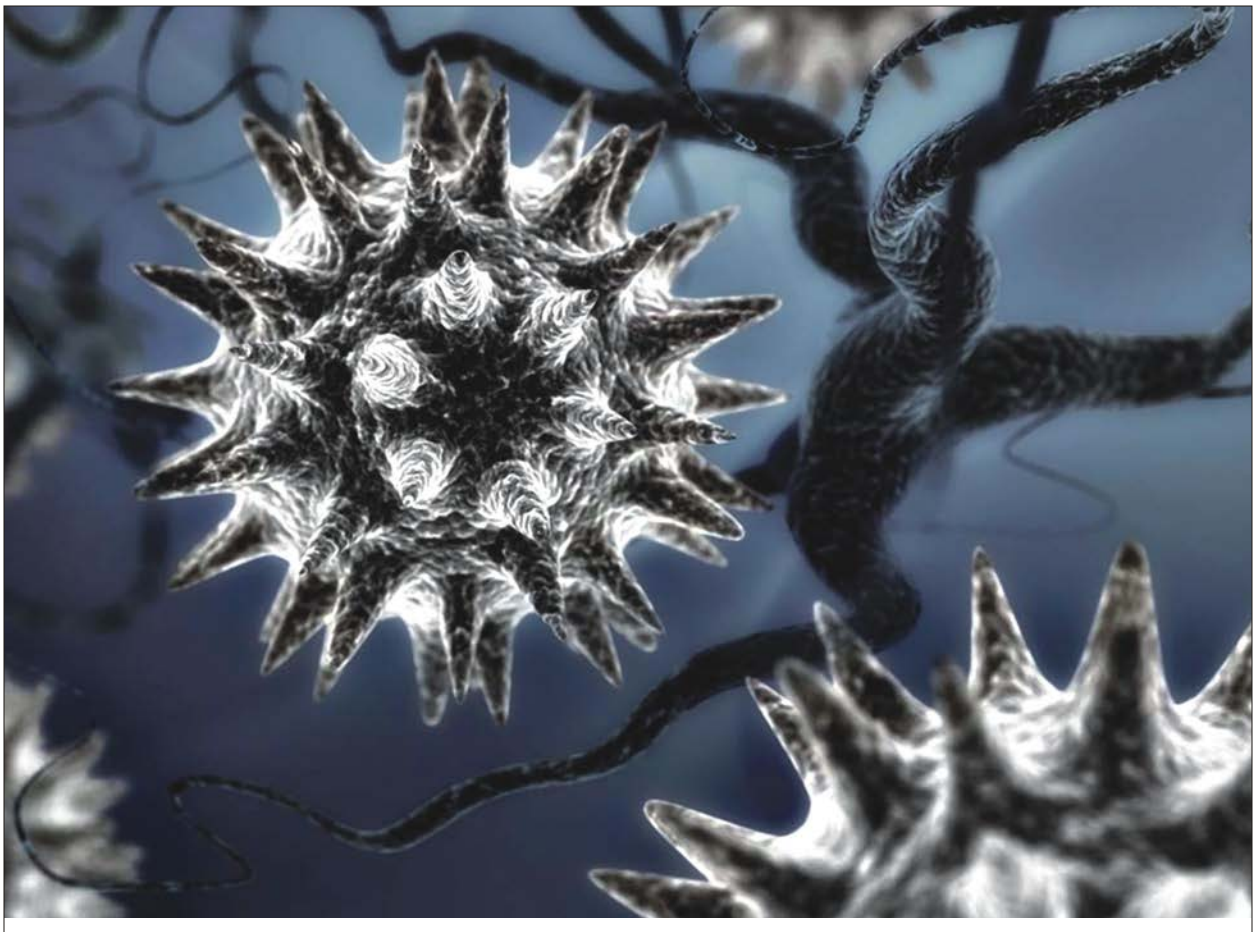

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Potential of Insect Vector Screening Method for Development of Durable Resistant Cultivars to *Rice yellow mottle virus* Disease

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Abstract: The study aimed to investigate the potential of insect vector *Rice yellow mottle virus* (RYMV) cultivar screening method. Screening rice cultivars against RYMV under artificial conditions is usually carried out inside the screen house by mechanical inoculation of RYMV isolates. Such an approach may be highly criticized as not fully representative of how RYMV disease is spread or transmitted under field conditions. Consequently, the potential of three RYMV insect vectors, *Oxya hyla*, *Locris rubra* and *Chnootriba similes*, was evaluated in comparing the cultivar screening method with mechanical transmission using eight differential rice genotypes against a highly virulent RYMV Nigerian isolate. The study revealed that each of the three insect vector methods is different from the mechanical transmission method and all methods screened rice cultivars in the same way. This study revealed the potential of the insect vector screening method to provide a basis not only for the development of durable resistant cultivars to RYMV disease but also for further investigation on vectors, virus and rice plants interaction.

Key words: Rice, RYMV, screening, mechanical transmission, insect vectors

INTRODUCTION

Rice yellow mottle virus (RYMV), genus sobemovirus, remains one of the major constraints to rice production in rainfed lowland and irrigated ecosystems in Africa. The virus is now found in most parts of Africa (Séré *et al*., 2008) with host range being restricted to gramineous species, mainly in the genus Oryzae and Eragrostidae. RYMV is mechanically transmissible and insect vectors play a major role in its transmission (Abo *et al*., 2000a). The regular occurrence of insects in rice fields in West Africa has prompted close examination of these species as possible vectors (Nwilene, 1999). *Chaetocnema pulla*, *Dicladispa gestroi*, *Trichispa sericea* and *Sessilia pusilla* are some of the important vectors of RYMV (Abo *et al*., 1998). *Chaetocnema abyssinica*, *C. kenyensis* and *C. pallidipes* are also capable of transmitting the disease.

Resistant varieties are the main component of an integrated management system for RYMV. Screening rice cultivars against RYMV under artificial conditions is usually carried out by mechanical inoculation of RYMV isolates into the plant tissues and allowing the inoculated young plant to grow on so that RYMV disease symptom scores and serological diagnostic tests can be conducted at least 14 days after viral inoculation (Onasanya *et al*., 2004, 2006). Such an approach is open to criticism as not being fully representative of how RYMV disease is spread or transmitted under field conditions where the insects play an important role in initiating disease transferred from infected weeds or wild rice surrounding farmers' fields.

The study aimed to investigate the potential of insect vector RYMV cultivar screening method. In line with the study objective, insect vector transmission was investigated in comparison with the

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mechanical inoculation method to establish if a method of viral transmission or inoculation by insect vectors could be as effective and reliable as the mechanical inoculation method for screening rice cultivars for RYMV resistance.

MATERIALS AND METHODS

Rice Genotypes

Eight differential rice genotypes (Table 1) used in this study were obtained from the WARDA Plant Pathology Unit.

RYMV Isolate

The highly virulent Nigerian isolate of RYMV 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 screenhouse at the WARDA Nigeria Station, Ibadan, Nigeria. 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

The study was conducted inside the screenhouse between 2006 and 2007 at the WARDA Nigeria Station, Ibadan, Nigeria. For the infected row, BG 90-2, a RYMV susceptible variety, was first sown in 5 L plastic pots at 1 m distance 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 screenhouse 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. 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-days-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 RCB design with three replications, each in a separate insect-proof screenhouse.

Data Collection

At 56 days after the introduction of the insect vector, chlorophyll content was measured using a SPAD 502 Chlorophyll Meter (Monje and Bugbee, 1992; Martines and Guiamet, 2004), Disease Incidence (DI) was evaluated according to Onasanya *et al*. (2004) and Viral Content (VC) was determined using enzyme linked immunosorbent assay (Séré *et al*., 2007). SPAD measurement and viral contain were obtained both for test and control genotypes.

Table 1: Identity of differential rice genotypes used

Code	Genotype	Variety type
V1	Gigante	Indica
V2	Bouake189	Indica
V3	Faro11 (Os 6)	Japonica
V4	Moroberekan	Japonica
V5	Lac23	Japonica
V6	ITA235	Japonica
V7	PNA647F4-56	Japonica
V8	H232-44-1-1	Indica

Data Analysis

Based on the SPAD readings and viral content, the percentages of SPAD reduction and viral increase were calculated. IRRISTAT statistical software was used for all the analyses. Variance and mean comparison of percentage disease incidence, viral content and SPAD reductions were performed. Potential of RYMV cultivar screening fitness of the three insect vectors (*Oxya hyla*, *Locris rubra* and *Chnootriba similes*) and mechanical methods was plotted using regression analysis.

RESULTS AND DISCUSSION

The comparison between the classical mechanical screening method and the potential method of screening with RYMV vectors (*Oxya hyla*, *Locris rubra and Chnootriba similes*) was done using a highly virulent RYMV Nigerian isolate against eight differential rice genotypes. Comparing each insect vector's potential to the classical mechanical method, the analysis of variance (ANOVA) revealed no significant interaction between both methods thereby indicating that the three insect vectors (*O. hyla*, *L. rubra* and *C. similes*) used in this study screened rice cultivars in the same way in terms of disease incidence, plant chlorophyll reduction (SPADR) and Viral Content (VC) (Table 2).

The ANOVA also revealed that for each of the three insect vectors the method was different from mechanical transmission method (Table 2). For example, *L. rubra* and *C. similis* gave higher DI (74.2 and 69%), SPADR (35.1 and 36.5%) and VC (12.5 and 9.5%), with fitness above 65% than was obtained using the mechanical method to screen the eight genotypes (Table 3-5, Fig. 1).

The study revealed that *O. hyla*, was able to transmit higher viral content into the eight rice genotypes than did the mechanical inoculation method, but virus pathogenicity is reduced which results in lower incidence of plant disease and chlorophyll reductions than with mechanical transmission.

The high disease incidence, chlorophyll reduction and viral content across the eight differential rice genotypes due to RYMV transmitted by *L. rubra* and *C. similis* strongly revealed the potential and possible use of these vectors in screening rice genotypes for RYMV resistance. However, the behavior of the rice genotypes tested indicated that the differences between the insect vector method and the classical mechanical inoculation are significant for some varieties and not for others (Table 3-5).

Virus transmission by insects is a common way for viruses to travel between different host plants and this is possibly as a result of a protein that plant viruses attach to as they hitch an insect ride between plants (Uzest *et al*., 2007). Protocols for the whitefly transmission test (Brown and Nelson, 1988) have also been used for cultivar screening. Studying interaction between virus and insect vectors, the authors reported a case of dose virus effect. Similar approaches have been used to screen cultivars for resistance to *Cauliflower mosaic virus* (CaMV) aphid transmission (Blanc *et al*., 1993), *Maize chlorotic dwarf virus* (MCDV) leafhopper transmission in maize (Gingery *et al*., 2004) and *Maize streak virus* (MSV) *Cicadulina mbila* and *Cicadulina storeyi* transmission in maize (Reynaud and Peterschmitt, 1992; Sunday, 2006). Variable virus transmission efficiency by vector species has been demonstrated. For instance, *Frankliniella schultzei* was more efficient in transmitting Tomato spotted *wilt virus* (TSWV) than Scirtotrips dorsalis (Amin *et al*., 1981). Burrow *et al*. (2006) indicated that the difference between populations in their ability to transmit virus was demonstrated for the first time with *Ciccadiluna mbila* and the *Maize streak* virus. Gray *et al*. (2002) indicated that clonal populations of *Schizaphis graminum*, a vector of *Barley yellow dwarf virus*, can differ in their ability to transmit viruses. However, there is no available information or report on using insect vectors to screen against RYMV. Studies carried out by Abo *et al*. (1998, 2000a, 2000b) to understand the epidemiology of the disease concentrated on feeding insects on diseased rice plants where the insects collect the virus and then pass it on to the next healthy plants on which they feed.

Table 2: Analysis of variance comparing each insect vector with mechanical viral transmission for percentage RYMV Disease Incidence (DI), SPAD reduction (SPADR) and Viral Content (VC)

		Locris rubra			*Chnootriba similis*			*Chnootriba similis*		
Source	DF	DI	SPADR	VC	DI	SPADR	VC	DI	SPADR	VC
		F	F	F	F	F	F	F	F	F
Rep (R)	2	<1	<1	3.5*	<1	<1	3.6*	<1	<1	<1
Variety (V)	7	1.3ns	2.4*	1.1ns	1.6ns	2.8*	1.8ns	3.3*	3.0*	1.3ns
Method (M)	1	52.5**	8.7**	8.0**	38.9**	13.9**	1.3ns	29.2**	3.6ns	967.2**
VxM	7	1.7ns	1.6ns	<1	1.6ns	1.9ns	1.6ns	1.8ns	2.3ns	1.2

**Significant at 1% level; *Significant at 5% level; ns = Not significant

Table 3: Comparison, for each variety screened, of the difference between the classical mechanical method (CM) and the method using *Chnootriba similis* (CS) as vectors

	Incidence			SPADR			Viral content		
Varieties	CM	CS	Diff[1)]	CM	CS	Diff[1)]	CM	CS	Diff[1)]
Gigante	22.2	66.7	-44.5**	16.2	47.0	-30.8**	9.6	13.8	-4.2ns
Bouake189	66.7	69.5	-2.8ns	47.1	40.5	6.6ns	14.2	6.2	7.9ns
Faro11	44.6	69.5	-24.5*	16.1	35.7	-19.6*	6.4	9.5	-3.2ns
Moroberekan	44.9	69.5	-21.4ns	14.3	25.7	-11.4ns	5.8	10.2	-4.3ns
Lac23	45.3	66.7	-34.4**	33.5	28.0	5.6ns	6.0	2.6	3.4ns
ITA235	36.7	71.1	-27.8*	12.5	33.6	-21.1*	6.8	6.2	0.6ns
PNA647F4-56	58.9	66.7	-23.5**	26.1	38.4	-12.4ns	4.6	9.7	-5.0ns
H232-44-1-1	44.4	72.2	-7.8ns	30.4	43.4	-13.0 ns	8.5	17.4	-9.0*
Mean	45.5	69.0	-24.9*	24.5	36.5	-12.0**	7.7	9.5	-1.7ns

[1)]Diff = Differences; **Significant at 1% level; *Significant at 5% level; ns = Not significant

Table 4: Comparison, for each variety screened, of the difference between the classical mechanical method (CM) and the method using *Locris rubra* (LR) as vectors

	Incidence			SPADR			Viral content		
Varieties	CM	KR	Diff[1)]	CM	LR	Diff[1)]	CM	LR	Diff[1)]
Gigante	22.2	-78.3	56.1**	16.2	40.0	-23.8*	9.6	9.7	-0.1ns
Bouake189	66.7	75.6	-8.9ns	47.1	41.7	5.5ns	14.2	15.4	-4.6ns
Faro11	44.6	75.6	-31.0**	16.1	33.5	-17.4ns	6.4	4.8	1.6ns
Moroberekan	44.9	69.5	-24.5*	14.3	25.7	-11.4ns	5.8	12.0	-6.2ns
Lac23	45.3	73.9	-28.6*	33.5	26.2	7.4ns	6.0	13.9	-7.9ns
ITA235	36.7	75.6	-38.9**	12.5	34.1	-21.6*	6.8	11.0	-4.2ns
PNA647F4-56	58.9	73.9	-15.0ns	26.1	47.4	-21.4*	4.6	13.0	-8.4ns
H232-44-1-1	44.4	71.1	-26.7*	30.4	31.8	-1.4ns	8.5	20.0	-11.5*
M-MEAN	45.5	74.2	-28.7**	24.5	35.0	-10.5**	7.7	12.5	-5.1**

[1)]Diff = Differences; **Significant at 1% level; *Significant at 5% level; ns = Not significant

Table 5: Comparison, for each variety screened, of the difference between the classical mechanical method (CM) and the method using *Oxyla hyla* (OH) as vectors

	Incidence			SPADR			Viral content		
Varieties	OH	CM	Diff[1)]	OH	CM	Diff[1)]	OH	CM	Diff[1)]
Gigante	24.7	22.2	2.5ns	11.2	16.2	-5.0ns	47.1	9.6	37.5**
Bouake189	19.7	66.7	-47.0**	18.4	47.1	-28.7**	43.5	14.2	29.4**
Faro11	9.9	44.6	-34.7**	14.5	16.1	-1.6ns	43.2	6.4	36.8**
Moroberekan	14.8	44.9	-30.1*	24.9	14.3	10.6ns	43.6	5.8	41.6**
Lac23	19.7	45.3	-25.5*	28.7	33.5	-4.8ns	47.6	6.0	41.4**
ITA235	19.7	36.7	-17.0ns	15.9	12.5	3.5ns	48.2	6.8	37.4**
PNA647F4-56	55.6	58.9	-3.3ns	27.1	26.1	1.1ns	42.0	4.6	38.1**
H232-44-1-1	9.9	44.4	-34.6**	8.3	30.4	-22.0*	46.6	8.5	37.8**
M-MEAN	21.7	45.5	-23.7**	18.6	24.5	-5.9ns	45.2	7.7	37.5**

[1)]Diff = Differences; **Significant at 1% level; *Significant at 5% level; ns = Not significant

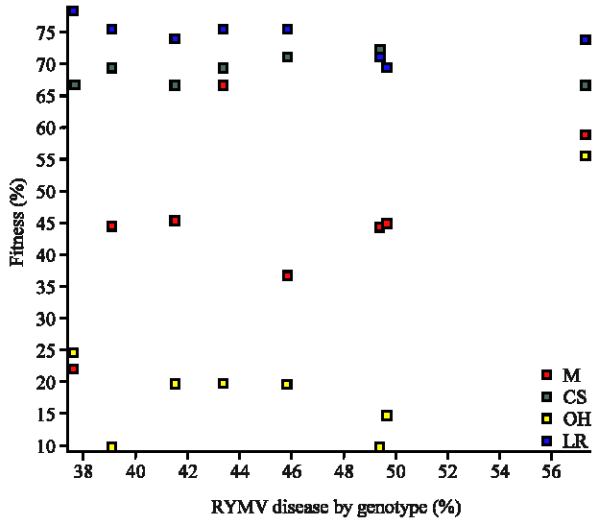

Fig. 1: Comparing the potential fitness of four different RYMV inoculation methods for screening eight differential rice genotypes M=Mechanical; CS= *Chnootriba similes*; OH= *Oxya hyla*; LR.= *Locris rubra* % RYMV disease by genotype

The potential to use insect vectors to screen rice varieties for resistance/tolerance to RYMV was reported for the first time in the present study. The study has also revealed the vectoral capacity of insects found in and around rice fields. This insect vector role has seriously hindered progress towards controlling the disease.

Following the RYMV epidemic outbreak of the 1990s, research activities focused on the population structure of the virus (Konaté *et al*., 1997; N'Guessan *et al*., 2000; Fargette *et al*., 2004, 2008; Traoré *et al*., 2005; Sorho *et al*., 2005) and the resistance of rice varieties (Pressoir *et al*., 1998; Ndjiondjop *et al*., 2001; Albar *et al*., 2003; Ioannidou *et al*., 2003). They provided a good understanding on the relationship between the virus and the rice plant. However, no investigation was undertaken on the interaction between the virus, the vectors and rice plants. There is a need to investigate the population diversity of the RYMV vectors in order to determine its contribution to virus spread in farmers' fields. Some questions remain to be clarified. Are there significant differences between RYMV vectors of the same species and biotypes to transmit RYMV disease? Is there any resistance/tolerance in rice plants against the insect vector species and/or biotypes? Such questions are important not only to improving the utilization of vectors to screen rice varieties but also to better understand the disease epidemic in farmers' fields and make progress in controlling the disease.

CONCLUSION

The present study was able to provide information on the potential of screening for RYMV using insect vectors and this could provide the basis for investigating the relationship between different vectors, the virus and the rice plants.

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