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Symptoms Diversity Elicited by *Rice Yellow Mottle Virus* (RYMV) Infection on *Oryza* Species in Nigeria

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ABSTRACT

RYMV is endemic in Africa and assumes great economic challenge to rice production in the region where rice is a staple food. The disease is available in all rice growing areas in Nigeria, and constitutes great threat to self-sufficiency in the country's rice development sector. Rice genotypes were screened for resistance to RYMV infection in the screenhouse at Badeggi, Niger State between 2008 and 2016, using virus isolates from farmers' fields in north central Nigeria. Based on the Standard Evaluation Scale (SES) score, Back Inoculation Test and Enzyme Linked Immunosorbent Assay (ELISA) results, the disease was present in rice genotypes evaluated and elicited diversity of symptoms. Symptom expression varied from 2 to 4 weeks in all infected plants; producing usual yellow mottle symptoms, bunchy or dispersed tiller formation, orange or pink coloration, stunted growth (height reduction) streak and dots formation as well as hypersensitive reactions on leaves. RYMV infections appeared to have also delayed senescence leading to multiple unproductive tillering. Irrespective of the levels of symptom expression based on the SES score, leaf extracts of all inoculated entries were highly infectious, and elicited similar symptoms on SES score of 7 on FARO 29 (BG 90-2) in a back inoculation test. Leaf extract from Gigante, which showed symptoms of localized infection to the virus was also infectious and elicited clear RYMV symptoms on back inoculation to BG 90-2. Degree of symptom expression did not correlate with virus content and all screened rice genotypes contained varying levels of the virus (RYMV) based on ELISA. NERICA-L 22 had the highest virus content of 62.5%, followed by NERICA-L 21 (56.3%) and NERICA-L 14 (52.1%); whereas, a low virus content (%) of 2.1%, 4.2% and 14.6% were recorded by NERICA-L 17, 23, 26, 36, & 42, FARO 44, NCRO 48 and Gigante respectively. The result showed that Gigante and NIL 54 are the most reliable resistant rice genotypes against the virus.
Keywords: RYMV; Symptomatology; ELISA; Hypersensitivity; Badeggi

INTRODUCTION

Rice has traditionally been an important basic food commodity for a large part of the world's human population, especially in East, South, Southeast Asia, the Middle East, Latin America, and the West Indies. It has also been the most important staple food for certain populations in Sub-Saharan Africa, and West Africa in particular. In Nigeria, rice is an important cereal, with the share of rice in cereals consumed increasing from 15% in the 1970s to 26% in the early 1990s (Kebbah *et al.,* 2003). The overall interest of the Federal Ministry of Agriculture in Nigeria is to promote agricultural food and cash crops which hold great prospects in view of obvious need for diversification of Nigerian economy. However, RYMV has become one of the major limiting factors to rice production for lowland and irrigated ecosystems (Abo *et al.,* 1998; Salaudeen *et al.,* 2008a). Severe yield losses of 50 – 100% have been recorded on rice crops in farmers' field in Africa and adjoining islands (John *et al.,* 1984; Fomba, 1988; Taylor *et al.,* 1990; Abo *et al.,* 2002; Salaudeen *et al.,* 2008b).

RYMV belongs to *Sobemovirus* group, a single stranded positive sense RNA (Bakker, 1975; Hull, 1988). It was first noticed in November 1966 along the shores of the Kavirondo Gulf of Lake Victoria Kenya (East Africa) (Bakker, 1970). The disease was first reported in West Africa in 1975 (John *et al.,* 1984), and has since been reported to occur in almost all the West African countries where rice production is intensified (Abo *et al.,* 2002). RYMV is indigenous to Africa and has come to limelight with the introduction of exotic rice varieties from Asia, coupled with intensification of cropping practices without dry season gaps (Rossel *et al.,* 1982a, b; Ou, 1985). It is characterized by mottling and yellowing or orange coloration of the leaves of the infected plants (Bakker, 1970, 1974). The intensity of symptom expression depends on the genotype especially the lowland *indica* types. Variety of symptoms including delayed flowering with poorly exerted panicles and bearing sterile and discoloured spikelets (Abo *et al.,* 2001). Severely infected plants will be stunted and death may also occur in very susceptible varieties.

Transmission of the virus is by some insect vectors, and through mechanical contact (Bakker, 1975; Abo *et al.*, 2000; Nwilene *et al.*, 2009), but not through seed (Konate *et al.*, 2001; Abo *et al.*, 2004; Allarangaye *et al.*, 2006). It has been transmitted in irrigated rice crops by grazing cows, donkeys and grass rats (Sarra and Peters, 2003). Infection may appear at the edges (border) of the fields, within the field, and on ratooned/volunteer rice as well as weeds or wild rice species (Abo *et al.*, 2002; Salaudeen *et al.*, 2008b; Nwilene *et al.*, 2009), which serve as sources of inoculum for fresh infection in the field.

RYMV disease has assumed great economic importance throughout Africa especially in Nigeria, where it has been reported in every rice growing environment since 1980s following intensification of rice production in the country. Symptomatology of RYMV infection in the field is vital to the disease management and epidemiology. This investigation will help in no small measure to validate records on symptom expressions of the disease on rice, as well as serve to bridge the information gap that exists in terms of RYMV symptomatology in Nigeria.

MATERIALS AND METHODS

The experiment was conducted in the screenhouse at the National Cereals Research Institute (NCRI) Badeggi, Niger State between August 2008 and August 2016. The rice genotypes were obtained from Africa Rice Center (Africa Rice) Cotonou, Benin Republic, and National Cereals Research Institute (NCRI), Badeggi, Nigeria within the period. Bouake 189 and FARO 29 (BG 90-2) were used as susceptible checks (SCK), whereas Moroberekan and Gigante served as resistant checks (RCK) in the first trial. In the confirmatory trial in 2016, four near isogenic lines (NILs), two farmer's conventional genotypes and the checks – Gigante (RCK) and FKR 28 (SCK) were also evaluated.

Maintenance and Propagation of RYMV

RYMV infected plants collected from farmer's fields across the north central States of Niger, Kogi, Benue and Nasarawa between 2008 and 2015 were prepared (6% w/v) for serial inoculation on the highly susceptible Bouake 189 in the screenhouse. Typical yellow and mottle symptoms of the RYMV were reproduced in all inoculated samples, which served as a fresh source of inoculum throughout the experiment.

Experimental Design, Treatments and Treatment Combinations

The factorial experiment laid out in a Completely Randomized Design (CRD), with virus isolates (RYMV) and rice genotypes constituting the two factors. One level of factor A (RYMV) represents the control with zero doses (Little and Hills, 1978; Obi, 2001). The 2 x 35 factorial (70 treatment combinations) was replicated thrice, and treatment allocation was at random using the paper disc method, in which treatments were allotted without replacement (Gumez and Gumez, 1984; Obi, 2002). Two hundred and ten plastic buckets, each measuring 16 cm diameter and filled with 2.5kg sterilized fadama soil was used. The pots were laid 0.5m apart on screenhouse tables (1m above the ground level).

Planting Operation

Three (3) seeds each of test genotypes were seeded directly in the plastic buckets (16cm in diameter) containing 2.5kg freshly collected fadama soil samples. Seedlings were later thinned down to one seedling at 21 Days after Planting (DAP) to maintain equal number of seedlings per pot for all entries.

Water and Fertilizer Application

Pots were constantly supplied with fresh tap water in the mornings and evenings until maturity (i.e. 2 weeks before harvest). 2g of N: P: K 16:16:16 was applied to the plants at 28DAP to enhance tiller establishment, followed by split application of Urea (2g per pot) at 45DAP and early flowering stages respectively.

Virus Extract Preparation and Inoculation Procedure

Fresh leaf samples of virus infected Bouake 189 raised in the screenhouse were collected, chopped into pieces and stored overnight in a refrigerator (4°C). Six grammes (6g) of infected leaf-tissues was ground with 100ml-distilled water (i.e. 6% w/v) in an electric blender. The resulting homogenate filtered through cheesecloth was used for mechanical inoculation. Virus isolate was mixed with an abrasive – carborundum (600 mesh), to aid virus penetration into leaf tissues. Stroking technique, which involves holding the leaves together and gently robbing the leaves of the test plant with the virus isolate beginning from the base, was adopted. The mechanical inoculation was done at 40DAP. Three seedlings each of every sample was inoculated with the RYMV (V_1), whereas another three seedlings were left un-inoculated and served as a control (V_0). To avoid possible escapes from infection all the inoculated test plants were re-inoculated 24 hours after the first inoculation (Thottappilly and Rossel, 1993).

Data Collection

Beginning from three weeks after inoculation, samples were closely observed for symptom expressions, and disease assessments including date to symptom expression, and leaf death or desiccation score at maturity were recorded. The leaf death score was based on scale of 0 – 5 (Lafitte *et al.,* 2003), where 0 = no senescence; 1 = < 25% dead or dried leaf area; 2 = > 50% dead or dried leaf area; 3 = < 75% dead or dried leaf area; 4 = < 100% dead or dried leaf area; 5 = whole plant dead.

Disease Diagnostic Methods and Detection

Visual score based on the Standard Evaluation Scale (SES) of 1 – 9 for rice at five (5) weeks after inoculation was adopted (IRRI, 1996). A score of 1 = no symptoms observed; 3 = leaves green but with sparse dots or streaks and less than 5% of height reduction; 5 = leaves green or pale green with mottling and 6 to 25% of height reduction, flowering slightly delayed; 7 = leaves pale yellow or yellow and 26 – 75% of height

reduction, flowering delayed; whereas, score of 9 = leaves turn yellow or orange, more than 75% of height reduction, no flowering or some plants dead. Based on the SES, varieties/lines evaluated were classified as resistant (R) at SES of 1 - 3, moderately resistant (MR) at SES of 5, moderately susceptible (MS) at SES of 7 or highly susceptible (HS) at SES of 9.

Enzyme Linked Immunosorbent Assay (ELISA) Procedure

The ELISA of leaf samples was carried out to evaluate and determine virus titre in the inoculated rice plant. The indirect Antigen coated-plate ELISA (ACP-ELISA) as described by Koenig and Paul (1982) was followed. 200µl of sap macerated from 1g leaf in 100ml coating buffer (i.e. 1.5g sodium carbonate, 2.93g sodium bicarbonate, 0.20g sodium azide dissolved in 900ml H_2O and adjusted to P^H 9.6 with HCL to make up to 1 litre) was added to duplicate wells of a microtitre plate, and left over night in the refrigerator at 4°C. Using a wash bottle, the plates were washed three times with phosphate buffered saline–Tween (PBS–Tween) (i.e. 8.0g sodium chloride (NaCl), 0.2g monobasic potassium phosphate (KH_2PO_4), 1.15 g dibasic sodium phosphate (Na_2HPO_4), 0.2 g potassium chloride (KCl), 0.2g sodium azide (NaN_3), dissolved in 900 ml H_2O and adjusted to P^H 7.4 with HCl to make up to 1 litre + 0.5ml Tween 20 per litre) and, blotted dry by tapping upside down on tissue paper. Blocking of the wells was by use of 200µl of 1% bovine serum albumin (BSA) dissolved in phosphate buffered saline (PBS) (i.e. 8.0g NaCl, 0.2g KH_2PO_4, 1.15g Na_2HPO_4, 0.2g KCl, & 0.2g NaN_3, dissolved in 900ml H_2O and adjusted to P^H 7.4 with HCl to make up to 1 litre) and incubated at 37°C for 1 hour. Unabsorbed BSA is poured off, and plates were blotted dry by tapping upside down. 200µl/well of polyclonal antibodies raised in rabbits against RYMV at 1:1000 dilutions in PBS was added to the plates and left over night in the refrigerator at 4°C. Again, the plates were washed three times with PBS-Tween using wash bottle, and blotted dry by tapping upside down on tissue paper. Then, 200µl of goat anti-rabbit alkaline phosphatase

(Sigma) diluted to 1:1000 in conjugate buffer (i.e. PBS-Tween + 2% egg albumin) was added to each well, and incubated at 4°C overnight in a refrigerator. The plates were again washed three times with PBS-Tween and tapped dry on tissue paper. Then, 200µl of freshly prepared substrate (1mg/ml of p-nitrophenyl phosphate dissolved in substrate buffer (i.e. 97ml diethanolamine, 600 ml H_2O, 0.2g NaN_3, adjusted to P^H 9.6 with HCl and made up to 1 litre with H_2O) was added to each well and incubated at room temperature for 1 hour. The colour change in the substrate assessed by spectrophotometric measurement of absorbance at 405 nm was accepted as positive when the reading was at least greater or equal to twice the mean absorbance of the non-infected control rice sample.

Back-inoculation Test

Back inoculation test on FARO 29 (BG 90-2), a susceptible variety (check) was carried out at 45 days after inoculation (i.e. 85DAP). Sap from leaves of every test plant previously infected (inoculated) with RYMV isolate was prepared as described for virus extract preparation and inoculation procedure above, and inoculated to carborundum dusted 25-day-old seedlings of FARO 29 in the screenhouse. Symptom expression RYMV was observed and scored after 5 weeks from inoculation date based on SES score of 1 -9 (IRRI, 1996).

RESULTS AND DISCUSSION

The reaction levels of the first 35 rice genotypes to RYMV as determined by Standard Evaluation Scale, ELISA and Back inoculation test (BIT) in a screenhouse condition is shown on Table 1. The results indicated that 3 rice genotypes were highly susceptible (HS) at SES score of 9, 13 were moderately susceptible (MS) at SES score of 7, and 11 were moderately resistant (MR) at SES score of 5, whereas 8 rice varieties were shown to be resistant at SES of 1- 3.

The symptom expressions on rice genotypes and the leaf desiccation score at maturity for virus inoculated plants were also investigated; and virus inoculated entries (V_1) gave a lower mean leaf desiccation (dead) score of 1.2 at maturity against the un-inoculated control entries (V_0), which gave 1.7. RYMV symptoms were expressed on rice plants at about 3 weeks after mechanical inoculation in the screenhouse. Symptom expression is a visible indication of the establishment of the virus, and all inoculated rice genotypes showed obvious typical symptoms of RYMV except Gigante – a resistant *Oryza sativa*indica, which only expressed a hypersensitive reaction with the virus. Similar hypersensitive reactions were observed in NCRO 48, NERICA-L 21, NERICA-L 4, and CG-14, whereas NERICA-L 4, NERICA-L 36, FARO 52 and Bouake 189 exhibited unusually dispersed or bunchy tiller formation, that contributed to higher number of unproductive tiller formation. The resistance (hypersensitivity) expressed by Gigante is in non-expression of typical symptoms and its ability to withstand obvious damaging effects of the disease, but not by non-detection of the virus in leaf sap of inoculated plants. Irrespective of the levels of symptom expression based on the SES score, leaf extracts of all the screened rice genotypes were highly infectious, and elicited similar symptoms on SES score of 7 on FARO 29 (BG 90-2) in a back inoculation test (Table 1). Leaf extract from Gigante, which earlier showed symptom of localized infection (hypersensitive reaction) to the virus in the experiment was equally infectious and elicited clear RYMV symptoms on back inoculation to BG 90-2.

The result of ELISA test showed that leaf extract of screened rice genotypes contained varying levels of the virus (RYMV). The ELISA values (OD 405nm) indicated that NERICA-L 22 recorded the highest virus content of 0.078, followed by NERICA-L 21 (0.075) and then, NERICA-L 14 (0.073); a low virus content (%) of 0.048, 0.049, 0.050 and 0.055 were recorded by NERICA-L 17, 23, 26, 36, & 42, FARO 44, NCRO 48 and Gigante in the experiment (Table 1). NERICA-L 49

and 42 among others expressed some levels of resistance to the virus when compared to other screened rice genotypes. Ndjiondjop-Nzenkam *et al.* (2006) explained that the genetic basis of resistance to RYMV was determined through inter-specific and intra-specific crosses using TOG 5681 (*O. glaberrima*) and Gigante as RYMV resistance donors and IR 64 as the susceptible parent. The resistance was due to the presence of a single recessive gene with probably different resistance alleles in TOG 5681 and Gigante. However, according to Ebdon and Gauch (2002), population resistance is enhanced by gene polymorphism that may result in short-term selection of more tolerant genotypes in a stressful virus environment. Thus rice varieties in this experiment produced different levels of resistance to RYMV based on symptomatology, although the ELISA test indicated presence of the virus at various quantities in all inoculated rice samples. Differences in the quantities of virus regardless of symptoms expressed by infected plants might be an indication of variations in mechanism to fight virus infection among rice genotypes. Future work in molecular biology could be beneficial as well for development of resistant varieties.

The result of the confirmatory trial (Table 3) also showed that lower ELISA (OD 405nm) values were obtained for NIL 54, NIL 2, NIL 130 and FARO 52 than what was recorded in the resistant check. NIL 54 also indicated the lowest virus indexing that correlated with non-observation of visual symptoms on the plant after serial inoculation (Table 4).

The mean leaf desiccation score was lower for virus-infected plants (1.2) compared to the control, which recorded mean value of 1.7. Symptom expression on virus-inoculated rice plants in the screen house experiment was observed between 2 - 4 weeks depending on rice genotype. According to Ou (1985), the diseased plants in the field may be observed 3 – 4weeks after transplanting and could be noticed because of their yellowish appearance. Symptom expression of RYMV may be strongly

influenced by light intensity, day length, humidity, temperature, and growth stage of plant among other factors (Bakker, 1974; Albar *et al.*, 1995). Apart from the usual typical yellow mottle symptom and characteristic height reduction in all entries with the virus, 4 rice genotypes expressed bunchy/dispersed tiller formation, and the other 5 showed a characteristic hypersensitive reaction (localized infection) after 2-3 weeks of mechanical inoculation. Orange and pink coloration of leaves was observed in 2 entries, whereas streak, dots and green mottles were observed in another 3 genotypes. Virus infection also caused stunted growth and death of tillers in three rice genotypes.

CONCLUSION

Gigante and NIL 54 displayed high levels of resistance to RYMV; however, there are indications that such resistance is in the non-expression of typical symptoms and ability to withstand obvious damaging effects of the disease, but not in a total absence of the virus pathogen in the plant tissues. Based on the SES score for rice at 5 weeks after inoculation date, Back inoculation test, and the ELISA (A 405nm) results, there is indication that the RYMV was present in plant samples and elicited variety of symptoms ranging from classical yellow mottle symptoms to stunted growth and bunchy or dispersed tiller formation as well as orange or pink coloration and green mottling of the youngest leaves of infected plants. NIL 54 indicated the lowest virus indexing that correlated with non-observation of visual symptoms on the plant after serial inoculation.

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TABLE 1: Reaction Levels of Thirty-five Rice Genotypes to *Rice Yellow Mottle Virus* (RYMV) as determined by Standard Evaluation Scale, Elisa and back Inoculation Test (BIT) in a Screenhouse Condition

Rice Varieties	Disease Reaction	OD 405nm	RYMV indexing	BIT
NERICA-L 4	HS	0.065	+	+++
NERICA-L 6	MR	0.055	+	+++
NERICA-L 7	MR	0.068	+	+++
NERICA-L 8	MS	0.070	++	+++
NERICA-L 10	MS	0.072	++	+++
NERICA-L 14	R	0.073	++	+++
NERICA-L 15	R	0.064	+	+++
NERICA-L 17	MS	0.048	-	+++
NERICA-L 19	MR	0.064	+	+++
NERICA-L 21	MR	0.075	++	+++
NERICA-L 22	MR	0.078	++	+++
NERICA-L 23	MS	0.049	-	+++
NERICA-L 24	MS	0.068	+	+++
NERICA-L 25	MR	0.064	+	+++
NERICA-L 26	MS	0.049	-	+++
NERICA-L 34	MR	0.070	++	+++
NERICA-L 36	MR	0.049	-	+++

NERICA-L 38	R	0.067	+	+++
NERICA-L 41	MS	0.071	++	+++
NERICA-L 42	R	0.050	-	+++
NERICA-L 43	MR	0.068	+	+++
NERICA-L 47	R	0.072	++	+++
NERICA-L 49	MR	0.066	+	+++
FARO 8	MS	0.062	+	+++
FARO 29	HS	0.072	++	+++
FARO 44	MS	0.049	-	+++
FARO 51	MS	0.069	+	+++
FARO 52	MS	0.067	+	+++
NCRO 26	MR	0.067	+	+++
NCRO 36	MS	0.060	+	+++
NCRO 48	MS	0.049	-	+++
Gigante	R	0.055	+	+++
Moroberekan	R	0.060	+	+++
CG-14	R	0.068	+	+++
Bouake 189	HS	0.059	+	+++

SES: Standard Evaluation scale for rice at 5 weeks after inoculation (IRRI, 1996); where, 1 - 3 = R (resistant), 5 = MR (moderately resistant), 7 = MS (moderately susceptible), and 9 = HS (highly susceptible);
ELISA: Enzyme Linked Immunosorbent; BIT = Back Inoculation Test; + = ≥ positive control (OD 405nm = 0.069), - = ≤ Negative control (OD 405nm) = 0.049

TABLE 2: Symptom expression and Leaf Desiccation score at Maturity in Thirty-five Rice Genotypes in Screenhouse Condition at Badeggi

Rice varieties/lines	Leaf dead score		Date to symptom expression (days)	Expressed symptoms
	V_1	V_0		
NERICA-L 4	2	3	19	Dispersed tillers; Hypersensitive reaction; XX
NERICA-L 6	2	3	19	XX
NERICA-L 7	1	2	16	XX
NERICA-L 8	1	2	19	XX
NERICA-L 10	1	2	16	XX
NERICA-L 14	2	2	21	XX
NERICA-L 15	1	2	28	XX
NERICA-L 17	2	3	16	Streak; dead; XX
NERICA-L 19	1	2	19	XX
NERICA-L 21	1	1	16	Hypersensitive reaction; XX
NERICA-L 22	1	2	19	XX

NERICA-L 23	2	2	28	Orange/pink; XX
NERICA-L 24	2	2	19	XX
NERICA-L 25	1	1	16	XX
NERICA-L 26	1	1	19	Streak/dots; XX
NERICA-L 34	2	2	19	XX
NERICA-L 36	1	1	16	Bunchy/dispersed tillers; XX
NERICA-L 38	1	1	28	XX
NERICA-L 41	1	1	19	XX
NERICA-L 42	1	1	14	XX
NERICA-L 43	1	1	23	XX
NERICA-L 47	1	1	23	XX
NERICA-L 49	1	1	28	Orange/pink; XX
FARO 8	1	2	19	XX
FARO 29	1	1	21	XX
FARO 44	1	2	19	XX
FARO 51	1	2	16	XX
FARO 52	1	2	26	Bunchy/dispersed tillers; dead; XX
NCRO 26	1	1	14	XX
NCRO 36	1	1	16	XX
NCRO 48	1	2	16	Hypersensitive reaction; XX
Gigante	1	2	12	Hypersensitive reaction
Moroberekan	1	2	14	Green mottles; XX
CG-14	1	2	23	Hypersensitive reaction; XX
Bouake 189	1	2	16	Dispersed tillers/stunted/ XX
Mean	1.2	1.7	19.2	

XX = Usual typical symptoms of RYMV (height reduction, yellow mottles, some levels of spikelets sterility);0 = no senescence; 1 = < 25% dead/dried leaf area; 2 = > 50% dead/dried leaf area; 3 = < 75% dead/dried leaf area; 4 = < 100% dead/dried leaf area; 5 = whole plant dead (Lafitte *et al.,* 2003); V_1 = inoculated entries; V_0 = un-inoculated control entries

Table 3: Reaction Levels of Eight Confirmatory Rice Genotypes to Rice Yellow Mottle Virus (RYMV) as determined by Standard Evaluation Scale, ELISA and Back Inoculation Test (BIT) in a Screenhouse condition

Rice Varieties	Disease Reaction	OD 405nm	RYMV indexing	BIT
NIL 130	MR	0.900	++	+++
NIL 16	MR	1.233	+++	+++
NIL 54	R	0.170	-	+++
NIL 2	MR	0.273	+	+++
FARO 44	MS	1.448	+++	+++
FARO 52	MS	0.543	+	+++
FKR 28 (SCK)	HS	1.450	+++	+++
Gigante (RCK)	MR	1.460	+++	+++

SES: Standard Evaluation scale for rice at 5 weeks after inoculation (IRRI, 1996); where, 1 - 3 = R (resistant), 5 = MR (moderately resistant), 7 = MS (moderately susceptible), and 9 = HS (highly susceptible); ELISA: Enzyme Linked Immunosorbent; BIT = Back Inoculation Test; + = ≥ positive control (OD 405nm = 0.176), - = ≤ Negative control (OD 405nm) = 0.527

TABLE 4: Symptom expression and Leaf Desiccation Score at Maturity in a Confirmatory Trial using Eight Rice Genotypes

Rice varieties/lines	Leaf dead score		Date to symptom expression (days)	Expressed symptoms
	V_1	V_0		
NIL 130	1	3	28	Dispersed tillers; Hypersensitive reaction; XX
NIL 16	2	3	28	XX
NIL 54	1	2	-	XX
NIL 2	1	2	28	XX
FARO 44	1	2	21	XX
FARO 52	1	2	14	Bunchy/dispersed tillers; dead; XX
FKR 28 (SCK)	1	2	14	Dispersed tillers/stunted/ XX
Gigante (RCK)	1	3	21	Hypersensitive reaction; XX
Mean	1.13	2.38	22.00	

0 = no senescence; 1 = < 25% dead/dried leaf area; 2 = > 50% dead/dried leaf area; 3 = < 75% dead/dried leaf area; 4 = < 100% dead/dried leaf area; 5 = whole plant dead (Lafitte *et al.,* 2003); V_1 = inoculated entries; V_0 = un-inoculated control entries

Plate 1: Spikelets sterility (a) and dead tillers (b) on RYMV inoculated rice plants in the screenhouse at Badeggi

Plate 2: Pink coloration (a) and obvious height reduction (b) due to RYMV infection in the screenhouse at Badeggi

Plate 3: Hypersensitivity reactions/poor panicle exertion on Gigante due to RYMV infection in the screenhouse at Badeggi

Plate 4: Mottling/twisting of youngest leaf (a) and dispersed/bunchy tillers formation (b) due to RYMV infection: (B-C = Control and B_2V = inoculated Bouake 189 rice variety).