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RYMV Serological Detection in Insect Vector, Distribution and Transmission to Rice Cultivars

¹A. Onasanya, ^{3,4}A. Joseph, ⁴D.B. Olufolaji, ⁴M.M. Ekperigin, ²Y. Sere, ³F.E. Nwilene, ¹P. Kiepe and ^{1,4}R.O. Onasanya

¹Africa Rice Center (AfricaRice), P.O. Box 33581, Dares-Salaam, Tanzania

²Africa Rice Center (AfricaRice), 01 BP 2031, Cotonou, Benin

³Africa Rice Center (AfricaRice), PMB 5320, Ibadan, Nigeria

⁴Federal University of Technology, PMB 704, Akure, Ondo State, Nigeria

Corresponding Author: A. Onasanya, Plant Pathology Unit, Africa Rice Center (AfricaRice), P.O. Box 33581, Dares-Salaam, Tanzania Tel: +255222780768

ABSTRACT

RYMV transmission by insect vectors is considered to fully represent how RYMV disease is spread under natural field conditions. The present study aimed to use *Oxya hyla*, *Locris rubra* and *Chnootriba similes* vectors after acquisition of the virus to determine RYMV movement and distribution in insect body and transmission to rice cultivars. RYMV susceptible BG 90-2 was sown in 5 L plastic pots each at 0.5, 1 and 1.5 m distance from test entries and seedlings were mechanically inoculated with a highly virulent RYMV Nigerian isolate 14 days after sowing. Seven days after inoculation of BG 90-2, test entries were sown in 5-litre plastic pots and same day *Oxya hyla*, *Locris rubra* and *Chnootriba similes* vectors were introduced into the screen house to feed on RYMV infected BG 90-2. RYMV content in *Oxya hyla*, *Locris rubra* and *Chnootriba similes* whole body was 71.8, 44.1 and 50 and head part was 42, 44.6 and 10.1%. RYMV incidence at 0.5, 1.0 and 1.5 m vector migration distance was 14.6, 16.0 and 19.0% for *Oxya hyla*, 31.3, 35.2 and 39.6% for *Locris rubra* and 13.7, 16.2 and 19.9% for *Chnootriba similes*. Cluster dendrogram revealed three groups (GrpA, GrpB, GrpC) of RYMV cultivar screening methods. GrpA was typical of *Locris rubra*, GrpB has mechanical and *Oxya hyla* while *Chnootriba similes* formed GrpC. The information reported in this study would help to better understand RYMV disease epidemic in farmers' fields and to develop durable resistant rice varieties against the disease.

Key words: Rice variety, RYMV disease, cultivar screening method, vector migration distance, Insect vectors, Mechanical transmission

INTRODUCTION

Rice production in Africa is seriously affected by diseases. Rice Yellow Mottle Virus (RYMV) remains a major constraint to rice production in Africa (Sere *et al.*, 2008a; Ochola and Tusiime, 2011b), especially in the lowland and irrigated rice ecologies (Banwo *et al.*, 2004). The virus is widely spread, indigenous to Africa and very infectious to rice with varying mottle and yellowing symptoms (Banwo *et al.*, 2004; Onasanya *et al.*, 2006; Gnanamanickam, 2009). Different serotypes (Sere *et al.*, 2007) and pathotypes (Onasanya *et al.*, 2004; Ochola and Tusiime, 2011a) of RYMV isolates are known to exist. Yield losses of between 4-90% have been reported which depend on

genotype and infection time (Onwughalu *et al.*, 2010, 2011). There is need to replace existing susceptible rice varieties with new varieties that are better adapted to lowland and irrigated conditions (Sie *et al.*, 2008).

RYMV transmission and spread under field condition is usually by insect vectors and mechanical inoculations (Nwilene *et al.*, 2009). Research study has revealed that cows, donkeys and grass rats transmit the virus in irrigated rice fields (Sarra and Peters, 2003) while the disease is not transmitted by seed or nematode (Abo *et al.*, 2004). Abiotic transmission of RYMV through soil and contact between plants now revealed (Traore *et al.*, 2005, 2008). The virus has also been observed on some grasses, cultivated rice and wild rice (Abo *et al.*, 2000a). Previous studies on insect vectors have led to the identification of *Chaetocnema pulla*, *Dicladispa gestroi*, *Trichispa sericea*, *Sessilia pusilla*, *Chaetocnema abyssinica*, *C. kenyensis* and *C. pallidipes* *Oxya hyla*, *Locris rubra* and *Chnootriba similis* as important vectors of RYMV (Abo *et al.*, 1998; Sere *et al.*, 2008b; Nwilene *et al.*, 2009). In natural condition, the insect vectors played a significant role in disease epidemic as they are able to transfer RYMV from surrounding infected fields and contaminated weeds to the new rice fields.

The use of resistant varieties has been considered as most reliable control measure to RYMV disease. In order to identify resistant material to be released or to be used as donor in breeding programs, rice varieties are usually screened under artificial conditions by mechanical inoculation of RYMV isolates into the rice plants (Onasanya *et al.*, 2004, 2006). Such screening method does not demonstrate how RYMV disease is spread or transmitted under natural or field environment (Sere *et al.*, 2008b). Most importantly, there is need to know the vector that acquired or retained more RYMV in their body parts (head, thorax and abdomen) before transmission to rice plants. Therefore, the objective of the current study was to identify among *Oxya hyla*, *Locris rubra* and *Chnootriba similes* insect vectors RYMV contents after acquiring the virus, determine the virus movement and distribution across the insect head, thorax and abdomen and finally investigate their RYMV transmission potential to rice cultivars as compared to mechanical transmission approach.

MATERIALS AND METHODS

Research location: The study was conducted between April to October 2009 at Africa Rice Center, Nigeria Station, Ibadan, Oyo State, Nigeria.

Rice genotypes: Eight differential rice genotypes (Table 1) used in this study were obtained from the Africa Rice Center (AfricaRice), Plant Pathology Unit, Cotonou, Benin Republic.

Table 1: Identity of differential rice genotypes used for the study

| Code | Genotype | Sub-species |
|------|--------------|-------------|
| V1 | Gigante | indica |
| V2 | Bouake 189 | indica |
| V3 | Faro 11 | japonica |
| V4 | Moroberekan | japonica |
| V5 | Lac 23 | japonica |
| V6 | ITA 235 | japonica |
| V7 | PNA 647F4-56 | japonica |
| V8 | H 232-44-1-1 | indica |

RYMV isolate: The highly virulent Nigerian isolate of RYMV (Sere *et al.*, 2008b) used for this study was first propagated in the susceptible rice variety BG 90-2 following mechanical inoculation of 21-day-old rice seedlings in the screen house. Four weeks after inoculation, leaves bearing typical yellow mottle symptoms were harvested and used to prepare the viral inoculum. The viral inoculum was prepared by grinding the RYMV-infected leaf samples in 0.01 M phosphate buffer pH 7.0 at the ratio of 1:10 (w/v) and the resulting homogenate filtered through cheesecloth. Carborundum powder (600 mesh) was added to the inoculum to aid the penetration of the virus into leaf tissues during mechanical inoculation.

Insect vector RYMV inoculation: For the infected row, the susceptible variety BG 90-2, was first sown in 5 litre plastic pots each at 0.5, 1 and 1.5 m distance respectively from test entries. The rice seedlings in the infected row were mechanically inoculated with a highly virulent RYMV Nigerian isolate 14 days after sowing and 7 days later, the test entries (Table 1) were sown in 5 L plastic pots. Insect vectors were introduced into the screen house on the development of the first symptoms at 14 days after mechanical inoculation to allow them to feed on the infected row of rice plants. Five days after feeding some insect vectors were removed for RYMV serological diagnosis. One species of insect vector per experiment was used and a total of three species (*Oxya hyla*, *Locris rubra* and *Chnootriba similis*) were tested. In another experiments 14-day-old test entries of rice seedlings were mechanically inoculated with the same RYMV isolate while controls were not inoculated. Each of the experiments was laid down on a Randomized Complete Block Design (RCBD) with three replications, each in a separate insect-proofed screenhouse.

Insect vectors RYMV serological diagnosis: Indirect-antigen coated-plate enzyme-linked immunosorbent assay (ACP-ELISA) was performed on head, thorax, abdomen and whole insect as described by Sere *et al.* (2007).

Data collection: At 42 days after the introduction of the insect vector, chlorophyll content was measured using a SPAD 502 Chlorophyll Meter (Martines and Guiamet, 2004) and Disease Incidence (DI) was evaluated according to Onasanya *et al.* (2004). Insect vector viral (head, thorax, abdomen and whole insect) content (VC) was determined using enzyme linked immunosorbent assay (Sere *et al.*, 2007). SPAD measurement was obtained both for test and control genotypes.

Data analysis: Based on the SPAD readings and viral content, the percentages of chlorophyll reduction and viral content were calculated. IRRISTAT statistical software was used for all the analyses (Zhu and Kuljaca, 2005). Variance of percentage disease incidence and chlorophyll reductions were performed. RYMV cultivar transmission fitness of the three insect vectors (*Oxya hyla*, *Locris rubra* and *Chnootriba similes*) and migration distance (0.5, 1 and 1.5 m) was plotted using regression analysis (Sere *et al.*, 2008b). Additive Main Effect and Multiplicative Interaction (AMMI) analysis was performed to identify the insect vector that screened rice cultivars like the mechanical method (Ebdon and Gauch, 2002).

RESULTS

Insect vector RYMV serological detection and distribution: After acquired RYMV by feeding on leaves of RYMV inoculated BG 90-2 seedlings, RYMV was detected on selected *Oxya hyla*, *Locris rubra* and *Chnootriba similes* after serological test. RYMV content in *Oxya hyla*,

Table 2: Analysis of variance for cultivar percentage chlorophyll reduction (%SPADR42) and percentage disease incidence (%DI) due to RYMV transmitted by *Oxya hyla*, *Locris rubra* and *Chnootriba similes*

| Source | DF | F value | | | | | |
|--------------|----|---------------------|--------------------|---------------------------|--------------------|--------------------|--------------------|
| | | <i>Locris rubra</i> | | <i>Chnootriba similes</i> | | <i>Oxya hyla</i> | |
| | | % SPADR42 | % DI42 | % SPADR42 | % DI42 | % SPADR42 | % DI42 |
| R (Rep) | 2 | 0.79 ^{ns} | 0.0 ^{ns} | 0.49 ^{ns} | 2.42 ^{ns} | 0.56 ^{ns} | 2.35 ^{ns} |
| V (Variety) | 7 | 2.19* | 5.58** | 2.62* | 2.37* | 11.24** | 3.92** |
| D (Distance) | 2 | 5.47** | 2.95 ^{ns} | 3.05* | 10.54** | 2.67 ^{ns} | 10.39** |
| A (Age) | 1 | 0.56 ^{ns} | 14.02** | 3.51 ^{ns} | 0.02 ^{ns} | 28.24** | 179.92** |
| V×D | 14 | 0.82 ^{ns} | 1.28 ^{ns} | 0.95 ^{ns} | 2.5** | 1.08 ^{ns} | 0.95 ^{ns} |
| V×A | 7 | 0.12 ^{ns} | 0.2 ^{ns} | 0.87 ^{ns} | 0.15 ^{ns} | 1.67 ^{ns} | 3.92* |
| D×A | 2 | 0.18 ^{ns} | 0.22 ^{ns} | 0.42 ^{ns} | 0.71 ^{ns} | 4.14* | 10.39** |
| V×D×A | 14 | 0.18 ^{ns} | 0.18 ^{ns} | 0.42 ^{ns} | 0.12 ^{ns} | 0.38 ^{ns} | 0.95 ^{ns} |

** : Significant at 1% level, * : Significant at 5% level, NS: Not significant

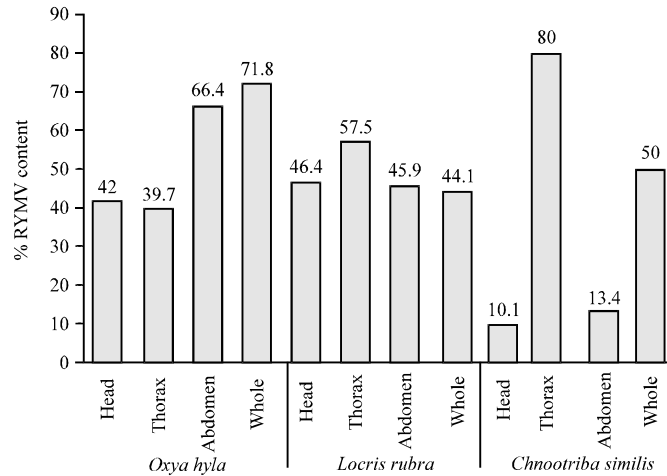


Fig. 1: RYMV content acquired by *Oxya hyla*, *Locris rubra* and *Chnootriba similes* and distribution within the insect vector body

Locris rubra and *Chnootriba similes* whole body was 71.8, 44.1 and 50%, respectively (Fig. 1). On the head part, RYMV content in *Oxya hyla*, *Locris rubra* and *Chnootriba similes* was 42, 44.6 and 10.1%, respectively (Fig. 1). The thorax part has RYMV content of 39.7, 57.5 and 80% for *Oxya hyla*, *Locris rubra* and *Chnootriba similes*, respectively while abdomen part has 66.4, 45.9 and 13.4% RYMV content for *Oxya hyla*, *Locris rubra* and *Chnootriba similes*, respectively (Fig. 1). *Oxya hyla* and *Locris rubra* have more RYMV in their body parts (head and abdomen) than *Chnootriba similes* which has more viral content only in thorax part.

Insect vector RYMV transmission potential to rice cultivars: Analysis of variance for percentage chlorophyll reduction (%SPADR42) and percentage disease incidence (%DI) revealed significant ($p = 0.01$; $p = 0.05$) RYMV transmission by *Oxya hyla*, *Locris rubra* and *Chnootriba similes* into the 8 rice cultivars (Table 2). The insect vector viral transmission was significantly

Table 3: Analysis of variance for cultivar percentage chlorophyll reduction (%SPADR42) and percentage disease incidence (%DI) due to combined effect of RYMV transmitted by *Oxya hyla*, *Locris rubra*, *Chnootriba similes* and mechanical method

| Source | DF | % SPADR42 | % DI42 |
|--------------|----|--------------------|--------------------|
| R (Rep) | 2 | 0.47 ^{ns} | 0.93 ^{ns} |
| V (Variety) | 7 | 1.59 ^{ns} | 2.18* |
| D (Distance) | 3 | 12.57** | 5.22** |
| A (Age) | 1 | 0.88 ^{ns} | 31.64** |
| M (Method) | 3 | 19.44** | 65.13** |
| V×D | 31 | 2.32** | 1.26 ^{ns} |
| V×A | 15 | 0.92 ^{ns} | 3.25** |
| V×M | 31 | 4.61** | 9.56** |

** : Significant at 1% level, * : Significant at 5% level, NS: Not significant

Table 4: Cultivar age mean comparison effect on percentage chlorophyll reduction (%SPADR42) and percentage disease incidence (%DI) due to RYMV infection by *Oxya hyla*, *Locris rubra* and *Chnootriba similes*

| Age | <i>Locris rubra</i> | | <i>Chnootriba similes</i> | | <i>Oxya hyla</i> | |
|--------------|---------------------|-------------------|---------------------------|-------------------|-------------------|-------------------|
| | % SPADR42 | % DI42 | % SPADR42 | % DI42 | % SPADR42 | % DI42 |
| Young leaves | 35.3 ^a | 30.1 ^b | 29.2 ^a | 16.5 ^a | 26.2 ^b | 11.1 ^b |
| Old leaves | 37.9 ^a | 40.6 ^a | 22.7 ^a | 16.7 ^a | 36.0 ^a | 21.9 ^a |

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

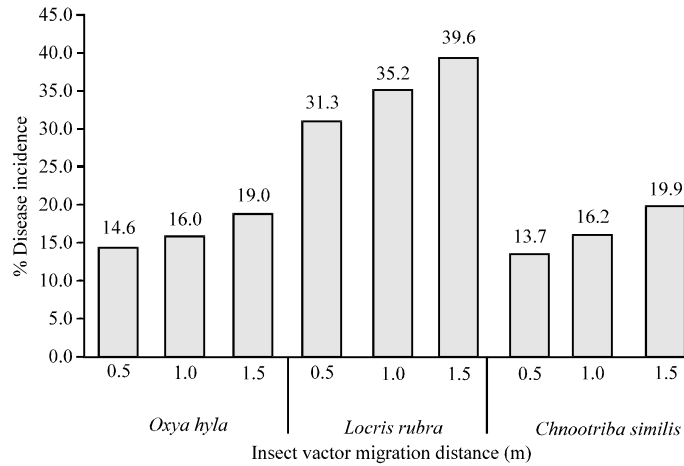


Fig. 2: *Oxya hyla*, *Locris rubra*, *Chnootriba similes* migration distance relative to rice cultivar % disease incidence

different ($p = 0.01$; $p = 0.05$) at different migration distance (0.5, 1.0 and 1.5 m) (Table 2). Rice cultivar mean percentage disease incidence (%DI) increased as insect vector migration distance increased (Fig. 2). Rice cultivar mean %DI at 0.5, 1.0 and 1.5 m vector migration distance was between 14.6-19.0% for *Oxya hyla*, 31.3-39.6% for *Locris rubra* and 13.7-19.9% for *Chnootriba similes* (Fig. 2). The highest %DI was caused by *Locris rubra* (39.6%), followed by *Chnootriba similes* (19.9%) and the least was *Oxya hyla* (19.0%) (Fig. 2). On the cultivar age, RYMV infection on young and old leaves caused by *Oxya hyla* and *Locris rubra* was significant ($p = 0.01$) and not significant with *Chnootriba similes* (Table 2). Percentage DI of young and old leaves was between 11.2-21.9% for *Oxya hyla*, 30.1-40.6% for *Locris rubra* and 16.5-16.7% for *Chnootriba similes*

Table 5: Rice cultivar and viral transmission method mean comparison on percentage chlorophyll reduction (%SPADR42)

| Variety | Screening Method (SM) | | | |
|--------------|-----------------------|-------------------|-------------------|-------------------|
| | LR | M | CS | OH |
| Gigante | 28.6 | 45.2 | 23.3 | 45.6 |
| Bouake 189 | 40.3 | 42.3 | 21.2 | 21.3 |
| Faro 11 | 39.3 | 36.9 | 32.6 | 35.5 |
| Moroberekan | 35.6 | 45.3 | 42.4 | 17.0 |
| Lac 23 | 37.0 | 46.4 | 23.2 | 33.9 |
| ITA 235 | 45.6 | 44.6 | 25.7 | 33.0 |
| PNA 647F4-56 | 42.8 | 37.1 | 20.7 | 30.4 |
| H 232-44-1-1 | 23.7 | 39.1 | 18.5 | 32.2 |
| SM-mean | 36.6 ^{ab} | 42.1 ^a | 25.9 ^c | 31.1 ^b |

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test. OH: *Oxya hyla*, LR: *Locris rubra*, CS: *Chnootriba similes*, M: Mechanical

Table 6: Rice cultivar and viral transmission method mean comparison on percentage disease incidence (%DI)

| Variety | Screening Method (SM) | | | |
|--------------|-----------------------|-------------------|-------------------|-------------------|
| | LR | M | CS | OH |
| Gigante | 18.5 | 22.8 | 19.8 | 18.5 |
| Bouake 189 | 40.7 | 28.4 | 19.8 | 16.1 |
| Faro 11 | 35.2 | 27.2 | 16.7 | 14.8 |
| Moroberekan | 41.4 | 23.5 | 16.1 | 12.4 |
| Lac 23 | 35.8 | 25.9 | 16.7 | 15.4 |
| ITA 235 | 42.0 | 26.5 | 12.4 | 17.9 |
| PNA 647F4-56 | 45.1 | 27.8 | 16.7 | 18.5 |
| H 232-44-1-1 | 24.1 | 21.0 | 14.8 | 18.5 |
| SM-mean | 35.3 ^a | 25.4 ^b | 16.6 ^c | 16.5 ^c |

In a column, means followed by a common letter are not significantly different at the 5% level by duncan's multiple range test. OH: *Oxya hyla*, LR: *Locris rubra*, CS: *Chnootriba similes*, M: Mechanical

(Table 4). This revealed that *Oxya hyla* and *Locris rubra* RYMV transmission to rice cultivar is dependent on cultivar age while *Chnootriba similes* viral transmission is independent of cultivar age. Besides, analysis of variance also revealed significant interaction ($p = 0.01$) between rice cultivars and viral transmission methods (*Oxya hyla*, *Locris rubra*, *Chnootriba similes* and mechanical) (Table 3). Cultivar mean chlorophyll reduction (%SPADR) caused by viral transmission methods was 42.1 a for mechanical, 36.6 ab for *Locris rubra*, 31.1b for *Oxya hyla* and 25.9 c for *Chnootriba similes* (Table 5). Besides, cultivar mean %DI caused by viral transmission methods was 35.3 a for *Locris rubra*, 25.4 b for mechanical, 16.6 c for *Chnootriba simile* and 16.5 c for *Oxya hyla* (Table 6). Indicating that *Oxya hyla*, *Locris rubra*, *Chnootriba similes* and mechanical method screened the 8 rice cultivars differently.

Classification of RYMV cultivar screening methods: Cluster dendrogram revealed three main groups (GrpA, GrpB, GrpC) of RYMV cultivar screening methods. GrpA was typical of *Locris rubra* cultivar screening method. GrpB was made up of mechanical and *Oxya hyla* cultivar screening methods while *Chnootriba similes* cultivar screening methods dominated GrpC (Fig. 3).

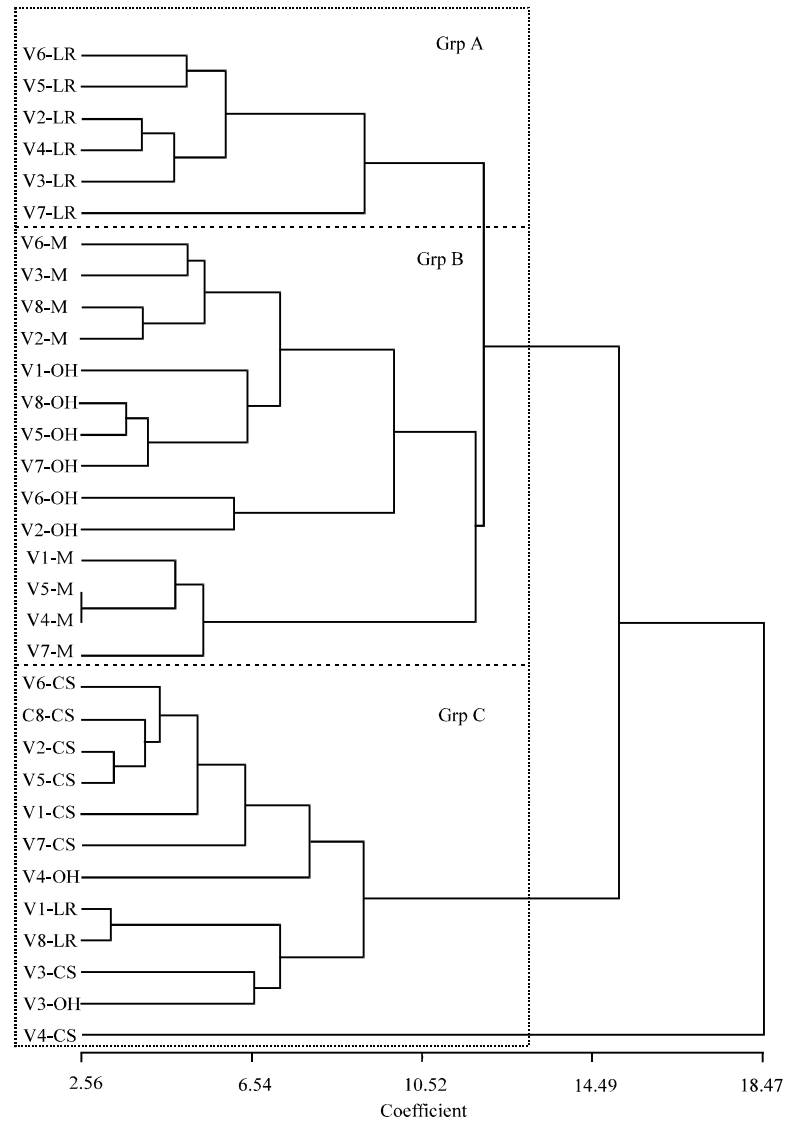


Fig. 3: Cluster dendrogram showing diversity among the four screening methods using additive main effects and multiplicative interaction (AMMI) analysis. V: Rice variety, CS: *Chnootriba similes*, OH: *Oxya hyla*, LR: *Locris rubra*, M: Mechanical

DISCUSSION

In terms of epidemiology, insects are the most important factors in plant virus disease. Approximately 80% of the plant viruses depend on insect vectors for transmission (other vectors can be nematodes and fungi) and the plant virus vector interactions are very specific (Hohn, 2007; Uzest *et al.*, 2007; Nwilene *et al.*, 2009; Sere *et al.*, 2008b). Thus, the recent spreading of RYMV throughout Africa might be caused by the known and unknown RYMV insect vectors (Nwilene *et al.*, 2009; Sere *et al.*, 2008b). This spreading by insect vectors provides the opportunity for the virus to be transmitted to a variety of rice cultivars in Africa (Nwilene *et al.*, 2009). The present study did not only support these previous findings but also provides new knowledge into how vectors could transmit RYMV under rice field conditions irrespective of their distance from the

rice fields. It revealed RYMV transmitted by *Oxya hyla*, *Locris rubra* and *Chnootriba similes* into the 8 rice cultivars has led to significant chlorophyll reduction and increased disease incidence among rice cultivars. Our study has demonstrated the importance of insect vector migration distance and effective viral transmission to rice cultivars. Rice cultivar RYMV disease incidence increases as insect vector migration distance increases. Short migration distance by insect vector reduced infection while long migration distance increase infection in the rice cultivars. There is evidence that *Oxya hyla* and *Locris rubra* transmit RYMV to young and old rice plants discriminately and cause different infection rates similar to mechanical transmission method. This evidence revealed that *Oxya hyla* and *Locris rubra* RYMV transmission rate was different in young and old rice cultivar while viral transmission rate was the same as with *Chnootriba similes*. It was very cleared in our study that *Oxya hyla*, *Locris rubra*, *Chnootriba similes* and mechanical method screened the 8 rice cultivars differently.

Non-circulative transmission has been considered the most adopted strategy for virus vector interaction. Having fed on infected plant, the vector acquired the virus and moved to inoculate a new host plant. The viral components involved in this interaction have been established in Cucumovirus, Potyvirus and Caulimovirus where domains of the viral coat protein directly recognize unknown retention sites in the vector mouthparts (Hohn, 2007; Uzest *et al.*, 2007; Ziegler-Graff and Brault, 2008). In the present study, after acquired RYMV by feeding on leaves of RYMV inoculated BG 90-2 seedlings, RYMV was detected on selected *Oxya hyla*, *Locris rubra* and *Chnootriba similes* after serological test. This gave the evidence that the three vectors actually acquired the virus which confirmed what was obtained in previous studies with Cucumovirus, Potyvirus and Caulimovirus vectors (Hohn, 2007; Uzest *et al.*, 2007; Ziegler-Graff and Brault, 2008). *Oxya hyla* and *Locris rubra* have more RYMV in their body parts (head and abdomen) than *Chnootriba similes* which has more viral content only in the thorax part. The presence of more RYMV content in *Oxya hyla* and *Locris rubra* mouthparts might possibly explained the reason for their efficient rice cultivar viral transmission (Uzest *et al.*, 2007). This efficient viral transmission from *Oxya hyla* mouthparts could possibly explained why it was similar to mechanical viral transmission method in their cultivar screening potential as revealed in our study. The implication of this finding is that *Oxya hyla* can be used in place of mechanical method for routine RYMV cultivar screening for durable resistance in order to demonstrate natural field RYMV infection pattern.

In past RYMV vector studies researchers focused on vectors feeding on infected plants, acquired the virus and moved to inoculate a new host plants (Abo *et al.*, 2000b; Sere *et al.*, 2008b; Nwilene *et al.*, 2009). Besides, high concentrations have been given also to researches on the population structure of the virus and cultivar resistance (N'Guessan *et al.*, 2000; Fargette *et al.*, 2004; Traore *et al.*, 2005; Sorho *et al.*, 2005; Fargette *et al.*, 2008; Ndjiondjop *et al.*, 2001; Albar *et al.*, 2003; Ioannidou *et al.*, 2003; Onasanya *et al.*, 2004, 2006). However, no investigation was carried out on RYMV content acquired by vector species and vector migration distance as demonstrated in the present study.

CONCLUSION

RYMV content in insect vectors, viral distribution within the insect vectors and vector migration distance were reported for the first time in the present study. The study linked insect vector viral transmission efficiency to higher RYMV content in the vector mouthparts and wider migration distance. RYMV movement and distribution in vector body now understood. There is need to

investigate the population structure and genetic diversity of RYMV vectors species and biotypes with specific linkage to RYMV strains carried by different vectors. This would help to better understand RYMV disease epidemic in farmers' fields and develop durable resistant rice varieties against the disease.

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