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Modes of transmission and stability of *Rice yellow mottle virus*

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Abstract: *Rice yellow mottle virus* (RYMV) is the most important rice virus in Africa. We examined RYMV transmission via soil and water contaminated with RYMV-infected rice plants and by serial cutting with RYMV-contaminated scissors. Transmission of RYMV via dried rice straw kept at 27°C was also examined. The results showed the virus could be transmitted via soil and water, and by scissors. Rice straw that was RYMV-infected was not infective if it was dried and was kept longer than 42 days. By insect transmission experiments and ELISA, long-horned grasshoppers (*Conocephalus* spp.) were found to be a possible vector of RYMV in Uganda.

Key words: *Rice yellow mottle virus*, stability, transmission, vector

Introduction

Rice yellow mottle virus (RYMV), a member of the family Sobemoviridae (Seghal 1981), was first described in Kenya in 1966 (Bakker 1970). Since then, it has been found and isolated in East and in West Africa (Kouassi *et al*. 2005). Streaking, mottling, yellowing, and malformation of leaves, and death of infected young plants are all typical signs of RYMV infection (Bakker 1970, 1974; Fauquet and Thouvenel 1977). The virus is transmissible by animals, by wind-mediated leaf contact, and by abiotic factors (e.g. irrigation water) (Abo *et al*. 2000; Sarra and Peters 2003; Sarra *et al*. 2004; Traoré and Traoré 2008). Infection via farm equipment has also been confirmed (Sarra 2005). Transmission of RYMV via guttation fluid from rice paddy fields has also been confirmed experimentally (Traoré and Traoré 2008).

Fauquet and Thouvenel (1977) examined longevity *in vitro* (LIV) and found that RYMV can remain viable in crude extract for at least 34 days at 27°C. This result suggests RYMV transmission via contaminated straw, but the longevity of RYMV in dried straw should also be examined.

Insect species in the families Chrysomelidae, Coccinelidae, and Tettigoniidae are important vectors of RYMV in Africa (Bakker 1971, 1974; Breniere 1983; Reckhaus and Adamou 1986; Nwilene 1999; Abo *et al*. 2000). The major vector species may vary in each of the countries and localities, so insect transmission experiments performed in Uganda are important for effective vector insect management.

We performed soil and water transmission experiments and transmission experiments using scissors artificially contaminated with RYMV as an alternative to agricultural equipment. The stability of RYMV in dried straw was examined, as well as new potential vectors in Uganda.

Materials and Methods

Virus source and inoculum preparation

The RYMV isolate collected in Uganda (U12) was used in the experiments. This was inoculated onto susceptible rice cultivar IR64 (*Oryza sativa indica*). Seedlings of IR64 were inoculated 21 days after sowing (DAS). As for the transmission-by-infected-rice-straw experiment, the Tanzania (Tz10-36) isolate was used. We confirmed their serotype of U12 and Tz10-36 as Serotype 4 and they belong to the same phylogenic group (data not shown). For all the experiments, the same variety of rice was used for inoculation and RYMV infection. The results were confirmed using ELISA.

Detection of RYMV in plants was confirmed using double antibody sandwich ELISA (DAS-ELISA) (Clark and Adams 1977) and reverse transcription polymerase chain reaction (RT-PCR). The ELISA was performed using an antibody kit (NEOGEN Europe Ltd., Ayr, Scotland, UK). For the RT-PCR, total RNA was extracted using TRIzol® (Invitrogen, Carlsbad, CA, USA), and first-

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strand cDNA was synthesised using a ReverTra Ace-α-® kit (TOYOBO, Osaka, Japan). The first-strand cDNA was amplified by PCR using a TaKaRa Ex™ kit (TaKaRa, Otsu, Japan). Primers that amplify the *CP* gene (720 bp) and the 3' untranslated region (3'UTR) of RYMV (Pinel *et al*. 2000) were also used. Amplification was performed under initial denaturation conditions of 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1.5 min, and then a final extension at 72°C for 10 min.

Soil and water transmission

Soil and water from pots (33.5 cm diameter and 35 cm height) with two or three rice plants infected with RYMV for > 2 months were taken and used as contaminated soil/ /water in the experiments. The contaminated soil (100 ml) was added to the top of RYMV-free soil (300 ml) which was in plastic cups. One healthy IR64 seedling of approximately 21 DAS, was then transplanted to each cup. The contaminated water (approximately 100 ml) was tested by adding RYMV-free soil (300 ml) to individual plastic cups. Three healthy IR64 seedlings were then planted in each cup. RYMV infection was detected using ELISA for soil transmission, and RT-PCR for water transmission, 14 DAS. Because the concentration of RYMV in water transmission seemed lower than the level detected by ELISA, we confirmed RYMV by RT-PCR as a more sensitive detection technique. To test for transmission via soil mixed with RYMV-infected root sap, fresh roots were taken from RYMV-infected rice plants and ground in water at a 1/10 (w/v) ratio. One, 10, or 100 ml of sap were added to 200 g of dried, RYMV-free soil which was in a plastic cup containing transplanted, healthy, IR64 seedlings. The seedlings were examined for the presence of virus at 14 days post inoculation (DPI) using ELISA.

Transmission by scissors

Pairs of scissors with blades approximately 0.1 cm in width and 3 cm in length were used as a substitute for agricultural equipment to examine the transmission of virus. Transmission of RYMV via scissors was tested using two methods. Experiment 1 was designed to determine the maximum number of plants that could be infected by cutting with a pair of contaminated scissors. A pair of scissors was contaminated via a single cut of RYMV-infected rice leaves. Twelve healthy rice seedlings were then each cut once with the scissors. Experiment 2 was designed to determine how the number of cuts with contaminated scissors affected infection with virus. Individual healthy rice seedlings were cut a different number of times (from one to five times) using scissors contaminated with a single cut of RYMV-infected rice leaves. The plants were examined for the presence of RYMV at 14 DPI using ELISA.

Transmission by infected rice straw

Inoculation of IR64 seedlings (21 DAS) was done with RYMV isolate Tz-10-36. They were maintained at 27°C for three weeks to allow for complete virus replication. Seedlings which were RYMV-infected (infection confirmed by ELISA) were harvested using clean scissors at 21 DPI. The leaves and stems were then chopped into 5–10 mm pieces and thoroughly mixed to ensure consistency. The chopped materials were then air dried at room temperature (25°C) for 3 days. The drying process resulted in a loss of approximately 93% of the original plant weight. Dried materials were then divided into 1.5 g lots in clean 10 ml plastic tubes and were immediately incubated at 27°C for 7, 14, 21, 28, 35, 42, 49, 56, 65, 90, or 120 days. After each incubation period, the samples were kept at –30°C and were subsequently used to inoculate six IR64 seedlings (21 DAS). They were then examined for signs of infection, and two samples from each lot were assayed using ELISA at 14 DPI.

Transmission by insect

One to three insects fed on RYMV-infected IR64 seedlings for 48 h were transferred onto healthy IR64 seedlings and allowed to feed for 48 h. After 14 DPI, the inoculated IR64 seedlings were assayed using ELISA. Several beetles (Crysomelidae), short-horned grasshoppers (Acrididae), long horned grasshoppers (Tettigoniidae), and several Hemipteran insects were used for this experiment though they were not identified to the species level. These insects were chosen because survey results indicated that they are major rice pests in Uganda (Fujiie *et al*., unpublished data).

Results

Soil and water transmission

In the soil transmission experiment, RYMV was detected from two out of five seedlings by ELISA (data not shown). The ELISA results for transmission via water from RYMV-infested pots indicated that all seedlings were negative for RYMV. However, the RT-PCR results indicated that one of the five seedlings was positive. The results of the experiment using soil mixed with different volumes of sap from RYMV-infected roots, indicated that RYMV could be transmitted from root sap volumes that were as small as 1 ml (Table 1).

Transmission by scissors

Experiment 1 was performed twice. The virus was transmitted via cuts of at least 12 seedlings (Table 2). It seems infectivity was lower in the second Experiment 1; some of the seedlings did not become infected. There was no rela-

Table 1. Results of transmission via soil mixed with root sap infected with RYMV

Volume of sap mixed [ml]	Infection	
	1st test	2nd test
1	2/10*	3/5
10	3/10	3/5
100	4/6	0/3

*infected/inoculated numbers tested by ELISA

Table 2. Maximum number of plants infected via cutting with a pair of scissors contaminated with RYMV

Seedling	Infection	
	1st test	2nd test
1st	–	–
2nd	–	–
3rd	+	–
4th	+	–
5th	–	–
6th	–	–
7th	+	–
8th	+	–
9th	+	+
10th	+	–
11th	+	–
12th	+	+

"+" and "–" indicate infected and not infected confirmed by ELISA

Table 3. Relationship between the number of cuttings and infection

No. of cuttings to a single plant	Infection
1	0/4*
2	1/4
3	1/4
4	2/4
5	2/4

*infected/inoculated numbers tested by ELISA

tionship between cutting order and whether infection occurred. The results for Experiment 2 indicated that when a single plant was cut repeatedly with a contaminated pair of scissors, infection occurred, and it occurred when the plant was cut two to five times (Table 3). A single cut was not sufficient to transmit the virus to healthy rice seedlings in this experiment.

Transmission of RYMV via infected rice straw

RYMV infection via dried straw did not occur beyond 49 days at 27°C (Table 4). Development of symptoms at 14 DPI was much slower when rice sap kept longer than 35 days was used. These results were confirmed with the use of ELISA.

RYMV vectors in Uganda

Four of the nine insect species used vectored RYMV (Table 5). Consistent with the reports by Bakker (1974) and Abo *et al.* (2000), short-horned grasshoppers might be vectors of RYMV in Uganda.

Table 4. Inoculation of seedlings via straw infected with RYMV that was kept for different number of days at 27°C

No. of days	RYMV-infection	
	Symptom[a]	ELISA[b]
7	6/6	2/2
14	6/6	2/2
21	6/6	2/2
28	6/6	2/2
35	3/6	2/2
42	2/6	2/2
49	0/6	0/2
56	0/6	0/2
65	0/6	0/2
90	0/6	0/2
120	0/6	0/2

[a] number of symptomatic seedlings/total number of inoculated seedlings

[b] number of plants confirmed positive by ELISA/total number of plants tested

Table 5. Transmission of RYMV via insect species present in Uganda

Insect tested	No. of inoculated plants	No. of infected plants	Infection [%]
Flea beetles (*Chaetocnema* sp.)	18	5	27.7
Hispid beetle (*Chrysispa viridicyanea*)	1	0	0
Leaf beetles (*Altica* spp.)	22	0	0
Ladybird beetle (*Chnootriba similis*)	4	1	25
Short-horned grasshoppers (*Coryphosima centralis*)*	16	6	37.5
Long-horned grasshoppers (*Conocephalus* spp.)	15	3	20
Spittle bug (*Loris* sp.)	6	0	0
Stink bug (*Aspavia* sp.)	9	0	0
Leafhopper (*Nephotettix* sp.)	1	0	0

**C. centralis* and other unidentified spp.

Discussion

Determination of various modes of transmission using artificial inoculation and ELISA is very important for the development of strategies for RYMV protection. The results of this study indicated that contact with only a small volume of contaminated soil could cause RYMV infection. Appropriate management of ratoons that can cause soil and water contamination in heavily affected fields

will be important for RYMV control. Transmission via soil contaminated with RYMV-leaves was shown to be positive by Traoré and Traoré (2008). The results of our soil transmission experiments indicated that roots, ratoons, and debris of RYMV-infected seedlings after harvesting can be a source of RYMV via contact and contamination of soil and water.

In addition, RYMV-contaminated scissors with small blades can transmit the virus to up to 12 seedlings during continuous cutting. This result strongly suggests that the use of hatchets and other agricultural equipment with blades larger than the scissors used in the experiment by RYMV, can be contaminated and will mechanically transmit the virus in the field.

Rice straw is left in the rice field or used in a variety of ways after harvest in Uganda. Compared with the LIV results obtained by Fauquet and Thouvenel (1977), the stability and infectivity of RYMV in rice straw persists longer, but not as long as other contact transmissible plant viruses such as *Tobacco mosaic virus* (TMV) (Okada *et al*. 1999). This result suggests the risk of infection via debris from RYMV-infected seedlings, may be reduced by drying the paddy fields or using longer furrow periods. Rotation of paddy rice with upland crops may also be effective.

Because RYMV is transmitted by several vectors, the vector potential of virus transmission may vary by season, location, and cropping patterns. Using ELISA, we confirmed RYMV infection at approximately 21 DPI. We are the first investigators to find that four species of insects (i.e. flea beetles, ladybird beetle, short-horned grasshoppers and long-horned grasshoppers) can be vectors of RYMV in Uganda. The flea beetle population in Uganda is not large, but this beetle has been found to be one of the major insects present in the paddy fields tested (NaCRRI, Namulonge, Uganda). Because grasshoppers have only chewing mouth parts, RYMV is most likely mechanically inoculated into healthy seedlings via the chewing action of these insects. This mechanism of RYMV transmission is different from transmission by beetles, which have sucking mouth parts. *Trichispa sericea* can maintain the ability to transmit the virus for 1–2 days, and *Chaetocnema similis* can maintain it for up to 3 days (Bakker 1974; Abo *et al*. 2000). Because the virus can be transmitted by *C. pulla* for up to 6 days, it is regarded as a persistent transmitter of virus (Abo *et al*. 2000). The mechanism of mechanical inoculation via the chewing action of short- and long-horned grasshoppers is similar to transmission of TMV by the bumblebee (*Bumbus terrestris*) (Okada *et al*. 1999).

The importance of managing RYMV will increase as the promotion of rice production increases in Africa. An improved understanding of the transmission modes is important for the protection of rice culture. Chemical control of vectors may be necessary once there is a better understanding of the vector population dynamics in each country.

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