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Short communication

Rice yellow mottle virus is seed-borne but not seed transmitted in rice seeds

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Abstract

Seed transmission of two pathogroups of rice yellow mottle virus from Soudano-Sahelian areas of West Africa was studied in several rice genotypes. The virus was detected by enzyme-linked immunosorbent assay and the highly susceptible rice variety BG 90-2 was used for infectivity assays. In most of the rice genotypes studied (17 out of 21), rice yellow mottle virus was detected in all seed parts including glumella, endosperm and embryo at a rate ranging from 65 to 100%. Nevertheless, no seed-borne infection was found. Infectivity of the virus decreased throughout the process of seed formation suggesting inactivation of the virus as a result of seed maturation and desiccation. It was concluded that rice yellow mottle epidemics do not develop from seed-borne infections in rice seeds.

Rice yellow mottle virus (RYMV), genus *Sobemovirus*, causes a severe disease of rice (*Oryza sativa*) in most countries of Africa. Depending on date of infection and/or rice genotype, yield losses ranging from 25 to 100% have been observed (Awoderu, 1991). No reliable means of control is available, probably because little is known about RYMV epidemics, and most widely grown rice varieties are susceptible to the virus.

RYMV is mechanically transmissible and naturally transmitted by several species of beetles (Bakker, 1975). It was not found to be seed transmissible (Bakker, 1974; Fauquet and Thouvenel, 1977) but more recently, some authors noted that there is some evidence for RYMV to be seed-transmitted and suggested that the question should be re-examined (Awoderu, 1991). Furthermore, only relatively small numbers of seeds (less than 350) were tested in Bakker's studies (Bakker, 1974) and it has been shown for some viruses that seed transmission may occur at very low incidence (Goodman and Oard, 1980; Mikel et al., 1984; Jensen et al., 1991). Low rates of seed transmission in conjunction with secondary spread by insect vectors can produce viral disease epidemics (Ryder, 1973; Dinant and Lot, 1992; Maule and Wang, 1996). This work was undertaken to further evaluate RYMV transmission through rice seed.

Two rice species, *Oryza glaberrima* (16 varieties) and *Oryza sativa* (5 varieties), were used. All plants were grown in 10-litre plastic buckets and maintained in an insect-proof greenhouse at 25–30 °C and relative humidity of 80–90%. RYMV isolates included pathogroups A and B originating from West Africa (Konaté et al., 1997). They differ in the capacity of pathogroup B to break down the high resistance of rice variety TOG 5681. Each pathogroup of the virus was mechanically inoculated to rice seedlings four weeks after germination. Inoculum was obtained by grinding one gram of infected rice leaves in 10 ml of 50 mM phosphate buffer pH 7.0. The homogenate was filtered through two layers of cheesecloth, and carborundum (600 mesh) was added. Seeds were collected from infected plants and used in subsequent tests.

RYMV was detected using indirect double antibody sandwich (IDAS)-ELISA. All buffer systems and incubation times were as described previously (Konaté et al., 1997). The coating and detecting antibody was a polyclonal antibody raised against a mixture of three RYMV serogroups (Konaté et al., 1997). Ninety-two whole seeds per rice variety were assayed individually for virus presence. Each seed was ground in 300 µl of buffer, and the homogenate was decanted and incubated in microplate wells previously coated with polyclonal rabbit antibody. Biotinylated gamma globulins were incubated and streptavidin–alkaline phosphatase conjugate was added. Optical density at 405 nm was recorded 1 h after substrate incubation using a Metertech Σ960 microplate reader. Extracts of seeds collected from healthy plants were incubated in triplicate as negative control. The mean absorbance value plus three times the standard deviation was taken as negative–positive threshold.

Both RYMV pathogroups were detected in rice seeds from all susceptible and resistant varieties (Table 1). All absorbance values were at least six times the negative threshold (data not shown). Background values were less than 0.06. Using the Student's t-test, no significant difference in seed contamination was observed between the two RYMV pathogroups ($t = 0.122$, $P > 0.90$). As expected, no virus was detected in seeds of the immune variety TOG5672. Diverse situations were observed for highly resistant rice varieties. Thus no seed or a small proportion of seeds was contaminated in TOG5681 and TOG5675 whereas high percentages of contamination were obtained for other varieties, e.g. VL6 and VL123 with similar resistance ratings to RYMV (Coulibaly et al., 1999). The high variability of seed contamination levels among rice varieties with different resistance ratings to RYMV suggests that in rice, resistance to seed contamination, if any, is independent of resistance to infection by the virus.

RYMV was also detected in dissected seeds. For each rice variety, 140 seeds were dissected into three parts (glumella, endosperm and embryo) which were pooled by lots of 20 and assayed as above. The virus was detected in all seed parts for almost all rice varieties regardless of the virus pathogroup (Table 2). Contamination of each seed part by the two RYMV pathogroups was found to be similar as determined by means of the χ^2 test ($\chi^2 = 1.463$, $P = 0.481$). However, when considering both pathogroups together, differences in RYMV detection between the seed parts were highly significant ($\chi^2 = 137.53$, $P < 0.001$). The virus was detected in all glumellas lots whereas only 64% and 34% of 140 lots were positive, respectively for embryo and endosperm. The higher proportion of contaminated glumellas compared to embryos and endosperm indicate that in some seeds, the virus invades the glumella but not the other seed parts.

Table 1. Detection of RYMV in rice seeds and seedlings

Rice variety	Resistance scoring*	Contaminated seeds (%)	
BG 90-2	S	97.80**	64.73***
Bouaké 189	S	98.90	98.91
Kogoni	S	97.80	98.91
Metica	S	78.26	76.08
TOG 7214	S	92.39	94.56
TOG 7217	S	91.30	91.30
TOS 3554	S	65.21	65.21
TOS 16101	S	95.65	97.82
VL 17	S	66.30	75.00
VL 166	S	69.56	81.52
Wita 7	S	91.30	89.13
Wita 8	S	96.70	97.82
Wita 9	S	51.06	47.82
FKR 33	R	93.50	78.26
IRAT 104	R	100.0	100.0
Morobérékan	R	100.0	100.0
TOG 5681	HR	0.00	0.00
TOG 5675	HR	0.00	4.33
VL 6	HR	95.65	98.91
VL 123	HR	75.00	69.56
TOG 5672	I	0.00	0.00

*I, immune; HR, highly resistant; R, resistant; S, susceptible.
**pathogroup A.
***pathogroup B.

Table 2. Detection of RYMV in rice seed parts using DAS-ELISA

Rice variety	Virus detection					
	Glumella		Embryo		Endosperm	
Kogoni 91-1	7/7*	7/7**	7/7*	7/7**	4/7*	3/7**
Bouaké 189	7/7	7/7	1/7	6/7	1/7	6/7
IRAT 104	7/7	7/7	4/7	2/7	1/7	2/7
Moroberekan	7/7	7/7	7/7	7/7	7/7	7/7
TOS 16101	7/7	7/7	3/7	7/7	2/7	2/7
TOS 3554	7/7	7/7	6/7	6/7	2/7	4/7
TOG 5675	7/7	7/7	0/7	2/7	0/7	0/7
TOG 7214	7/7	7/7	4/7	1/7	1/7	0/7
VL 123	7/7	7/7	2/7	4/7	1/7	4/7
Metica	7/7	7/7	6/7	7/7	0/7	0/7

*Number of seed parts lots tested positive for virus/number tested (pathogroup A).
**Number of seed parts lots tested for virus/number tested (pathogroup B).

Seed invasion by the virus is likely to start in the glumella before reaching the embryo and lastly the endosperm.

Seed-borne infection was tested by sowing 1000 seeds per variety in sterilised soil. Six weeks after sowing, seedlings were collected, and groups of 20 were pooled and assayed for virus using ELISA as described above. The dilution of the tissue extracts was 1/10. No seedling grown from seeds contaminated by either RYMV pathogroups developed rice yellow mottle symptoms six weeks after germination. In addition, all ELISA results were negative. Thus RYMV could not be detected in any seedling of the 21 rice varieties tested, supporting the evidence from the lack of symptoms. Embryo invasion is a common feature of most seed-transmitted viruses (Mathews, 1991; Maule and Wang, 1996) but finding the virus in the embryo does not always lead to seed transmission. There may be some inactivation of the virus infectivity (de Assis Filho and Sherwood, 2000).

To ascertain this hypothesis for RYMV, infectivity was monitored during five stages of seed formation: (i) flowering; (ii) soft immature seeds with green glumella; (iii) hard immature seeds with pale yellow glumella; (iv) mature seeds at harvest; (v) desiccated seeds stored for one month. For each stage, 100–120 seeds of variety Bouaké 189 infected with either RYMV pathogroups were tested individually. Seed extracts were inoculated to seedlings of the highly susceptible rice variety BG 90-2. Inoculation was done as above except that 0.1 M thioglycollic acid or 0.1 M sodium diethyldithiocarbamate were added to the buffer as stabilising agents. Inoculation of crude seed extracts at any stage of seed formation to rice seedlings resulted in infection. No significant difference was observed between the two RYMV pathogroups (data not shown). Infection rates were high at stages I and II when the seeds still remained green (Figure 1). As the colour turned to yellow and the seeds started to lose water (stage III), infectivity decreased. For completely desiccated seeds (stage V) infection rates were very low (3.4–3.7%) despite high proportions of seed contamination (Table 1). These results suggest that during the seed maturation, the virus is strongly inactivated. This inactivation may be responsible for the absence of seed-borne infection of RYMV in rice as reported for some host plant/virus systems (Bowers and Goodman, 1979; Bailiss and Offei, 1990). Other factors may also be involved since the virus remained infectious in some dry seeds, although at low rates (Figure 1), and this did not result in any seed-borne infection. In this case, absence of seed transmission may be due to the activity of the cell or the location of the virus within the embryo that hampers seedling infection (de Assis Filho and Sherwood, 2000). Altogether, our results indicate that RYMV is one of the few non-seed-borne viruses found in embryo (Bowers and Goodman, 1979). Further investigation at viral RNA level may be useful for better understanding of the decrease of infectivity during seed maturation.

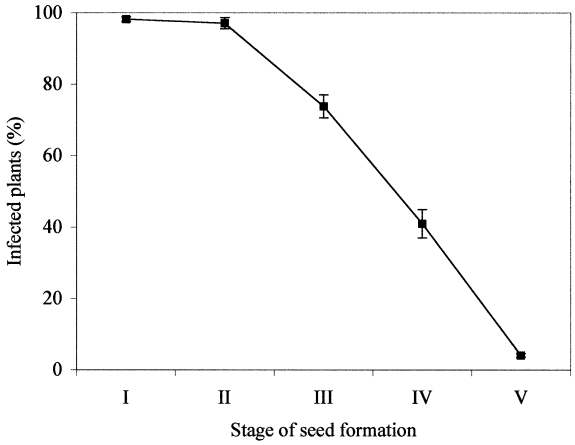

Figure 1. Infectivity of RYMV in rice seeds during the course of seed formation: I, flowering stage; II, soft seeds with green glumellas; III, hard seeds with pale yellow glumellas; IV, mature seeds at harvest; V, desiccated seeds in storage. Error bars represent the standard error of the mean of three replicates for each stage.

References

Awoderu VA (1991) Rice yellow mottle virus in west Africa. Tropical pest management 37: 356–362

Bakker W (1974) Characterisation and ecological aspects of rice yellow mottle virus in Kenya. Agricultural Research Reports, Wageningen 829: 1–152

Bakker W (1975) Rice yellow mottle virus. CMI/AAB Descriptions of Plant Viruses 149: 1–4

Bailiss KW and Offei SK (1990) Alfalfa mosaic virus in lucerne seed during seed maturation and storage, and in seedlings. Plant Pathology 39: 539–547

Bowers GR Jr and Goodman RM (1979) Soybean mosaic virus: infection of soybean seed parts and seed transmission. Phytopathology 69: 569–572

Coulibaly MM, Konate G and Zongo JD (1999). Criblage variétal du riz pour la resistance au RYMV au Sahel. Revue du Cames 01 (01) (in press).

de Assis Filho FM and Sherwood JL (2000) Evaluation of seed transmission of *Turnip yellow mosaic virus* and *Tobacco mosaic virus* in *Arabidopsis thaliana*. Phytopathology 90: 1233–1238

Dinant S and Lot H (1992) Lettuce mosaic virus. Plant Pathology 41: 528–542

Fauquet C and Thouvenel JC (1977) Isolation of rice yellow mottle virus in the Ivory Coast. Plant Disease Reporter 61: 443–446

Goodman RM and Oard JH (1980) Seed transmission and yield losses in tropical soybean infected by soybean mosaic virus. Plant Disease 64: 913–914

Jensen SG, Wysong DS, Ball EM and Higley PM (1991) Seed transmission of maize chlorotic mottle virus. Plant Disease 75: 497–498

Konaté G, Traoré O and Coulibaly MM (1997) Characterisation of rice yellow mottle virus isolates in Soudano-Sahelian areas. Archives of virology 142: 1117–1124

Mathews REF (1991) Plant virology. Third Edition. (835 pp.) Academic Press, Inc.

Maule J and Wang D (1996) Seed transmission of plant viruses: a lesson in biological complexity. Trends in Microbiology 4: 153–158

Mikel MA, D'Arcy CJ and Ford RE (1984) Seed transmission of maize dwarf mosaic virus in sweet corn. Phytopathologische Zeitschrift 110: 185–191

Ryder EJ (1973) Seed transmission of lettuce mosaic virus in mosaic resistant lettuce. Journal of American Society of Horticultural Science 98: 610–614