



VARIABILITY OF *RICE YELLOW MOTTLE VIRUS* IN A SET OF ISOLATES ORIGINATING FROM NORTH-CENTRAL NIGERIA

*Onwughalu, J. T.¹ & Abo, M. E.¹

¹National Cereals Research Institute (NCRI), Badeggi, Niger State, Nigeria

²Chukwuemeka Odumegwu Ojukwu University, Igbariam Anambra State

*Email: tobechi44@yahoo.com

ABSTRACT

The study was carried out to determine the resistance-breaking ability of six RYMV isolates collected from the north central Nigeria. Seven rice genotypes obtained from AfricaRice Center were used for the experiment in a Split-plot design with three replications under screenhouse environment at Badeggi, Niger State. The result showed that the resistant alleles - *rymv1-1* was circumvented by the six virus isolates, which also expressed the characteristic symptoms of RYMV at 42 Days after inoculation (DAI). The *rymv1-4/rymv2* and *rymv1-3* were showed to be moderately resistant with isolates from Mararaba-Obi and Gulu at 42 DAI respectively. The breakdown of resistance observed in the traditional resistant rice genotype (Gigante) appeared first as hypersensitivity reaction and later (≥ 42 DAI) as symptom expressed breakdown. *Rymv1-5* found in Tog 5674 was not affected by the six virus isolates in terms of symptom expression; however, saps from earlier inoculated test plants (Tog 5674) were infective on the susceptible genotype (Bouake 189). Virus isolates from Obubu-Ofu and Makurdi, belonging to Ser1 reacted differently and circumvented the resistance in the conventional resistant check (Gigante) with obvious yellow mottle symptoms of RYMV at 42 DAI. The serological profile analysis of the virus isolates indicated they belonged to Serogroups 1 and 2 (Ser1 and Ser2). The present study has reported significant differences in the aggressiveness of isolates of same serogroup, and the overall RYMV isolates existing in north central Nigeria. The pathogenicity effect of the virus isolates on number of panicles and yield at harvest for susceptible test plant indicated 100 % yield loss with Gulu, Makurdi and Edozhigi isolates. Moreover, the four virus isolates from Mararaba-Obi, Edozhigi, Gulu and Obubu-Ofu contributed more than 60 % yield loss in Tog 5672, which only showed moderate resistance with Mararaba-obi and Gulu isolates. This study has reported the resistance gene/alleles holding in north-central Nigeria to include *rymv1-5*, *rymv1-3*, and *rymv1-4+rymv2*.

Keywords: Rice yellow mottle virus; Resistance-breaking; isolates; Nigeria

INTRODUCTION

Rice yellow mottle virus (RYMV) is fast becoming a serious viral threat to rice production in Nigeria as it has caused important epidemics (Rossel *et al.*, 1982; Alegbejo *et al.*, 2006; Onwughalu *et al.*, 2009; Abo, 2010; Alkali *et al.*, 2014; Odedara *et al.*, 2016). Baker (1974) first reported the virus in Kenya, East Africa in mid 1960s, and numerous reports of the virus have since emerged from major lowland rice growing countries in Africa (Kouassi *et al.*, 2005). In Nigeria, RYMV has been reported virtually in all rice growing areas in the country. The virus was earlier found in rice-leaf samples collected in the southeastern states of Delta, Imo and Anambra (Awoderu, 1991). Singh *et al.* (1997) also reported the disease in Akwa-Ibom, Ebonyi and Sokoto States; whereas Alegbejo *et al.* (2006) reported its incidence in Zamfara State. The virus has also been reported in rice fields at tillering and panicle initiation stages in Niger, Kano, Bauchi, Gombe, and Benue States in the north-central zone of Nigeria between 2000 and 2008 (Abo *et al.*, 2002; Onwughalu *et al.*, 2009; 2018). Surveys in five southwest states of Nigeria, indicated disease incidence of 15 – 70% on farmer's fields in Lagos, Oyo, Ogun, Ekiti and Ondo States (Onasanya *et al.*, 2011). There are other reports of RYMV incidence on farmers' fields in the Northern Nigeria (Abo, 2010; Alkali *et al.*, 2014). The serogroups found in the country are those commonly reported in other West African countries, namely Ser 1, 2 and 3 (Fargette *et al.*, 2002; Kanyeka *et al.*, 2007). The characteristic symptoms of RYMV include yellow mottles, plant height reduction, spikelet's sterility and in severely infected plants, dispersed and bunchy tiller formation may occur with orange coloration (Onwughalu *et al.*, 2017). The top management strategies for RYMV in the field include good farm sanitation to eliminate weed hosts and vectors and also, maintaining a clean working implements as well as use of resistant varieties.

Earlier studies have identified RYMV resistance in the African traditional rice species, *Oryza glaberrima*, especially in cultivars Tog 5307, Tog 5681, Tog 5672, Tog 5675, Tog 7991 and Tog 5674 (Ndijiondjop *et al.*, 1999; Traore *et al.*, 2006; Orjuela *et al.*, 2013; Pidon *et al.*, 2017). To date, the following accessions are known to harbor respective resistance



genes/alleles viz; IR 64 (rymv 1-1), Gigante (rymv 1-2)Tog 5681 (rymv 1-3), Tog 5672 (rymv 1-4 & RYMV 2), Tog 5674 (rymv 1-5), Gigante and Bekarosaka (rymv 1-2), Tog5307(RYMV3) as described by the following authors (Ndjiondjop *et al.*, 1999; Thiémélé *et al.*, 2010; Rakotomalala *et al.*, 2008; Pidon *et al.*, 1017). However, N'Guessanet *et al.* (2001) had reported negligible yield losses with the resistant cultivars Gigante and Tog 5675, when challenged with 15 RYMV isolates under field conditions. In addition, reports on resistance breaking RYMV isolates were published in the early and mid-2000s in some countries of Africa (Fargette *et al.*, 2002; Hebrard *et al.*, 2006; Traore *et al.*, 2006) hence, raising serious concern on the fact that the development of durable resistance to RYMV in the region might be challenging. It is therefore necessary to identify, characterize and determine the resistance-breaking ability of RYMV isolates commonly found in north-central Nigeria.

MATERIALS AND METHODS

The experiment was conducted in a screen house at National Cereals Research Institute NCRI, Badeggi in 2016. The rice genotypes used included IR 64 (rymv 1-1), Tog 5681 (rymv 1-3), Tog 5672 (rymv 1-4 & RYMV2), Tog 5674 (rymv 1-5), Gigante (rymv 1-2), BG 90-2 and Bouake 189 with no known gene/allele for these later two. All these accessions were obtained from the Plant Pathology laboratory of AfricaRice Center then situated in Benin Republic. The experiment was laid out in a Split-plot design, with virus isolates and rice genotypes constituting the two factors A (Main-Plot) and B (Sub-Plot) respectively. The levels of factor A (six RYMV isolates) included a control (i.e. pure distilled water inoculants). The level of Factor B (seven rice genotypes) included IR64, BG 90-2 and Bouake 189 (susceptible checks), and Gigante (resistant check). The 7 x 7 factorial composed of 49 experimental units, which was replicated three times.

The six isolates were collected between 2014 and 2015 from Makurdi, Benue State (09° 66.456'N/06°10.621'E), Gulu, Niger State (08°19.112'N/06°40.962'E), Wuya, Niger State (09°08.456'N/05°85.723'E), Edozhigi, Niger State (09°05.596'N/05°50.910'E), Mararaba-Obi, Nasarawa State (08°

38.396'N/08° 76.251'E) and Obubu-Ofu, Kogi State (07° 36.553'N/06° 74.478'E). The locations have been under rice cultivation for years, and RYMV incidences have been reported in the area (Abo *et al.*, 2002; Onwughalu *et al.*, 2009).

Rice genotypes were seeded in the pots (15 cm diameter buckets) in the screen house at Badeggi. Three seeds were sown and later thinned down to one seedling per pot at 21 days after sowing (DAS). Fresh leaf-extracts of Bouake 189 on which the RYMV isolates were multiplied, were harvested, crushed and the sap obtained was finger-rubbed on leaves of three test plants for each isolate. Carborundum (600 mesh) was added to leaf saps as an abrasive to aid virus penetration to test plants. Three leaves, including the flag leaf of the test plants were inoculated in each replication. For the control, only pure distilled water with carborundum was used to rub the plants. Visual assessment and scoring for RYMV symptom expression was done at 14, 28 and 42 days after inoculation (DAI) following the SES (1 – 9) score for rice at 35 DAI according to IRRI (1996). Disease severity (%) was calculated as described by Oludare *et al.*, 2016 as follows:

$$\text{Disease Severity (DS)} = \frac{(n_0 \times 0) + (n_1 \times 1) + (n_3 \times 3) + (n_5 \times 5) + (n_7 \times 7) + (n_9 \times 9) \times 100}{(n_0 + n_1 + n_3 + n_5 + n_7 + n_9) \times 9}$$

Where $n^0, n^1, n^3, n^5, n^7, n^9$ = number of leaves scored 0, 1, 3, 5, 7, and 9. The rice genotypes were then classified as resistant (R) for DS = 0 – 9%; moderately resistant (MR) for DS = 10 – 25%; moderately susceptible (MS) for DS = 26 – 39% and susceptible (S) for DS = 40% and above (Oludare *et al.*, 2016). Data analysis was done using Cropstat Windows Version 7.2 (IRRI, 2007), and significant differences between means were determined using Fishers Least Significant Difference Test at 1 and 5 % probability levels.

Serological test: The indirect triple antibody sandwich (TAS) ELISA as described by [Pinel-Galzi et al.](#) (2018) was followed. The wells of ELISA plates were coated with 100 μ L/well of polyclonal antibodies raised in rabbits against RYMV at 1/1000 dilution in coating buffer (1.59 g sodium



carbonate, 2.93 g sodium bicarbonate, 0.20 g sodium azide dissolved in 900 mL H₂O and adjusted to pH 9.6 with HCl to make up to 1 litre) and incubated at 37 °C for 2 hours. The plates were then washed three times with phosphate buffered saline-Tween (PBS-T) (8.0 g sodium chloride, 0.2 g monobasic potassium phosphate, 1.1 g dibasic sodium phosphate, and 0.2 g potassium chloride dissolved in 900 ml H₂O and adjusted to pH 7.4 with HCl to make up to 1 litre + 0.5 mL Tween 20 per litre) and tapped dry. The sites on the well where antibodies were not adsorbed were blocked with 200 µL of 3 % w/v solution of skimmed milk dissolved in distilled water and incubated at 37 °C for one hour. The plates were then washed once with PBS-T for 3 minutes and tapped to dry on tissue paper. Then, 100 µL of sap macerated from 1 g leaf (using mechanical blender) in 10 mL PBS-T were put in each well and incubated at 37 °C for 2 hours. The plates were again washed three times with PBS-T for 3 minutes and tapped dry on a tissue paper upside down. 100 µL of monoclonal antibody (Mab) raised against RYMV at a working dilution of 1:8000 diluted in PBS-T was added to each well of the plates and incubated overnight at 4 °C. The plates were then washed with PBS-T titre in 3 minutes and tapped dry.

Then, 100 µL of goat anti-rabbit alkaline phosphatase (Sigma) diluted to 1:1000 in conjugate buffer (i.e. PBS-Tween + 2 % egg albumin) were added to each well and incubated at 37 °C for 2 hours. The plates were again washed three times with PBS-T and tapped dry on tissue paper. Then, 200 µL of freshly prepared substrate (1 mg/mL of p-nitrophenyl phosphate dissolved in substrate buffer (i.e. 97 mL diethanolamine, 800 mL H₂O, 0.2 g NaN₃, adjusted to PH 9.6 with HCl and made up to 1 litre with H₂O) was added to each well and incubated in the dark at room temperature for 1 hour. The colour change in the substrate assessed by spectrophotometric measurement with ELISA reader (DYNEX MR ELISA) at absorbance (OD 405nm) after 3 hours 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.

RESULTS AND DISCUSSIONS

The result of serological analysis using TAS-ELISA, and the characterization of the six RYMV isolates indicated that four isolates from Obubu-Ofu (Kogi State), Wuya (Niger State), Makurdi (Benue State) and Mararaba-Obi (Nasarawa State), belonged to Ser1, while isolates from Edozhigi (Niger State) and Gulu (Niger State) belonged to Ser2 (Table 1). N'Guessan *et al.* (2001) had reported two different serotypes of the virus (S1 and S2) co-existing in nearby fields in Cote d'Ivoire, with S2 predominating overwhelmingly in the surveyed locations. The present study recorded more Ser1 than Ser2 (Table 1). However, Oludare *et al.* (2016) reported the prevalence of S1 in the West African Countries of Benin and Togo. An earlier report had indicated only S1 originating from three isolates collected in south-western Nigeria (Mansour and Baillis, 1994) but the serological study using 20 RYMV isolates from southwest states of Lagos, Oyo, Ogun, Ekiti and Ondo revealed two major Nigeria serogroup (NSg1 and NSg2) and four subgroups (NSg1a, NSg1b, NSg2a and NSg2b) in the area (Onasanya *et al.*, 2011). The present study has confirmed the presence of two serogroups in north-central Nigeria. Moreover, Salaudeen *et al.* (2008) have also reported that two serotypes co-existed within the same State of Kaduna in northern Nigeria. The identification of Ser1 and Ser2 in the four north-central states is in agreement with earlier reports that these serotypes are common in West African Countries (Fargette *et al.*, 2002; Kanyeka *et al.*, 2007; Oludare *et al.*, 2016).

Results in Table 2 indicated that resistance breakdown on traditional resistant check (Gigante) was a hypersensitive reaction at early infection state which later (≥ 42 DAI), became clear mottle symptoms. Similarly, symptoms of localized infections were observed on Tog 5681 and Tog 5672 with Mararaba-obi and Gulu isolates at 42 DAI. Kam *et al.* (2018) also reported that RYMV isolate (Ng122) overcame the resistance of Gigante with mild leaf symptom at 42 dpi. Virus disease severity (%) of 14.14 and 10.76 % were recorded on Tog 5672 with Gulu and Mararaba-Obi isolates respectively (Table 2). The pathogenicity effect of the isolates on growth and yield components measured at 14, 28 and 42 DAI, showed that virus



isolates from Mararaba-Obi (S1), Wuya (S1) and Edozhigi (S2) significantly reduced plant height (cm) of Tog 5681 progressively from 14 DAI; whereas Tog 5672 was affected significantly by virus isolates from Mararaba-Obi (S1), Gulu (S2), Makurdi (S1) and Edozhigi (S2) at 14, 28 and 42 DAI. Isolate's pathogenicity might be cultivar dependent (Oludare *et al.*, 2016); hence, S1 from Makurdi and Obubu-Ofu circumvented the high resistance in Gigante whereas the S1 strains from Mararaba-Obi and Wuya did not. 100% yield losses (%) at harvest were recorded on the susceptible Bouake 189 with Gulu (S2), Makurdi (S1) and Edozhigi (S2) isolates; whereas, the resistance breakdown in Gigante showed first as hypersensitive reaction and later (≥ 42 DAI) as symptom expressed breakdown (Table 2). Kam *et al.* (2018) also reported that RYMV isolate (Ng122) overcame the resistance of Gigante, with mild leaf symptom at 42 days post inoculation.

Several sources of varietal high resistance have been reported in Nigeria (Abo *et al.*, 2005; Onwughalu *et al.*, 2010; Salaudeen, 2014) and elsewhere in Africa (Oludare *et al.*, 2016); but the emergence of hyper-virulent pathotype (Pathotype T) that can overcome all known sources of high resistances partially localized in West-central Africa has been reported (Hebrard *et al.*, 2018). In East Africa, Hubert *et al.* (2017) reported that the Tanzanian strain (Tz445), which belonged to S5 overcame wide range of resistant alleles including rymv1-2, rymv1-3, rymv1-4 and rymv1-5 resistance, with exception of rymv1-4 + rymv2. Ochola and Tusiime (2011) had reported no significant differences in aggressiveness between isolates of RYMV in eastern Uganda. In the present study, failure of resistance and symptom expression on Gigante occurred with S1 from Makurdi and Obubu-Ofu; whereas the S1 strains from Mararaba-Obi and Wuya did not show obvious symptom on the test plant. This is an indication of variation in the virus pathogenicity and ability of virus strains reacting differently on different cultivars. Plant yield (g) at harvest was significantly low on the susceptible genotype (Bouake 189), which produced zero yield with Gulu, Makurdi and Edozhigi isolates; moreover, yield varied significantly ($P = 0.01$) across

the virus isolates inoculated to Tog 5674, Tog 5672 and Tog 5681 at harvest (Figure 1; Table 3).

The expression of yellow mottle symptom on the resistant Gigante in the present study shows that the resistant alleles *rymv1-2*, were circumvented by the six virus isolates; whereas *rymv1-4/rymv2* (Tog5672) and *rymv1-3* only failed with Mararaba-Obi and Gulu isolates at 42 DAI respectively. *Rymv1-5* found in Tog 5674 was not affected by the six virus isolates, but sap extract from the plant elicited characteristic symptoms of RYMV at SES score of 5 on highly susceptible Bouake 189 during back inoculation test (Table 2). In an earlier study, 148 Beninese and 27 Togolese isolates attacked the susceptible accession (IR 64), whereas the resistant accessions, TOG 5681 (*rymv 1-3*), TOG 5672 (*rymv 1-4* and RYMV 2), TOG 5674 (*rymv 1-5*) and Gigante (*rymv 1-2*) remained symptomless (Oludare *et al.*, 2016).

The result of Table 2 indicated that the resistant genes/alleles, *rymv1-5*, *rymv1-3*, *rymv1-4* and *rymv2* are holding in Nigeria. Yield losses due to RYMV infection that ranged from 17 – 100% were recorded among rice cultivars in Nigeria with the least yield loss effect on *O. sativa* Cv. Gigante and NERICA-L 42, and *O. glaberrima* Cv. Moroberekan (Onwughalu *et al.*, 2011). Salaudeen (2014) also reported partial tolerance in Cvs FARO 12, FARO 17, FARO 37 and FARO 52 in Nigeria. Elsewhere, six (NERICA 9, NERICA 12, NERICA 13, TOG 7291, WAB56-50, CG 14 and Moroberekan) were reported as being very resistant to wide range of isolates from Benin and Togo (Oludare *et al.*, 2016). The strategy for RYMV management through cultivar resistance in Nigeria should therefore involve the use of *rymv1-5*, *rymv1-4*, *rymv1-3* and *rymv2* resistance genes/alleles for the control of the disease.

CONCLUSION

The present study has reported significant differences in the aggressiveness of isolates of same sero group, and the overall RYMV isolates existing in north central Nigeria. The effects of isolates' pathogenicity on number of panicles per plant, and yield loss at harvest was 100 % loss on the



susceptible accessions with Gulu, Makurdi and Edozhigi isolates; however, the four virus isolates from Mararaba-Obi, Edozhigi, Gulu and Obubu-Ofu contributed more than 60 % yield loss in Tog 5672. The study has confirmed the resistance gene/alleles holding in Nigeria to include *rymv1-5*, *rymv1-3*, and *rymv1-4+rymv2*. The *rymv1-5* (Tog 5674) was not affected by the six virus isolates, although the sap extract from the plant also elicited characteristic symptoms of RYMV at SES score of 5 on highly susceptible Bouake 189 during back inoculation test.

REFERENCES

- Abo, M. E., Gana, A. S., Maji, A. T., Ukwungwu, M. N., and Imolehin, E. D. (2005). The resistance of farmers' rice varieties to Rice yellow mottle virus (RYMV) at Badeggi, Nigeria. *Tropicultura*, 23 (2): 100 – 104.
- Abo, M. E., Ukwungwu, M. N. and Onasanya, A. (2002). The Distribution, Incidence, Natural Reservoir Hosts and Insect Vectors of *Rice yellow mottle virus* (RYMV), Genus *Sobemovirus* in Northern Nigeria. *Tropicultura*, 20 (4): 198 – 202.
- Abo, M.E. (2010). Plant virus diseases of rice, sesame and sugarcane in twelve (12) Northern States of Nigeria – Technical Report submitted to International Institute of Tropical Agriculture, (IITA), Ibadan, Oyo State as Consultant Plant Virologist on a Nation-wide Pest Survey Project, August 2010, 70pp.
- Alegbejo, M. D., Raji, B. A., Abubakar, I. U., Banwo, O. O. (2006). *Rice yellow mottle virus*. A new disease of Rice in Zamfara. *International Rice Research Notes*, 31: 1.
- Alkali, G., Alegbejo, M. D., Kashina, B. D. and Banwo, O. O. (2014). First report of *Rice yellow mottle virus* Genus *Sobemovirus* in Borno State, Nigeria. *Nigerian Journal of Experimental and Applied Biology*, 15: 87 – 91.
- Awoderu, V. A., (1991). *Rice yellow mottle virus* in West Africa. *Tropical Pest Management*, 37: 356 – 362.

- Bakker, W. (1974). Characterization and ecological aspects of *Rice yellow mottle virus* in Kenya. Agricultural Research, Doctoral Thesis, University of Wageningen, The Netherlands, 152pp.
- Fargette, D., Pinel, A., Traore, O., Ghesquiere, A., and Konate, G. (2002). Emergence of resistance-breaking isolates of Rice yellow mottle virus during serial inoculations. *European Journal of Plant Pathology*, 108: 585 – 591.
- Hebrard, E., Pinel-Galzi, A., Oludare, A., Poulicard, N., Aribi, J., Fabre, A., Issaka, S., Mariac, C., Dereeper, A., Albar, L., Silue, D., Fargette, D. (2018). Identification of a hypervirulent pathotype of *Rice yellow mottle virus*: A threat to genetic resistance deployment in West-Central Africa. *Phytopathology*, 108 (2): 299– 307. <https://doi.org/10.1094/PHYTO-05-17-0190-R>
- Hebrard, E., Pinel-Galzi, A., Bersoult, A., Sire, C. and Fargette, D. (2006). Emergence of a resistance-breaking isolate of *Rice yellow mottle virus* during serial inoculations is due to a single substitution in the genome-linked viral protein VPg. *Journal of General Virology*, 87: 1369–1373.
- Hebrard, E., Pinel-Galzi, A., Oludare, A., Poulicard, N., Aribi, J., Fabre, A., Issaka, S., Mariac, C., Dereeper, A., Albar, L., Silue, D., Fargette, D. (2018). Identification of a Hypervirulent Pathotype of *Rice yellow mottle virus*: A threat to genetic resistance deployment in West-Central Africa. *Phytopathology*, 108 (2): 299– 307.
- Hubert, J., Lyimo, H.J.F. and Luzi-Kihupi, A. (2017) Pathogenic Variation and Occurrence of Multiple Resistance-Breaking Rice yellow mottle virus Strains in Tanzania. *American Journal of Plant Sciences*, 8, 1820–1841. <https://doi.org/10.4236/ajps.2017.88124>
- IRRI (International Rice Research Institute), (1996). The International Rice Testing Program – Standing Evaluation System (ITP-SES) for Rice, Los Banos, Laguna, The Philippines, 4th Edition, P.25.
- IRRI (International Rice Research Institute), (2007). Cropstat for Windows Version 7.2 2007.3. IRRI Metro Manila Philippines.



- Kam, H., Ndjionjop, M. N., Ouedraogo, N., Laing, M. D. and Ghesquiere, A. (2018). Evaluation of rice cultivars for resistance to *Rice yellow mottle virus*, *African Crop Science Journal*, 26 (1): 49 – 61.
- Kanyeka, Z.I., Sangu, E., Fargette, D., Pinel-Galzi, A. and Herbrard, E. (2007). Distribution and Diversity of Local Strains of Rice yellow mottle virus in Tanzania. *African Crop Science Journal*, 15 (4): 201 – 209
- Kouassi, N.K., N'Guessan, P., Albar, L., Fauquet, C.M. and Brugidou, C. (2005). Distribution and Characterization of Rice yellow mottle virus: A Threat to African Farmers. *Plant Disease*, 89 (2): 124-133. DOI: 10.1094/PD-89-0124.
- Mansour, A. N. and Baillis, K. W. (1994). Serological relationships among *Rice yellow mottle virus* isolates. *Ann. Appl. Biol.* 125: 133 – 140.
- N'Guessan, P., Pinel, A., Sy, A. A., Ghesquière, A., and Fargette, D. (2001). Distribution, pathogenicity, and interactions of two strains of *Rice yellow mottle virus* in forested and savannah zones of West Africa. *Plant Diseases*, 85:59 – 64.
- Ndjionjop, M. N., Albar, L., Fargette, D., Fauquet, C., and Ghesquière, A. (1999). The genetic basis of high resistance to *Rice yellow mottle virus* (RYMV) in cultivars of two cultivated rice species. *Plant Diseases*, 83:931-935.
- Ochola, D. and Tusiime, G. (2011). Pathogenicity of *Rice yellow mottle virus* and the potential sources of resistance against the disease in Eastern Uganda. *Asian Journal of Plant Pathology*, 5 (1): 1 – 15.
- Odedara, O. O., Ademolu, K. O., and Ayo-John, E. I. (2016). Prevalence of Rice yellow mottle virus (RYMV) on Rice plants grown in selected farms in Ogun State: Preliminary Results, *Nigerian Journal of Biotechnology*, 31: 96 – 102. Available online at <http://www.ajol.info/index.php/njb/index>
- Oludare A., Tossou T.H., Kini and K., Silué, D. 2016. Diversity of rice yellow mottle virus in Benin and Togo and screening for resistant accessions. *Journal of Phytopathology*, 164: 924 – 935.

- Onasanya, R.O., Olufolaji, D.B., Onasanya, A., Sere, Y., Nwilene, F.E., Wopereis, M. and Kiepe, P. (2011). Occurrence, Distribution and Characterization of *Rice yellow mottle virus* isolates Genus *Sobemovirus* in Southwest Nigeria. *Trends in Applied Sciences Research*, 6: 1301 – 1323.
- Onwughalu, J. T., Abo, M. E. and Okoro, J. K. (2009). Preliminary investigation on the occurrence of *Rice yellow mottle virus* (RYMV) and its insect vectors in Benue State, Nigeria. *Nigerian Journal of Plant Protection*, 23: 44 – 52.
- Onwughalu, J. T., Abo, M. E., Nwankiti, A. O. and Okoro, J. K. (2017). Symptoms diversity elicited by *Rice yellow mottle virus* (RYMV) infection on *Oryza* species in Nigeria. *International Journal of Agricultural Research and Food Production*, 2 (2): 61 – 81.
- Onwughalu, J.T., Okoro, J. K. and Abo, M.E. (2010). Comparative assessment of three rice genotypes as donor materials for durable resistance to rice yellow mottle virus (RYMV) infection on rice (*Oryza sativa* L.) *Crop resource India*. 40 (1, 2 & 3): 203 – 207.
- Onwughalu, J.T., Abo, M.E. and Okoro, J. K. (2011). *Rice yellow mottle virus* and reproductive losses in rice. *Trends in Applied Sciences Research*, 6 (2): 182 – 189.
- Onwughalu, J. T., Abo, M.E., Nwankiti, A. O. and Okoro, J.K. (2018). First report of two pathotypes of *Rice yellow mottle virus Sobemovirus* in Niger State, Nigeria. *Nigerian Journal of Plant Protection*, 32(1): 121 – 132.
- Orjuela, J. Deless, T.E.F., Kolade, O., Chéron, S., Ghesquière, A. and Albar, L. (2013). A recessive resistance to *Rice yellow mottle virus*s associated with a rice homolog of the *CPR5* gene, a regulator of active defense mechanisms. *Molecular Plant-Microbe Interactions* 26, 1455–1463.
- Pidon, H., Ghesquiere, A., Cheron, S., Issaka, S., Hebrard, E., Sabot, F., Kolade, O., Silue, D. and Albar, L. (2017). Fine mapping of *RYMV3*: a new resistance gene to *Rice yellow mottle virus* from



- Oryza glaberrima*. *Theoretical and Applied Genetics*, 130, 807–818.
- Pinel-Galzi, A. Hebrard, E., Traore, O., Silue, D., Albar, L. (2018). Protocol for RYMV inoculation and resistance evaluation in rice seedlings. *Bioprotocols* 8 e2863. DOI: 10.21769/BioProtoc.2863.
- Rakotomalala M, Pinel-Galzi A, Albar L, Ghesquière A, Rabenantoandro Y, Ramavovololona P. and Fargette D. (2008) Resistance to Rice yellow mottle virus in rice germplasm in Madagascar. *Eur J Plant Pathol* 122:277–286.
- Rossel
- Salaudeen M.T. (2014): Relative resistance to *Rice yellow mottle virus* in rice. *Plant Protect. Sci.*, 50: 1–7.
- Salaudeen, M. T., Banwo, O. O., Kashina B. D. and Alegbejo M. D. (2008). Preliminary studies on the serotypes and sero-distribution of Rice yellow mottle Sobemovirus in northern Nigeria. *Biological and Environmental Science Journal for the Tropics*, 5 (1): 23 – 26.
- Singh, B. N., Fagade, S., Ukwungwu, M. N., Williams, C., Jagtap, S. S., Oladimeji, C., Efisue, A. and Okhidievbie, O. (1997). Rice growing environments and biophysical constraints in different agro-ecological zones of Nigeria. *Meteorological Journal*, 2 (1): 35 – 44.
- Thiémélé, D., Boisnard, A., Ndjiondjop, M.-N., Chéron, S., Séré, Y., Aké, S., Ghesquière, A., and Albar, L. (2010). Identification of a second major resistance gene to *Rice yellow mottle virus*, RYMV2, in the African cultivated rice species, *O. glaberrima*. *Theor. Appl. Genet.* 121:169–179.
- Traoré O, Pinel A, Hébrard E, Gumedzo M, Fargette D, Traoré A, Konaté G (2006). Occurrence of Resistance-Breaking Isolates of *Rice yellow mottle virus* in West and Central Africa. *Plant Diseases*, 90(3): 259–263.

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Table 1: Serological analysis and characterization of RYMV isolates collected from six locations of Mararaba-Obi, Gulu, Makurdi, Wuya, Edozhigi and Obubu-Ofu.

RYMV Isolate	OD (405nm, 3hrs) using Monoclonal antibodies (Mabs)			Serological group
	Mabs A	Mabs G	Mabs D	
Mararaba-Obi	0.807 ⁺	3.232 ⁺	0.553 ⁻	S1
Gulu	0.374 ⁻	3.169 ⁺	0.474 ⁻	S2
Makurdi	0.605 ⁺	3.210 ⁺	0.458 ⁻	S1
Wuya	0.744 ⁺	3.196 ⁺	0.475 ⁻	S1
Edozhigi	0.566 ⁻	3.213 ⁺	0.469 ⁻	S2
Obubu-Ofu	0.758 ⁺	3.222 ⁺	0.481 ⁻	S1

- OD ≤ 0.31 ≤ 0.6 = 1 (negative); OD ≤ 0.61 ≤ 1.2 = 2 (positive); OD ≤ 1.21 ≤ 1.8 = 3 (positive); OD ≤ 1.81 = 4 (positive).
- If, Mabs A (+ve) and Mab D (-ve) = S1, Mab A (+ve) and Mab G (+ve) = S1, Mabs A (-ve) and Mabs D (+ve) = S2, Mab A (-ve) and Mabs G (+ve) = S2.
-

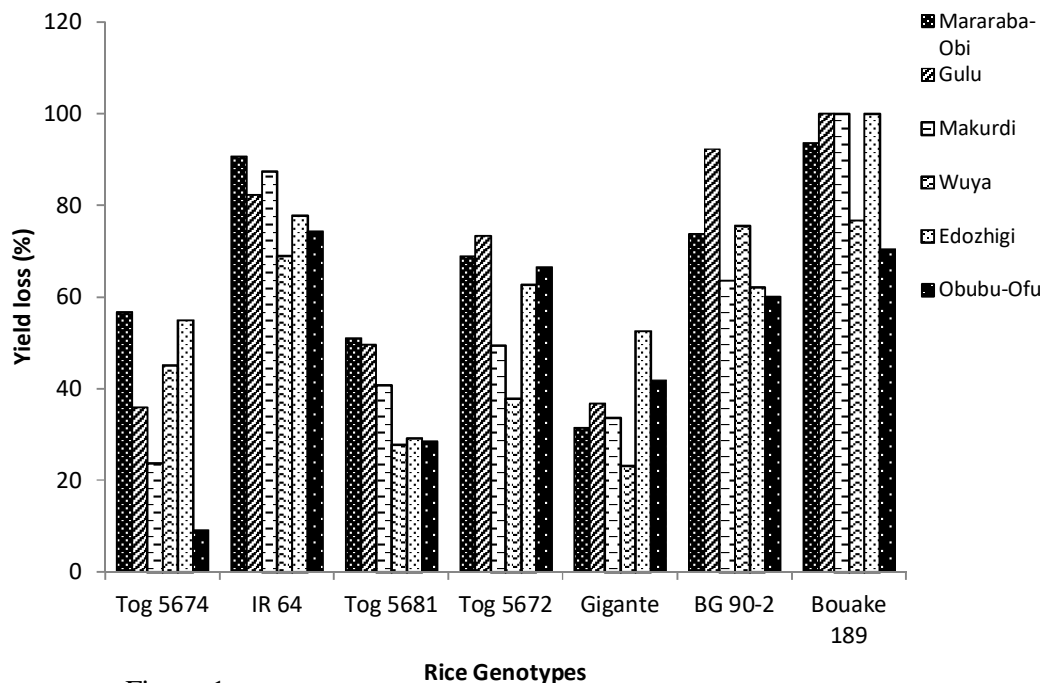


Figure 1: The contribution of RYMV isolates to yield losses (%) on the test rice genotypes in the screen house condition



Table 2: The virus disease reactions and severity on test rice genotypes in the screen house at Badeggi

Isolates	Rice Genotypes	Known Resistant gene/allele	Disease severity (%) at 42 DAI	Resistance gene/allele holding in N-C Nigeria	Disease reaction/Resistance level
Mararaba-Obi	Tog 5674	<i>rymv 1-5</i>	4.65	-	R
	IR 64	<i>rymv 1-1</i>	85.19	+	S
	Tog 5681	<i>rymv 1-3</i>	6.39	-	R
	Tog 5672	<i>rymv 1-4&RYMV2</i>	10.76	±	MR
	Gigante	<i>rymv 1-2</i>	23.36	±	MR
	BG 90-2	N	67.66	+	S
	Bouake 189	N	92.00	+	S
Gulu	Tog 5674	<i>rymv 1-5</i>	3.46	-	R
	IR 64	<i>rymv 1-1</i>	43.55	±	MS
	Tog 5681	<i>rymv 1-3</i>	9.61	±	MR
	Tog 5672	<i>rymv 1-4&RYMV2</i>	14.14	±	MR
	Gigante	<i>rymv 1-2</i>	28.10	±	MS
	BG 90-2	N	88.30	+	S
	Bouake 189	N	87.53	+	S
Makurdi	Tog 5674	<i>rymv 1-5</i>	9.04	-	R
	IR 64	<i>rymv 1-1</i>	59.05	+	S
	Tog 5681	<i>rymv 1-3</i>	55.55	+	S
	Tog 5672	<i>rymv 1-4&RYMV2</i>	5.90	-	R
	Gigante	<i>rymv 1-2</i>	55.16	+	S
	BG 90-2	N	76.40	+	S
	Bouake 189	N	92.34	+	S
Wuya	Tog 5674	<i>rymv 1-5</i>	4.85	-	R
	IR 64	<i>rymv 1-1</i>	42.47	+	S
	Tog 5681	<i>rymv 1-3</i>	7.28	-	R
	Tog 5672	<i>rymv 1-4&RYMV2</i>	7.26	-	R
	Gigante	<i>rymv 1-2</i>	32.32	±	MS
	BG 90-2	N	83.51	+	S
	Bouake 189	N	68.15	+	S
Edozhigi	Tog 5674	<i>rymv 1-5</i>	7.25	-	R
	IR 64	<i>rymv 1-1</i>	53.19	+	S
	Tog 5681	<i>rymv 1-3</i>	4.78	-	R
	Tog 5672	<i>rymv 1-4 & RYMV2</i>	5.37	-	R
	Gigante	<i>rymv 1-2</i>	9.40	-	R
	BG 90-2	N	95.96	+	S
	Bouake 189	N	81.22	+	S
Obubu-Ofu	Tog 5674	<i>rymv 1-5</i>	10.54	±	MR
	IR 64	<i>rymv 1-1</i>	42.25	+	S
	Tog 5681	<i>rymv 1-3</i>	9.07	-	R

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	Tog 5672	<i>rymv 1-4</i> & <i>RYMV2</i>	5.18	-	R
	Gigante	<i>rymv 1-2</i>	54.31	+	S
	BG 90-2	N	48.08	+	S
	Bouake 189	N	66.70	+	S
Control	Tog 5674	<i>rymv 1-5</i>	0.00	-	-
	IR 64	<i>rymv 1-1</i>	0.00	-	-
	Tog 5681	<i>rymv 1-3</i>	0.00	-	-
		<i>rymv 1-4</i> &	0.00	-	-
	Tog 5672	<i>RYMV2</i>			
	Gigante	<i>rymv 1-2</i>	0.00	-	-
	BG 90-2	NI	0.00	-	-
	Bouake 189	NI	0.00	-	-
		SE, Virus Isolates (VI):		2.648**	
	SE, Rice Genotypes (RG):		2.648**		
	SE(VI x RG):		7.006**		

- R (resistant), MR (moderately resistant), MS (moderately susceptible), S (susceptible); ** = highly significant (P = 0.01); NS = Non-significant; N-C = North Central
- Only mean disease scores (DS) at 42 DAI were used as follow: (-) for resistant (R) = DS ≤9; (±) for moderate resistant (MR) = DS 10 ≤25; (±) for Moderate susceptible (MS) = DS 26 ≤39; and (+) for susceptible: DS ≥40; N = not known gene/alleles



Table 3: Interaction of Virus Isolates and Rice Genotypes on Date to 50% Flowering (Days), Number of Panicles per Plant at Maturity and Yield (g) per Plant at Harvest

Disease Isolates	Rice Genotypes	Date to 50% flowering (days)	Number of panicles plant ⁻¹ at Maturity	Yield (g) per plant at harvest
Mararaba-Obi	Tog 5674	44.00	12.00	5.53
	IR 64	83.00	2.00	1.33
	Tog 5681	47.67	10.00	4.40
	Tog 5672	55.67	7.67	4.17
	Gigante	66.33	10.33	11.50
	BG 90-2	87.67	2.33	3.07
	Bouake 189	108.67	2.00	1.03
Gulu	Tog 5674	44.67	11.67	8.20
	IR 64	79.33	6.00	2.53
	Tog 5681	44.00	7.33	4.53
	Tog 5672	56.67	5.00	3.57
	Gigante	70.00	6.00	10.63
	BG 90-2	96.67	1.33	0.90
	Bouake 189	108.67	0.00	0.00
Makurdi	Tog 5674	44.00	15.00	9.77
	IR 64	80.67	1.67	1.80
	Tog 5681	47.67	9.67	5.33
	Tog 5672	57.67	7.67	6.77
	Gigante	64.00	11.33	11.13
	BG 90-2	89.00	4.00	4.27
	Bouake 189	106.33	0.00	0.00
Wuya	Tog 5674	44.33	10.67	7.03
	IR 64	75.67	10.67	4.43
	Tog 5681	44.00	18.67	6.50
	Tog 5672	56.00	16.00	8.33
	Gigante	65.67	11.00	12.90
	BG 90-2	95.67	2.00	2.87
	Bouake 189	102.00	2.33	3.73
Edozhigi	Tog 5674	47.00	9.33	5.77
	IR 64	72.67	5.33	3.17
	Tog 5681	45.00	14.00	6.37
	Tog 5672	52.67	17.00	5.00
	Gigante	70.00	13.00	7.97
	BG 90-2	85.33	4.00	4.43
	Bouake 189	103.33	0.00	0.00

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Obubu-Ofu	Tog 5674	46.67	14.33	11.63
	IR 64	70.00	13.67	3.67
	Tog 5681	48.00	8.00	6.43
	Tog 5672	51.00	14.00	4.47
	Gigante	66.33	11.67	9.77
	BG 90-2	83.00	6.00	4.67
	Bouake 189	100.00	3.67	4.73
Control	Tog 5674	42.67	12.00	12.80
	IR 64	44.33	11.00	14.30
	Tog 5681	42.00	13.00	9.00
	Tog 5672	41.33	14.33	13.40
	Gigante	60.33	14.00	16.80
	BG 90-2	68.67	11.00	11.73
	Bouake 189	72.33	14.33	16.03
SE, Virus Isolates (VI):		0.449**	0.190**	0.495**
SE, Rice Genotypes (RG):		0.652**	0.222**	0.507**
SE(VI x RG):		1.725**	0.586**	1.341**

** = Highly significant (P = 0.01); DAI = Day after Inoculation.