Relative Resistance to *Rice Yellow Mottle Virus* in Rice

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Abstract

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We identified sources of *Rice yellow mottle virus* (RYMV) resistance in rice cultivars. Eight cultivars together with susceptible and resistant controls were evaluated under screenhouse conditions as inoculated and uninoculated treatment in completely randomised design with three replications. Seedlings were inoculated with the virus by sap transmission at two weeks after sowing. Disease incidence and severity (scale 1–9: 1–3 = green leaves with sparse dots or streaks, 9 = yellow or orange leaves and some plant dead), yield, and agronomic traits were recorded. Data analyses included Area Under the Disease Progress Curve (AUDPC), independent *t*-test, and Analysis of Variance. According to differences in most measured traits control cultivars FARO 29 and Gigante were proved to be the most susceptible and partially tolerant ones, respectively. Cvs FARO 12, FARO 17, FARO 37, and FARO 52 were classified as partially tolerant. Uninoculated control plants performed better than the inoculated for all the yield and agronomic parameters. Reduction in plant height (6%) and number of tillers per plant (4.8%), increased days to heading (3 days), and reduction in paddy yield (6.5%) was lowest in cv. Gigante. Paddy yield per plant of the RYMV-inoculated was the highest in cv. Gigante (2.4 g). The rice cultivars which combined RYMV-resistance with high-yield could be utilised in rice breeding programmes in order to enhance food security.

Keywords: AUDPC; genetic resistance; rice; *Rice yellow mottle virus*; yield reduction

Rice (*Oryza sativa* L.) is a cereal crop consumed by millions of people in Sub-Saharan Africa including Nigeria. According to the FAOSTAT (2010), the world rice production in 2010 stood at 672 mil. tons. Nigeria produced 3.2 mil. t which accounted for 14.1% of the total output in Africa. In most parts of the African continent increase in area cultivated is not justified by the total production owing to several insect pests and pathogens at various growth stages of the crop. However, amongst the virus infections associated with low yield rice yellow mottle virus disease caused by *Rice yellow mottle virus* (RYMV; genus *Sobemovirus*; family *Sobemoviridae*) is the most economically important (Traoré *et al.* 2006). The origin of RYMV dates back to 1966 when it was first noticed at Otonglo, East Africa (Bakker 1970). Since then the pathogen has been encountered in several countries with attendant severe incidences ranging from 5–100% (Rossel *et al.* 1982; Alegbejo *et al.* 2006). In Nigeria it was first observed in 1975 in Niger and Oyo States (Raymundo & Buddenhagen 1976). Studies have shown that disease incidence is influenced by a number of factors including genetic background of the host plant, vector population and climatic conditions.

Symptoms induced by the virus are highly variable including leaf mottling and yellowing, stunting, reduced tillering, non-uniform flowering, and plant death. Disease severity depends on the genotype, virus strain, age of a plant at infection, and climatic factors (Bakker 1970). The pathogen is more deleterious on susceptible cultivars compared to those with broad genetic base for disease resistance. Both virulent and avirulent strains of RYMV have been reported (Banwo *et al.* 2004) with the former inducing a considerable level of severity. Temperature is the principal climatic condition influencing expression and intensity of foliar symptoms.

RYMV overseasons in weeds, infected plant residue, and cow dung. Additionally, dissemination by contact between healthy and infected plants, fluid from diseased plants, RYMV-contaminated hands, and chrysomelid beetles has been demonstrated (Konaté *et al.* 1997; Abo *et al.* 2003a; Sarra & Peters 2003; Traoré *et al.* 2006). The most important weed hosts include *Echinochloa colona* (L.) Link, *Panicum repens* L., and *Ischaemum rugosum* Salisb. The pathogen is often transmitted by the insect *Trichispa sericea*. Epidemics occur during the raining season and are mostly facilitated by these insects (Reckhaus & Andriamasintseheno 1995). The virus can be seed-borne but seed transmission is not feasible (Konaté *et al.* 2001). RYMV exhibits high rate of replication and mutation resulting in several strains. At present, six strains have been confirmed with specific geographical adaptations. Three strains, namely S1, S2, and S3, have been found in West Africa whereas S4, S5, S6 are present in East Africa (Pinel *et al.* 2000; Banwo 2002; Fargette *et al.* 2002). In Nigeria only S1 has been found originating from three isolates collected in south-western Nigeria (Mansour & Baillis 1994).

The virus can be managed through cultural practices such as early or late planting in order to avoid the peak vector population, and roguing of diseased plants. Insecticide application has also been recommended (Reckhaus & Adamou 1986; Reckhaus & Andriamasintseheno 1995). However, host plant resistance is the most effective option (Banwo *et al.* 2004) because it is sustainable with minimal deleterious effects on the environment. Investigations have revealed the existence of three types of resistance to RYMV: partial natural, high natural, and resistance obtained through genetic transformation (Sorho *et al.* 2005). The mechanism involved in partial resistance is retardation of virus movement, thereby lowering virus accumulation and symptom expression (Ioannidou *et al.* 2003). This type of resistance has been found in cv. Azucena and a few other cultivars of *Oryza sativa* subsp. *japonica* (Ndjiondjop *et al.* 1999). High resistance is conferred by a single recessive gene *Rymv* 1 (Albar *et a*l. 2003) which has been identified in the rice cv. Gigante. On the other hand, genetic transformation employs transgenic lines obtained by introducing the viral polymerase gene into the susceptible *O. indica* cv. Bouake 189 (Pinto *et al.* 1999). Screening of rice cultivars for resistance to RYMV in West Africa commenced at the Rice Research Station, Rokupr, Sierra Leone and the International Institute of Tropical Agriculture (IITA) in the 70's (Abo *et al.* 2003b). Studies have shown that most of the upland tropical japonica cultivars are resistant to the virus. These include OS 6, Morobérékan, IRAT 78, and FARO 2 (Raymundo & Buddenhagen 1976; Raymundo & Konteh 1980; Abo *et al.* 2005). This study was conducted to identify rice varieties which combined RYMV resistance with high-yield.

MATERIAL AND METHODS

Source of seeds, sowing, and agronomic practices. Rice seeds were obtained from the National Cereals Research Institute (NCRI), Badeggi, Nigeria. The trial was conducted under screenhouse conditions (23–35°C) between February and June (Trial 1) and repeated from July to November (Trial 2) within the same cropping year. Eight commonly grown rice cultivars (FARO 12, FARO 17, FARO 35, FARO 37, FARO 44, FARO 50, FARO 51, and FARO 52), FARO 29 (susceptible control), and Gigante (resistant control) were tested. Plastic pots measuring 18 cm in diameter filled with heat-sterilised soil were arranged in completely randomised design with three replications. Each genotype was evaluated as inoculated and uninoculated control. Seeds were sown at the rate of six seeds per pot and seedlings thinned to three plants per pot, giving a total of nine plants per cultivar. Compound fertiliser (NPK 15:15:15) was applied at 4 and 8 weeks after sowing (WAS) at the rate of 1.7 g per pot.

Virus source, maintenance, and inoculation. The isolate used was the severe Nigerian RYMV isolate (Abo *et al.* 2005). The isolate was kindly provided by Dr. M. E. Abo of the NCRI. The virus was maintained in 2-week-old FARO 29 rice plants by sap transmission. Seedlings were inoculated with the virus at 2 WAS. Inoculum was prepared by grinding infected leaf tissue with cold distilled water (Abo *et al.* 2005) at the ratio of 1:10 (w/v). Inoculations were accomplished by dipping a piece of cheesecloth in the virus extract and then rubbing it on the upper surface of carborundum (600 mesh; 5 mg/ml) dusted leaves (Konaté *et al.* 1997, 2001). Excess inoculum was washed off with distilled water (Noordam 1973). The inoculated plants were incubated in the screenhouse to be monitored for symptom expression.

Data collection and analyses. Disease incidence and severity, plant height, number of tillers per plant, number of days to heading, and paddy yield were recorded. Disease severity was assessed at weekly intervals for six weeks based on the intensity of leaf symptoms. Standard Evaluation System (scale 1–9) for rice was employed for severity scoring: 1–3 = green leaves with sparse dots or streaks, 5 = leaves are green or pale green with mottling, 7 = leaves are pale yellow or yellow, 9 = yellow or orange leaves and some plants dead (Anonymous 1996). The data on disease severity were subjected to Area Under the Disease Progress Curve (AUDPC) according to Shaner and Finney (1977):

$$\text{AUDPC} = \sum_{i=1}^{n} [(Y_{i+1} + Y_i)/2]\,[X_{i+1} - X_i]$$

where:

Y_i – disease severity at the i^{th} observation

X_i – time (weeks) at the i^{th} observation

n – total number of observations

Determination of resistance classes was done as detailed by Ariyo *et al*. (2002). The differences between inoculated and uninoculated plants were used to compute reductions and increase in the measured traits. Analysis of Variance was performed using the General Linear Models procedure of SAS (2008) and means were separated by Student-Newman-Keuls test at $P = 0.05$.

RESULTS

Infection was observed in all the inoculated plants but the rice cultivars differed significantly ($P < 0.01$) for their response to RYMV. Typical leaf mottling began at five days after inoculation. Subsequently, leaves turned yellow in the susceptible cultivars and the intensity of infection was very conspicuous. The severity of infection was relatively low in the partially tolerant genotypes. All the cultivars exhibited consistent reaction to the virus in both trials. According to the reaction to virus infection in most measured traits control cultivars (cvs) FARO 29 and Gigante were proved to be the most susceptible and partially tolerant ones, respectively. Cvs FARO 12, FARO 17, FARO 37, and FARO 52 were partially tolerant. Cvs FARO 35 and FARO 50 were moderately susceptible and susceptible, respectively. However, two genotypes (FARO 44 and FARO 51) were highly susceptible (Figure 1).

Generally, plants of uninoculated treatment were taller than their inoculated counterparts. Plants of the healthy control treatment showed normal growth and rapid development as opposed to the inoculated.

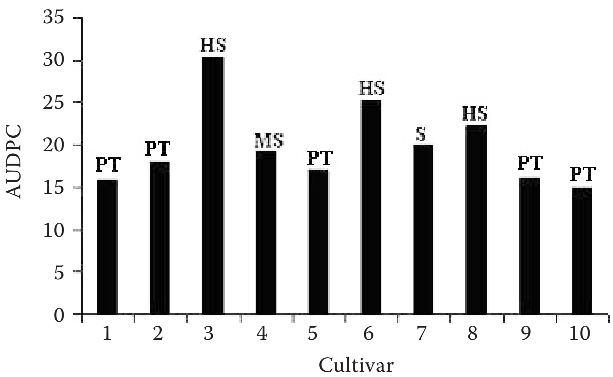

Figure 1. Average Area Under the Disease Progress Curve (AUDPC) and resistance classes of rice cultivars after inoculation with *Rice yellow mottle virus*, from two independent trials under screenhouse conditions

1 = FARO 12; 2 = FARO 17; 3 = FARO 29; 4 = FARO 35; 5 = FARO 37; 6 = FARO 44; 7 = FARO 50; 8 = FARO 51; 9 = FARO 52; 10 = Gigante; PT = partially tolerant; HS = highly susceptible; S = susceptible; MS = moderately susceptible

Some of the highly susceptible plants were stunted. In Trial 1, heights of uninoculated control plants varied from 54.8–73.6 cm as opposed to the 35.9–69.1 cm observed in their inoculated counterparts. In the latter the two extreme values came from the highly susceptible cv. FARO 44 and the resistant control cv. Gigante, respectively. When the heights of the inoculated plants were compared with their healthy control, height reductions varied between 6 and 41.4%. The highest came from the susceptible control cv. FARO 29, whereas the lowest occurred in the partially tolerant cv. Gigante. Furthermore, height reduction in cv. FARO 12 (13.1%) was comparable to the 12.6% recorded in cv. FARO 37. Similarly, there was no significant difference ($P > 0.05$) in height reduction between cvs FARO 29 and FARO 44 (41%). In Trial 2, plant heights of the healthy control ranged between 55.1 and 74.2 cm but in the inoculated plants these varied from 36.2 cm (FARO 44) to 69.5 cm (Gigante). Plants of cv. FARO 52 exhibited the lowest height reductions (6%) while the highest (41.7%) were detected in cv. FARO 44. There was no significant difference between cvs FARO 52 and Gigante for height reductions. Combined analysis of the trials indicated that the difference between heights of inoculated and uninoculated plants was significant in nine (90%) cultivars (Figure 2A). It also revealed the highest height reduction in cv. FARO 44 (Figure 2B).

There were differences among the cultivars with respect to tiller production. In all the genotypes uninoculated plants produced more tillers than the RYMV-inoculated. In Trial 1 plants of healthy control produced a range of 11–16 tillers per plant which was different from the 7–15 tillers obtained in the inoculated plants. Within the inoculated plants tiller production was the lowest in cv. FARO 29 while cv. FARO 52 produced the highest tiller number. The lowest reduction in tiller production was found in cv. Gigante (4.8%) whereas the highest reduction occurred in cv. FARO 29 (38.2%). However, there was no significant difference between cvs FARO 12 and FARO 52 for tiller reductions. In Trial 2, the number of tillers per plant varied from 12 to 16 in the healthy control, whereas the inoculated plants gave a range of 7–14. As observed in Trial 1, the lowest and highest numbers came from cvs FARO 29 and FARO 52, respectively. Consequently, tiller reductions ranged between 5.1 and 37.7%, in the same genotypes mentioned in Trial 1. The differences in tiller production among cvs FARO 12, FARO 52, and Gigante were not significant. Similarly, the variations between cvs FARO 35 and FARO 50, as well as between cvs FARO 44 and FARO 51 were not significant. Combined analysis

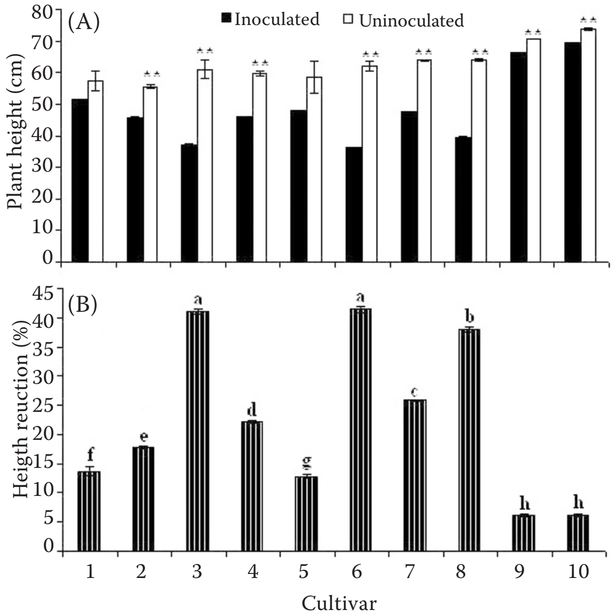

Figure 2. Average plant heights of *Rice yellow mottle virus*-inoculated and uninoculated rice cultivars (A), and height reductions after inoculation (B), from two independent trials under screenhouse conditions

For 1–10 see Figure 1; ± standard deviation; $^{**}P$ <0.01; means followed by the same letter are not significantly different according to Student-Newman-Keuls test at P = 0.05

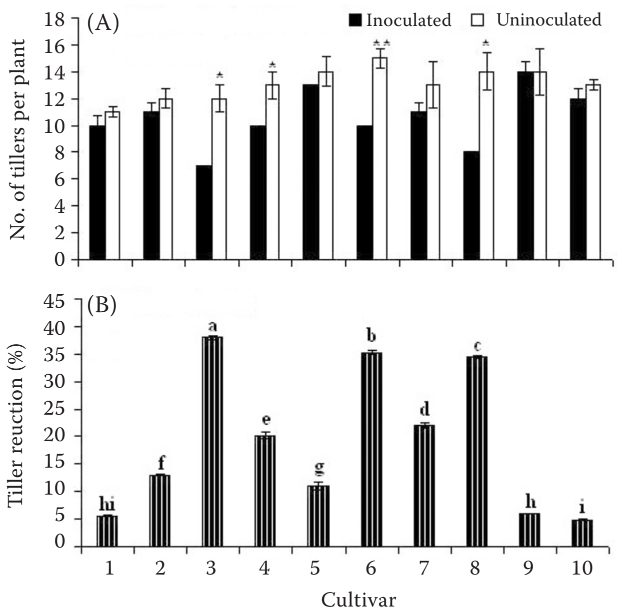

Figure 3. Average number of tillers per plant of *Rice yellow mottle virus*-inoculated and uninoculated rice cultivars (A), and tiller reductions after inoculation (B), from two independent trials under screenhouse conditions

For 1–10 see Figure 1; ± standard deviation; $^{*}P$ < 0.05; $^{**}P$ < 0.01; means followed by the same letter are not significantly different according to Student-Newman-Keuls test at P = 0.05

showed significant differences for tiller production in four (40%) cultivars, between the RYMV-inoculated and uninoculated plants (Figure 3A). Moreover, it indicated the highest tiller reduction in the susceptible control cv. FARO 29, whereas the lowest reduction occurred in cv. Gigante (Figure 3B).

Heading was earlier in all the healthy control plants compared to the inoculated ones. In Trial 1 plants of uninoculated treatment flowered between days 86–123 after planting, contrary to a range of 90 days to 131 days found in the inoculated. Gigante cultivar was the first to flower while cv. FARO 29 was the last. Prolonged time to heading varied from 4 days to 8 days in the RYMV-infected plants. Heading was delayed by 4 days in cvs FARO 12 and Gigante but extended by 8 days in cvs FARO 29, FARO 35, FARO 44, FARO 50, and FARO 51. There were no significant differences in time to heading among cvs FARO 12, FARO 17, FARO 37, FARO 52, and Gigante. Similarly, the differences in time to heading among cvs FARO 29, FARO 35, FARO 44, FARO 50, and FARO 51 were all at par. In Trial 2 number of days to heading in uninoculated plants varied from 87 to 124 but in the inoculated these ranged between 89 and 132. Heading was first noticed in cv. Gigante while cv. FARO 29 was the last to flower. Prolonged time to heading among the infected plants varied from 3 days in cv. Gigante to 9 days in cvs FARO 44 and FARO 50. However, there were no significant differences among cvs FARO 29, FARO 35, FARO 44, and FARO 50. Similarly, those of cvs FARO 17, FARO 37, and FARO 52 were not significant. Combined analysis indicated that the variations in the number of days to heading between the inoculated and uninoculated plants were significant in nine cultivars (Figure 4A). It also revealed that prolonged time to heading was the longest in cv. FARO 29 (Figure 4B).

Paddy yield per plant was higher in uninoculated plants compared to their infected counterparts. Some of the highly susceptible plants produced empty and poorly filled spikelets. In Trial 1 values ranged 1.3–2.6 g in the control plants as against the inoculated (1.1 to 2.4 g). Yield was the lowest in cv. FARO 37, whereas the highest came from cv. Gigante. The lowest yield reduction was found in cv. Gigante (7.1%) but the highest one came from cv. FARO 29 (28.4%). In Trial 2 paddy yield from the healthy control plants varied between 1.3 g and 2.6 g but in the inoculated a range of 1.1–2.4 g was found. As observed in Trial 1 for the infected plants, the lowest (1.1 g) and highest (2.4 g) yield came from cv. FARO 37 and cv. Gigante, respectively. Yield reductions were slightly lower compared to Trial 1. The lowest and highest reductions were 6.5%

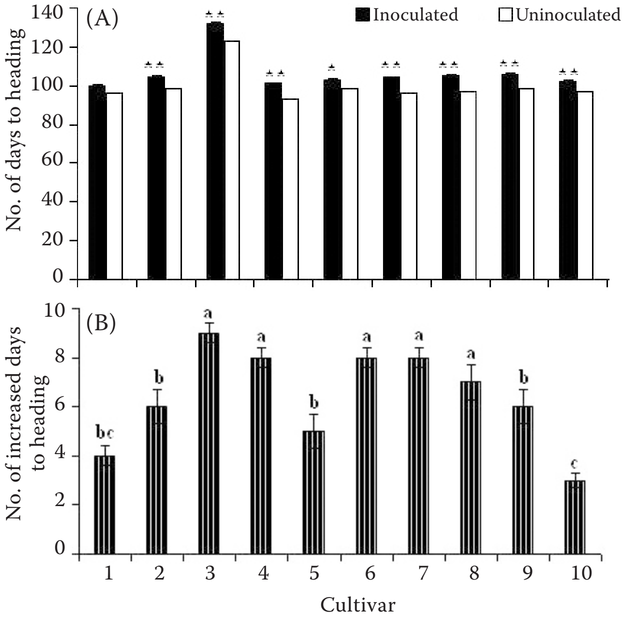

Figure 4. Average number of days to heading of *Rice yellow mottle virus*-inoculated and uninoculated rice cultivars (A), and increased days to heading (B) after inoculation, from two independent trials under screenhouse conditions

For 1–10 see Figure 1; ± standard deviation; $^*P < 0.05$; $^{**}P < 0.01$; means followed by the same letter are not significantly different according to Student-Newman-Keuls test at $P = 0.05$

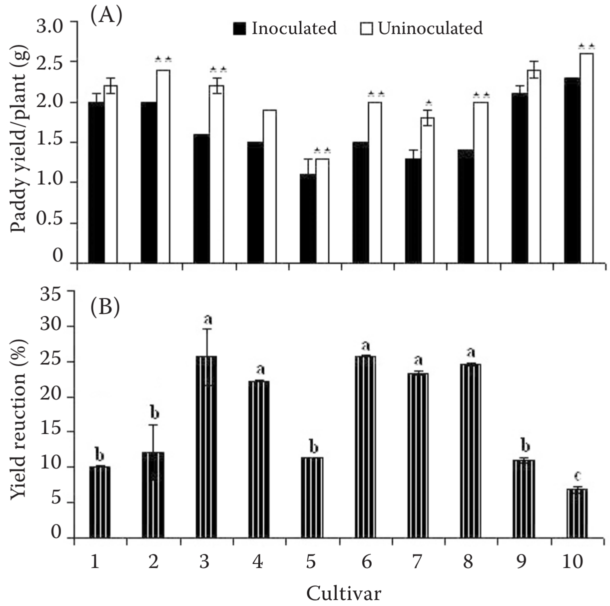

Figure 5. Average paddy yield per plant of *Rice yellow mottle virus*-inoculated and uninoculated rice cultivars (A), and yield reductions after inoculation (B), from two independent trials under screenhouse conditions

For 1–10 see Figure 1; ± standard deviation; $^*P < 0.05$; $^{**}P < 0.01$; means followed by the same letter are not significantly different according to Student-Newman-Keuls test at $P = 0.05$

and 25.6% from cvs Gigante and FARO 44, respectively. There were no significant differences in yield reductions among the partially tolerant cvs FARO 12, FARO 17, FARO 37, FARO 52, and Gigante as well as among highly susceptible, susceptible or moderately susceptible cvs FARO 29, FARO 35, FARO 44, FARO 50, and FARO 51. Combined analysis showed that the difference in paddy yield between the inoculated and uninoculated plants was significant in seven (70%) cultivars (Figure 5A). Also, it revealed that cv. FARO 44 suffered the highest paddy yield reduction (Figure 5B).

DISCUSSION AND CONCLUSION

Rice yellow mottle disease is a serious threat to rice productivity in Sub-Saharan Africa. Studies have shown that increased incidence and spread of the disease was due to several factors including annual double cropping and cultivation of high-yielding but highly susceptible varieties. Several management strategies have been recommended but the use of resistant cultivars is the most cost-effective, environment-friendly, and sustainable. Screening of rice genotypes for sources of resistance genes has gained importance owing to the severe losses caused by RYMV. The significant differences observed among the tested genotypes in response to RYMV inoculation indicate their genetic diversity to develop viral symptoms. The differences in susceptibility of many cultivated varieties have been attributed to different factors and, perhaps, the different numbers of resistance genes they possess. Since all the inoculated genotypes exhibited typical symptoms of RYMV disease, it can be concluded that there was no immunity against the pathogen. This corroborates the findings of Michel *et al.* (2008). That symptom of infection observed soon after inoculation is in consonance with the findings of Bakker (1970) who noticed typical symptom of RYMV disease about seven days after inoculation. The highly susceptible reactions of cvs FARO 29 and FARO 44 concur with the previous investigation reported by Abo *et al.* (2002). The partially tolerant cultivars performed better than the susceptible ones because of their strong genetic background to limit the deleterious impacts of the virus (Rakotomalala *et al.* 2008). Earlier, N'Guessan *et al.* (2001) encountered high genetic resistance to RYMV in cv. Gigante, in agreement with the results herein. The plants of uninoculated treatment were generally taller than their inoculated counterparts owing to the deleterious impact of the virus. This is consistent with the findings of Michel *et al.* (2008). The highest height reduction found in cv. FARO 29 can be attributed to its poor genetic background to resist RYMV infection. This agrees with the earlier work of Abo *et al*. (2002). The fact that the height reduction observed in cv. FARO 29 was similar to that in cv. FARO 44 was due to their vulnerability to the virus.

More tillers were produced by the healthy control plants compared to their infected counterparts and this indicates the negative significance of RYMV infection in rice productivity. Tiller production is of great importance in rice because of its direct relationship with yield. However, because of the genetic differences among the tested genotypes the level of tiller number reduction was the lowest in the partially tolerant Gigante cultivar. The highest percentage tiller reduction was found in cv. FARO 29 probably owing to its susceptibility to the pathogen. Reductions of tiller numbers was generally low among cvs FARO 12, FARO 52, and Gigante because they were partially tolerant to infection. Similarly, the poor genetic make-up of the susceptible cultivars resulted in the high tiller reductions. The same reasons accounted for the differences among the cultivars in time to heading. These observations agree with Bakker (1970). Non-uniform heading is of serious concern particularly in large scale rice farming as it does not encourage simultaneous harvesting of large fields.

The observation that paddy yield was higher in uninoculated plants than the RYMV-infected underscores the pathogenicity of the virus. The differences among the cultivars imply that genetic background is a critical factor influencing yield loss induced by RYMV. The lowest yield loss in the partially tolerant Gigante cultivar corroborates the findings of Onwughalu *et al*. (2010). Also, paddy yield was seriously reduced in cv. FARO 29 because of its high level of susceptibility to the virus. A similar result was encountered by Onwughalu *et al*. (2010) where > 90% yield loss was found in the RYMV-susceptible Bouake 189 cultivar. The small difference between the performance of the inoculated and uninoculated plants in some cultivars reveals that genes for RYMV resistance were tightly linked to poor yield. Linkage drag is a common phenomenon in quantitatively inherited traits such as disease resistance and yield. Because the yields of some susceptible genotypes were comparable to the resistant ones, it appears that cultivation of the former in RYMV-free fields is possible. However, the use of resistant varieties is a guarantee against total crop failure in case of disease epidemics.

It is important that rice growers give preference to rice cultivars with appreciable combination of RYMV-resistance and high-yield in order to enhance food security. Furthermore, the genotypes that meet these criteria could be used in rice breeding programmes to confer resistance on the high yielding but RYMV-susceptible cultivars. Therefore, cvs FARO 17 and FARO 52, which were partially tolerant to RYMV and produced paddy yields comparable to those of the resistant control cultivar Gigante, could be selected as alternative donors of RYMV resistance genes.

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