to the inoculum to aid the penetration of the virus into leaf tissues. Each rice plant was inoculated thrice same day.

SPAD and yield measurement: Chlorophyll (SPAD) and yield reductions due to RYMV disease were evaluated.

Table 1: Identity of differential rice genotype used

Code	Genotype	Variety
V_1	Gigante	Indica
V_2	Bouake 189	Indica
V_3	Faro 11	Japonica
V_4	Moroberekan	Japonica
V_5	Lac 23	Japonica
V_6	ITA 235	Japonica
V_7	PNA 647F4-56	Japonica
V_8	H 232-44-1-1	Indica

Table 2: Identity of RYMV isolates used for pathological study

S/N	Code	Isolate	Locality	Host plant	Ecology
1	MA	PR44	Kogoni	BG 90-2	Irrigated
2	MB	PR35	Macina	Kogoni 91	Irrigated
3	MC	PR28	N'Debougou	Kogoni 91	Irrigated
4	MD	PR17	Niono	Kogoni 91	Irrigated
5	ME	PR16	Selingue	Nil	Irrigated
6	MF	PR76	Selingue	ADNY 11	Irrigated
7	MG	PR92	Selingue	ADNY 11	Irrigated
8	MH	PR95	Selingue	ADNY 11	Irrigated
9	MI	PR121	Sikasso	Nil	Lowland
10	MJ	PR124	Sikasso	SIK 131	Lowland

Chlorophyll content was measured using SPAD 502 Chlorophyll Meter (Monje and Bugbee, 1992; Martines and Guiamet, 2004) at 42 days after inoculation. SPAD and yield measurement were obtained both for test and control genotypes.

Data analysis: Using SPAD and yield data from both test and control genotypes, percentage SPAD and yield reductions due to RYMV disease were determined for each genotype. IRRISTAT version 4.3 statistical software was used for all the analyses. Variance and mean comparison of percentage SPAD and yield reductions were performed. Genotype (cultivar) by environment (isolate) interaction effects on SPAD and yield reductions was carried out using additive main effect and multiplicative interaction (AMMI) analysis (Ebdon and Gauch, 2002). Cluster dendograms showing classification of genotype (cultivar) levels of resistance to environment (isolate) and classification of environment (isolate) pathogenic level to genotype (cultivar) were plotted using AMMI analysis.

RESULTS

Considerable difference was observed in the reactions of 8 rice genotypes to 10 RYMV isolates from 6 different localities in Mali in terms of SPAD and yield

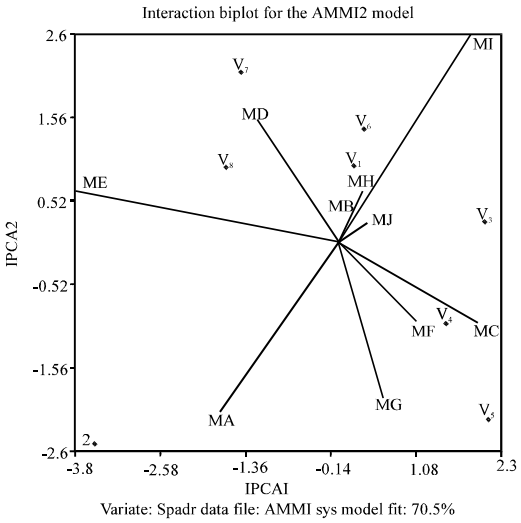

Fig. 1: Genotype (cultivar) by environment (isolate) interaction effects on SPAD reduction using additive main effects and multiplicate interaction (AMMI) analysis. Genotype: V_1 = Gigante; V_2 = Bouake189; V_3 = Faro11; V_4 = Moroberekan; V_5 = Lac 23; V_6 = ITA235; V_7 = PNA647F4-56; V_8 = H232-44-1-1. Environment: MA = PR44; MB = PR35; MC = PR28; MD = PR17; ME = PR16; MF = PR76; MG = PR92; MH = PR95; MI = PR121; MJ = PR124

Table 3: Analysis of means comparison for percentage SPAD reduction due to RYMV disease

	Genotype								
RYMV Isolate	Gigante	Bouake189	Faro11	Moroberekan	Lac 23	ITA235	PNA647F4-56	H232-44-1-1	I-MEAN
PR44	11.3a	36.5a	9.3a-c	18.3a	17.5ab	19.2a	13.2ab	11.7ab	17.1ab
PR35	18.3a	28.7a	11.3a-c	10.7ab	19.7a	14.2ab	21.0a	9.8ab	16.7ab
PR28	14.5a	28.2a	14.2ab	17.3ab	23.5a	9.8ab	18.0ab	11.3ab	17.1ab
PR17	15.7a	33.3a	13.5a-c	21.0a	13.3ab	11.2ab	21.8a	18.7a	18.6a
PR16	16.5a	39.2a	4.8bc	10.7ab	12.5ab	10.7ab	23.2a	15.2ab	16.6ab
PR76	13.3a	34.8a	13.0a	14.0ab	19.2a	12.2ab	16.7ab	8.7b	16.5ab
PR92	19.8a	38.2a	5.2c	14.8ab	17.5ab	8.2b	8.8b	11.2ab	15.5a-c
PR95	17.2a	34.7a	9.8a-c	16.8ab	16.0ab	14.5ab	13.8ab	14.0ab	17.1ab
PR121	18.8a	30.0a	9.5a-c	7.3b	9.0b	12.0ab	15.0ab	11.0ab	14.1bc
PR124	12.0a	26.0a	4.7a-c	14.5ab	12.3ab	6.3b	13.5ab	9.7ab	12.4c
V-MEAN	15.8	33	9.5	14.6	16.1	11.8	16.5	12.1	16.2

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test

Table 4: Analysis of means comparison for percentage yields reduction due to RYMV disease

	Genotype								
RYMV Isolate	Gigante	Bouake189	Faro11	Moroberekan	Lac 23	ITA235	PNA647F4-56	H232-44-1-1	I-MEAN
PR44	49.5ab	70.5a	56.4a	87.2a	36.7a	73.6a	86.8a	92.9a	69.2a
PR35	9.7c	37.8a	62.2a	81.6ab	46.4a	40.6ab	60.2a	86.4a	53.1b
PR28	31.9a-c	39.1a	70.8a	81.1ab	68.7a	46.2ab	77.2a	73.4a	61.0ab
PR17	44.4a-c	80.0a	58.3a	58.6ab	60.2a	69.1ab	81.3a	85.2a	67.1a
PR16	22.2bc	88.7a	44.8a	76.6ab	65.4a	34.9b	94.7a	94.8a	65.2ab
PR76	38.3a-c	58.0a	71.5a	72.5ab	59.3a	64.2ab	72.5a	92.5a	66.1a
PR92	59.5a	73.6a	71.6a	45.8b	52.1a	60.9ab	89.8a	92.6a	68.2a
PR95	31.9a-c	50.5a	71.1a	75.5ab	46.5a	62.0ab	92.0a	87.0a	64.6a
PR121	42.9a-c	65.2a	36.9a	68.4ab	44.5a	61.2ab	73.5a	77.4a	58.8ab
PR124	55.5ab	77.9a	65.6a	53.5ab	40.8a	45.4ab	78.3a	81.0a	62.3ab
V-MEAN	38.6	64.1	60.9	70.1	52.1	55.8	80.6	86.3	63.6

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test

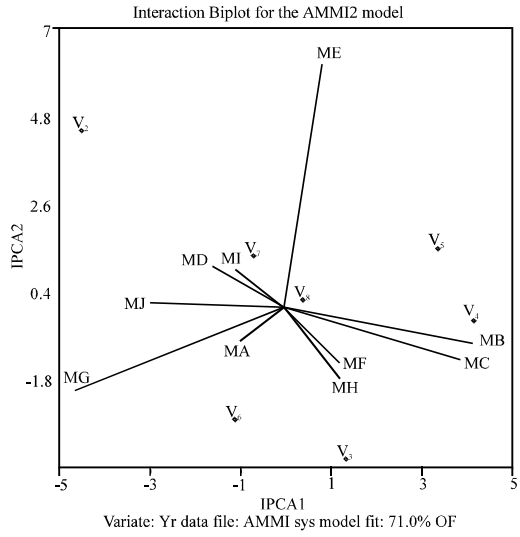

Fig. 2: Genotype (cultivar) by environment (isolate) interaction effects on yield reduction using additive main effects and multiplicate interaction (AMMI) analysis. Genotype: V_1 = Gigante; V_2 = Bouake189; V_3 = Faro11; V_4 = Moroberekan; V_5 = Lac 23; V_6 = ITA235; V_7 = PNA647F4-56; V_8 = H232 - 44-1-1. Environment: MA = PR44; MB = PR35; MC = PR28; MD = PR17; ME = PR16; MF = PR76; MG = PR92; MH = PR95; MI = PR121; MJ = PR124

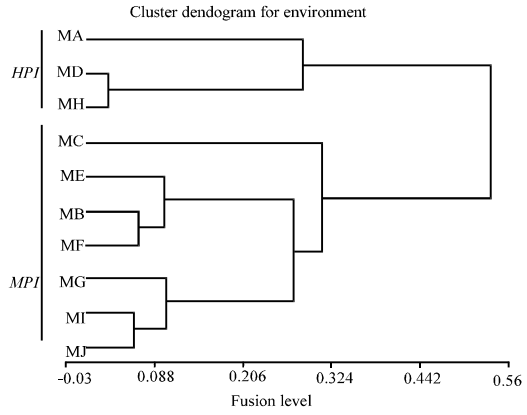

Fig. 3: Cluster dendogram showing classification of environment (isolate) pathogenic level to genotype (cultivar) using additive main effects and multiplicate interaction (AMMI) analysis. Environment: MA = PR44; MB = PR35; MC = PR28; MD = PR17; ME = PR16; MF = PR76; MG = PR92; MH = PR95; MI = PR121; MJ = PR124

reduction (Table 3). Percentage yield and SPAD reductions, due to RYMV disease, were between 38.6-86.3 and 9.5-33%, respectively (Tables 3 and 4). According to AMMI analysis, MA and MD isolates were responsible

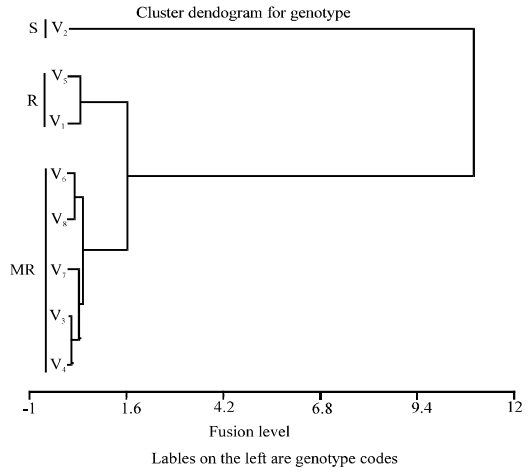

Fig. 4: Cluster dendogram showing classification of genotype (cultivar) level of resistance to environment (isolate) using additive main effects and multiplicate interaction (AMMI) analysis. Genotype: V_1 = Gigante; V_2 = Bouake189; V_3 = Faro11; V_4 = Moroberekan; V_5 = Lac 23; V_6 = ITA235; V_7= PNA647F4-56; V_8= H232-44-1-1. R = Resistant; MR = Moderately resistant; S = Susceptible

mainly for unfavorable interactive conditions leading to significant yield and SPAD reduction in all the rice cultivars (Fig. 1 and 2). Based on cluster dendrogram classification for isolates pathogenic and genotypes viral resistance levels, MA, MD, MH were classified as Highly Pathogenic Isolates (HPI) and MB, MC, ME, MF, MG, MI, MJ were classified as Mildly Pathogenic Isolates (MPI). Two genotypes (Gigante and Lac 23) were highly resistant, while the five others were moderately resistant and one genotype (Bouake 189) was susceptible (Fig. 3 and 4).

DISCUSSION

This study revealed the existence of two pathotypes of RYMV isolates in six localities in Mali. The two pathotypes consist of the Highly Pathogenic Isolates (HPI) and the Mildly Pathogenic Isolates (MPI). The Additive Main Effect and Multiplicative Interaction (AMMI) analysis seemed effective in understanding and explaining complex genotype by environment (GE) interactions between the rice genotypes and RYMV pathotypes (Ebdon and Gauch, 2002; Onasanya *et al*., 2004). Such interactions could generate complex data sets difficult to understand with ordinary analysis of variance (ANOVA). In the current study, 10 RYMV isolates used covered major rice ecologies from six different localities in Mali leading to very high RYMV interactions among rice genotypes. The existence of HPI and MPI RYMV pathotypes obtained in this study (N'Guessan *et al*., 2000) have led to differential interactions among genotypes with heavy implications on the genotype resistance and yield stability.

As revealed by this study, genotypes pathogenic resistance to HPI and MPI RYMV pathotypes first occurs at the level of the individual and involves physiological or behavioral tolerance or adaptability. Subsequent response to increasing viral pathogenicity may involve survival only of the better-adapted genotypes (Ebdon and Gauch, 2002; Barrett and Rosenberg, 1981). HPI pathotypes, which consist of three isolates, could be described as possessing both stable and high level of virulence affecting genotypes resistance to RYMV across 6 localities in Mali. Under different rice ecologies in Mali, V_1 and V_5 genotypes possessed heterogenous viral resistance characteristics making them to be more stable, adaptable and more resistant to stress induced by HPI pathotypes originated from different localities. Genotypes that have adapted to endure variable isolates or strains infestations are more likely to tolerate an independent stress compared to those genotypes that are only adapted to a fixed isolate or strain (Barrett and Rosenberg, 1981; Annicchiarico and Perenzin, 1994).

As RYMV isolates population increases, there is probability that MPI pathotypes population will be more than that of HPI and possible interactions between these two pathotypes could lead to the emergence of new highly virulent strains. The use of highly resistant genotypes (V_1, V_5) will potentially reduce HPI and MPI pathotypes population and their interactions. There is probability that the two resistant genotypes (V_1, V_5) obtained in this study will survive and evolve through combinations of genes present in the population (Barrett and Rosenberg, 1981; Crossa *et al*., 1990) since population resistance is enhanced by genes polymorphism that may result in short-term selection of more tolerant genotypes in stressful viral environments (Ebdon and Gauch, 2002; Barrett and Rosenberg, 1981).

Conclusively, the high genotypes by environment interactions in the reactions of rice genotypes to RYMV revealed the existence of two pathotypes of RYMV in Mali. This information could be useful in the rice breeding programs aiming at deployment of RYMV resistant genotypes to different rice ecologies and localities in Mali.

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