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Effect of Leaf Age on Rice Yellow Mottle Virus Severity and Chlorophyll Content with Mechanical Inoculation and Vector Transmission Method

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ABSTRACT

The physiological age of rice plant plays a significant role in Rice Yellow Mottle Virus (RYMV) infection. Both flag and old leaves of rice plant differ in their susceptibility to RYMV infection. The present study was, therefore, carried out to examine the effect of leaf age on RYMV severity and chlorophyll content in Moroberekan cultivar with mechanical inoculation and vector transmission method. Border row rice seedlings mechanically inoculated with RYMV isolate at 21 days after sowing metamorphosed into infected rows, following which seeds of Moroberekan were sown 15 days after. Eighty adults each of *Locris rubra* and *Oxya hyla* were released onto the infected rows inside separate screenhouse to acquire and transmit the virus to the rice plant. In another trial, three-week old seedlings of Moroberekan were mechanically inoculated with the RYMV isolate. In both experiments, RYMV severity and chlorophyll content of flag and old leaves were assessed at 42, 56 and 70 days after sowing. The highest disease severity (61.65%) was observed in the old leaves of mechanically inoculated plants at 42 DAS while the least disease severity (22.97%) was recorded in the flag leaves of *O. hyla* inoculated plants at 70 DAS. The highest chlorophyll content (49.29%) was observed at 70 DAS while the least (12.71%) was recorded at 56 DAS. Findings of this study showed that flag leaves of Moroberekan rice cultivar are more susceptible to RYMV infection than older leaves.

Key words: Rice yellow mottle virus, flag leaves, old leaves, plant age, disease severity, chlorophyll content

INTRODUCTION

Rice yellow mottle virus is the most devastating disease of rice in Africa (Kouassi *et al*., 2005; Abo, 2006) and has drawn considerable attention for research owing to its threat to rice production in the continent. The virus is both mechanically transmitted and insect-vectored (Nwilene, 1999; Abo *et al*., 2000; Kouassi *et al*., 2005).

After penetrating the host, pathogen spread through the plant (Chaerle *et al*., 2006). Based on the pathogen inciting a disease, there may be considerable variation in symptom expression. For instance, rice yellow mottle virus is characterized by yellowing and mottling of leaves and stunting of infected plants (Nwilene *et al*., 2009; Ndikumana *et al*., 2011; Ochola and Tusiime, 2011). Some cultivars produce nearly green leaves with faint streaking and mottling (Kouassi *et al.*, 2005). Narrow and very chlorotic leaves are pronounced in some, while others are characterized by distinct chlorosis.

The age of a plant plays a significant role in disease infection (Iqbal *et al.*, 2002; Agrios, 2005). Rice plant is composed of both flag (youngest) and old leaves that differ in contribution to the growth of the entire plant. The flag and old leaves of rice plant differ in their susceptibility to RYMV infection. The amount of chlorophyll in a plant varies with the stage of development of its leaves (Stone *et al.*, 2005).

Chlorophyll content is the primary index of assessing the photosynthetic capacity of a plant (Ramesh *et al.*, 2002). The chlorophyll content of the flag leaves is particularly important in determining the photosynthetic capacity of rice plant (Xie *et al.*, 2011). Visual (macroscopic) symptom assessment is insufficient to evaluate the degree of damage to physiological traits such as disease severity (Wagner *et al.*, 2007). Consequently, chlorophyll measurement is an ideal parameter for complementing disease severity assessment. The present study was therefore carried out to examine the effect of leaf age on RYMV severity and chlorophyll content in Moroberekan cultivar with mechanical inoculation and vector transmission method.

MATERIALS AND METHODS

Study site: The study was carried out in the screenhouse at Africa Rice Centre (Nigeria Station) Ibadan, between April 2008 and June 2009.

Isolate used: The RYMV isolate categorized as pathotype one, recommended for vector-rice screening experiment (Sere *et al.*, 2008; Nwilene *et al.*, 2009) was used in the study. This isolate was obtained from infected plants at the Plant Pathology Unit of Africa Rice Center, Cotonou, Republic of Benin.

Insect vectors used: Life adults of *Locris rubra* and *Oxya hyla* used for the trial were collected with a sweep net from Africa Rice Center fields at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The insects were caged in a screenhouse to feed on cowpea plants for three weeks in order to lose infectivity.

Rice cultivar evaluated: The rice cultivar evaluated in the trial was Moroberekan, a moderately susceptible cultivar to RYMV. This cultivar was obtained from AfricaRice Plant Pathology Unit, Cotonou.

Isolate propagation: The isolate was first propagated on BG 90-2 (a highly susceptible cultivar), following mechanical inoculation of 21-day-old rice seedlings in the screenhouse. Four weeks after inoculation, leaves showing typical yellow mottle symptoms characteristic of RYMV infection were harvested and used to prepare the viral inoculum. The inoculum was prepared by grinding infected leaf samples in 0.01 M phosphate buffer (pH 7) in the ratio of 1:10 weight by volume and the resulting homogenate filtered through cheesecloth.

Planting and inoculation of border rows: Seeds of BG90-2 were sown in plastic pots as border rows and the seedlings were mechanically inoculated with the viral suspension at three weeks after sowing. The inoculated seedlings metamorphosed to infected rows.

Planting of test cultivar and inoculation with vectors: Seeds of Moroberekan were planted two weeks after inoculating the border rows. Twenty plastic pots were used for sowing the rice cultivar. The experimental layout was a Complete Randomized Design (CRD) with three replicates. One week after planting the test cultivar, 80 adults each of non-viruliferous *O. hyla* and *L. rubra* were separately introduced onto the infected rows in each screenhouse to feed, acquire the virus and transmit to the rice plant.

In the experiment with mechanical inoculation, seedlings of Moroberekan were directly inoculated with the RYMV isolate at three weeks after sowing. Zero point five g of carborundum powder (600-mesh) was added to the inoculum to aid penetration of the virus into leaf tissues.

All recommended agronomic practices were carried out in both experiments.

Data collection: Data on disease severity and chlorophyll content were collected at 42, 56 and 70 days after sowing. These parameters were assessed on both the flag (youngest) and older leaves. The youngest leaf on each plant and three randomly selected old leaves per plant were evaluated for disease severity and chlorophyll content.

For disease severity, plants were scored based on a visual assessment of symptoms characteristic of RYMV infection on a Standard Evaluation System of 1, 3, 5, 7 and 9 according to IRRI (1996), where:

- Represents no symptoms (green leaves)
- Pale green leaves with sparse dots or streaks
- Pale leaves with mottling
- Pale yellow or yellowish-green leaves
- Yellow or orange leaves, coupled with height reduction and many dead plants

The chlorophyll contents of the leaves were measured using a SPAD 502 chlorophyll meter (Monje and Bugbee, 1992; Martines and Guiamet, 2004). SPAD-502 chlorophyll meter determines the relative amount of chlorophyll present in the leaves by measuring the absorbance of the leaf in two wavelength regions.

Data analysis: The percentage disease severity and chlorophyll content were calculated based on the visual scores and chlorophyll meter readings. Data on percentage disease severity and chlorophyll content were analysed using Statistical Analytical System (SAS, 2006). Mean separation was carried out using Duncan's Multiple Range Test (DMRT) at 5% probability level.

RESULTS

There were considerable variations in percentage RYMV severity and chlorophyll content in the leaves of Moroberekan at the three physiological stages of assessment i.e., vegetative (42 DAS), flowering (56 DAS) and maturity (70 DAS).

In Table 1, there was a significantly ($p<0.05$) higher disease severity in the flag leaves of *L. rubra* inoculated plants at 42 DAS. The highest disease severity (59.05%) was observed in the flag leaves of mechanically inoculated plants at 42 DAS. There was no significant difference in disease severity in the old leaves of mechanically inoculated plant at 42 and 56 DAS. The lowest disease severity (34.64%) was recorded in the flag leaves of *L. rubra* inoculated plants at 70 DAS.

In Table 2, there was no significant difference in disease severity in the old leaves of mechanically inoculated plants at 56 and 70 DAS. The disease severities in the flag leaves of *O. hyla* inoculated plants were significantly different ($p<0.05$) at 42, 56 and 70 DAS. The highest disease severity (61.65%) was recorded in the old leaves of mechanically inoculated plants at 42 DAS while the last disease severity (22.97%) was noticed in the flag leaves of *O. hyla* inoculated plants at 70 DAS.

In Table 3, chlorophyll reduction varied from 20.25% in the flag leaves of *L. rubra* inoculated plants at 42 DAS to 49.34% in the old leaves of mechanically inoculated plants at 56 DAS.

Table 4 showed that there was no significant difference in chlorophyll reduction in the flag leaves of mechanically inoculated plants at 42 and 56 DAS. No significant difference was also noticed in the leaves (flag and old) of *O. hyla* inoculated plants at 42 and 56 DAS, as well as in the flag leaves of mechanically inoculated plants at 42 and 56 DAS.

Table 1: Effect of plant age on percentage RYMV severity with mechanical and *L. rubra* inoculation methods

	Leaf age (DAS)		
Inoculation method	42	56	70
FLMC	59.05a	57.82a	37.04b
FLLR	44.05a	35.23b	34.64b
OLMC	59.02a	57.82a	38.33b
OLLR	48.43a	37.37ab	36.02b

FLMC: Flag leaves of mechanically inoculated plants, FLLR: Flag leaves of *L. rubra* inoculated plants, OLMC: Old leaves of mechanically inoculated plants, OLLR: Old leaves of *L. rubra* inoculated plants and DAS: Days after sowing. In a row, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test

Table 2: Effect of plant age on percentage RYMV severity with mechanical and *O. hyla* inoculation methods

	Leaf age (DAS)		
Inoculation method	42	56	70
FLMC	50.41a	36.30b	34.53c
FLOH	36.01a	27.97b	22.97c
OLMC	61.65a	52.65ab	40.13b
OLOH	46.41a	32.83b	33.60b

FLMC: Flag leaves of mechanically inoculated plants, FLOH: Flag leaves of *O. hyla* inoculated plants, OLMC: Old leaves of mechanically inoculated plants, OLOH: Old leaves of *O. hyla* inoculated plants and DAS: Days after sowing. In a row, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test

Table 3: Effect of plant age on percentage chlorophyll content with mechanical and *L. rubra* inoculation methods

	Leaf age (DAS)		
Inoculation method	42	56	70
FLMC	21.48b	27.34a	29.54a
FLLR	20.25b	21.48b	30.93a
OLMC	36.23a	49.34a	49.29a
OLLR	32.48b	39.28a	39.28a

FLMC: Flag leaves of mechanically inoculated plants, FLLR: Flag leaves of *L. rubra* inoculated plants, OLMC: Old leaves of mechanically inoculated plants, OLLR: Old leaves of *L. rubra* inoculated plants and DAS: Days after sowing. In a row, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test

Table 4: Effect of plant age on percentage chlorophyll content with mechanical and *O. hyla* inoculation methods

	Leaf age (DAS)		
Inoculation method	42	56	70
FLMC	14.28b	14.28b	36.96a
FLOH	12.75b	12.71b	23.61a
OLMC	24.51b	24.51b	47.19a
OLOH	19.71b	20.38ab	32.30a

FLMC: Flag leaves of mechanically inoculated plants, FLOH: Flag leaves of *O. hyla inoculated* plants, OLMC: Old leaves of mechanically inoculated plants, OLOH: Old leaves of *O. hyla* inoculated plants, DAS: Days after sowing . In a row, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test

DISCUSSION

This study revealed that RYMV severity and chlorophyll content of the leaves of Moroberekan are affected by age. Flag leaves were generally observed to be more susceptible to RYMV infection than older leaves. This observation corroborates the findings of Wamza *et al.* (2008) that reported the susceptibility of younger leaves of castor to leaf blight (*Fusarium pallidoroseum*) than older leaves, owing to more stomatal openings that permit pathogen penetration into the leaf tissues. The observation also agrees with the findings of Viruega and Trapero (2000) in which a negative correlation was reported between leaf age and disease severity in the development of olive leaf spot caused by *Spilocaea oleagina*. The result from this experiment is also consistent with the findings of Iqbal *et al.* (2002), who reported that two-week old seedlings of chickpea were more susceptible to blight (*Ascochyta rabiei*) than older plants. Similarly, the result is in agreement with the findings of Agrios (2005) who reported that plant age is important in disease infection, with younger plants being more susceptible.

Plants in their reaction (susceptibility or resistance) to viral diseases depend largely on age (Agrios, 2005). Host plants are susceptible mainly during the growing period and become resistant at maturity. Depending on the plant-pathogen combination, the age of the host plant at the time of arrival of the pathogen may considerably affect the development of infection and of an epidemic (Wamza *et al.*, 2008). As plants get older, the tissues become more lignified, thereby making it difficult for pathogen to penetrate.

However, the results of this trial contradict the findings of Kurt and Tok (2006) who reported that the susceptibility of Parsley to Septoria blight (*Septoria petroselini*) infection increases with increasing leaf age. Findings from this study is also at variance with the observation of Ross (2007) who reported that lesion size on senescing leaf of African violets (*Saintpaulia ionantha*) infected with Corynespora leaf spot were comparatively larger in size than on mature leaves. In the same vein, Nelson (2006) reported that older leaves of tomato showed higher level of susceptibility to the late blight pathogen, *Phytophthora infestans* than the younger leaves. The observation from the present study also disagrees with the result of Goldgerg (2006) that reported higher susceptibility of older leaves of turfgrass to powdery mildew (*Erysiphe graminis*) than younger leaves.

Generally, there is a paucity of information in literature on why some plants become more susceptible to certain pathogens as they increase in age. However, Miller (1983) and Kus *et al.* (2002) attributed the scenario to a stronger biochemical and molecular interactions between the pathogen and the host plant with increasing leaf age. There are variations of opinion as to whether virus destroys chlorophyll or inhibits its synthesis. Sinha and Srivastava (2010) attributed the reduction in chlorophyll content of virus-infected leaves to the synthesis of chlorophyll, a compound that inhibits the development of chloroplast. Secretion of this compound is triggered by the interruption of the function of chloroplast in the tissues of chlorosis-induced plants. Lindenthal *et al*. (2005) also opined that the colonization and subsequent spread of a pathogen as the plant ages could weaken the structural organization of the host. This effect could increase susceptibility to infection in older leaves.

CONCLUSION

Moroberekan exhibited a high level of susceptibility to RYMV disease at the vegetative stage compared with maturity stage. Thus, infection by RYMV at the early stage would have a more devastating effect on the rice plant than at maturity. On the other hand, leaf chlorophyll content increased with age of the rice plant. Findings of this study showed that flag leaves of Moroberekan rice cultivar are more susceptible to RYMV infection than older leaves.

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REFERENCES

Abo, M.E., M.D. Alegbejo, A.A. Sy and S.M. Misari, 2000. An overview of the mode of transmission, host plants and methods of detection of Rice yellow mottle virus. J. Sustain. Agric., 17: 19-36.

Abo, M.E., 2006. The tolerance/resistance levels of inter-and intra-specific lines and varieties to Rice Yellow Mottle Virus (RYMV) at Badeggi. Proceedings of the Africa Rice Congress, July 31-Aug. 4th, Dar es Salaam, Tanzania.

Agrios, G.N., 2005. Plant Pathology. 5th Edn., Elsevier Academic Press, London, ISBN: 9780120445653, Pages: 922.

Chaerle, L., M. Pineda, R. Romero-Aranda, D. Van Der Straeten and M. Baron, 2006. Robotized thermal and chlorophyll fluorescence imaging of pepper mild mottle virus infection in *Nicotiana benthamiana*. Plant Cell Physiol., 47: 1323-1336.

Goldgerg, N.P., 2006. Powdery mildew on turfgrass. Cooperative Extension Service, O and T Guide, TD-3, College of Agriculture and Home Economics, New Mexico State University, pp: 2.

IRRI, 1996. The International Rice Testing Program-standard Evaluation System (ITP-SES) for Rice. 4th Edn., Los Banos, Laguna, The Philippines, pp: 25.

Iqbal, S.M., A. Ghafoor, I. Ahmad and N. Ayub, 2002. Factors affecting blight disease development and severity by *Ascochyta rabiei* (Pass.) Lab in chickpea. Pak. J. Agric. Res., 17: 362-367.

Kouassi, N.K., P. N'Guessan. L. Albar, C.M. Fauquet and C. Brugidou, 2005. Distribution and characterization of Rice yellow mottle virus: A threat to African farmers. Plant Dis., 89: 124-133.

Kurt, S. and F.M. Tok, 2006. Influence of inoculum concentration, leaf age, temperature and duration of leaf wetness on Septoria blight of Parsely. Crop Prot., 26: 556-561.

Kus, J.V., K. Zaton, R. Sarkar and R.K. Cameron, 2002. Age-related resistance in *Arabidopsis* is a developmentally regulated defense to *Pseudomonas syringae*. Plant Cell, 14: 479-490.

Lindenthal, M., U. Steiner, H.W. Dehne and E.C. Oerke, 2005. Effect of downy mildew development on transpiration of cucumber leaves visualized by digital infrared thermography. Phytopathology, 95: 233-240.

Martines, D.E. and J.J. Guiamet, 2004. Distorsion of the SPAD 502 chlorophyll meter readings by changes in irradiance and leaf water status. Agronomie, 24: 41-46.

Miller, M.E., 1983. Relationship between onion leaf age and susceptibility to *Alternaria porri*. Plant Dis., 67: 284-286.

Monje, O.A. and B. Bugbee, 1992. Inherent limitations of non-destructive chlorophyll meters: A comparison of two meters. Hort. Sci., 27: 69-71.

Ndikumana, I., R. Gasore, S. Issaka, G. Pinel-Galzi and A. Onasanya *et al*., 2011. Rice yellow mottle virus in rice in Rwanda: First report and evidence of strain circulation. New Dis. Rep., 23: 18-18.

Nelson, H.E., 2006. Bioassay to detect small differences in resistance of tomato to late blight according to leaf age, leaf and leaflet position and plant age. Australasian Plant Pathol., 35: 297-301.

Nwilene, F.E., 1999. Current status and management of insect vectors of Rice Yellow Mottle Virus (RYMV) in Africa. Insect Sci. Appl., 19: 179-185.

Nwilene, F.E., A.K. Traore, A.N. Asidi, Y. Sere, A. Onasanya and M.E. Abo, 2009. New records of insect vectors of Rice Yellow Mottle Virus (RYMV) in cote d`Ivoire, West Africa. J. Entomol., 6: 198-206.

Ochola, D. and G. Tusiime, 2011. Survey on incidence and severity of rice yellow mottle virus disease in Eastern Uganda. Int. J. Plant Pathol., 2: 15-25.

Ramesh, K., B. Chandrasekaran, T.N. Balasubramanian, U. Bangarusamy, R. Sivasamy and N. Sankaran, 2002. Chlorophyll dynamics in rice (*Oryza sativa*) before and after flowering based on SPAD (chlorophyll) meter monitoring and its relation with grain yield. J. Agron. Crop Sci., 188: 102-105.

Ross, H.D., 2007. Cultural control methods that effect the development and spread of *Corynespora cassiicola* (Berk. And Curt.) Wei on African violet (*Saintpaulia ionantha* Wendl.). Master's Thesis, University of Tennessee, USA.

SAS, 2006. SAS Institute User's Guide. Statistical Version Package 9.1. SAS Institute, North Carolina, USA.

Sere, Y., A. Onasanya, F.E. Nwilene, M.E. Abo and K. Akator, 2008. Potential of insect vector screening method for development of durable resistant cultivars to Rice yellow mottle virus disease. Int. J. Virol., 4: 41-47.

Sinha, A. and M. Srivastava, 2010. Biochemical changes in mungbean plants infected by Mungbean yellow mosaic virus. Int. J. Virol., 6: 150-157.

Stone, C., L. Chisholm and S. McDonald, 2005. Effects of leaf age and psyllid damage on the spectral reflectance properties of *Eucalyptus saligna* foliage. Australasian J. Bot., 53: 45-54.

Viruega, J.R. and A. Trapero, 2000. Effect of temperature, wetness duration and leaf age on infection and development of olive leaf spot. International Society for Horticultural Science. Fourth International Symposium on Olive Growing. Acta Horticulturae, No. 586. http://www.actahort.org/

Wagner, A., A. Jamiolkowska and W. Michalek, 2007. Pathogenicity of *Fusarium oxysporum* from different soil environments and its effect on photosynthetic activity of tomato plants. EJPAU. Vol. 10.

Wamza, W.S., A.B. Zarafi and O. Alabi, 2008. Incidence and severity of leaf blight caused by *Fusarium pallidoroseum* on varied age of castor (*Ricinus communis*) inoculated using different methods. Afr. J. Gen. Agric., 4: 119-122.

Xie, X.J., S.H.H. Shen, Y.X. Li, X.Y. Xao, B.B. Li and D.F. Xu, 2011. Effect of photosynthetic characteristic and dry matter accumulation of rice under high temperature at heading stage. Afr. J. Agric. Res., 6: 1931-1940.