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Rice seedbeds as a source of primary infection by *Rice yellow mottle virus*

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Abstract

The effect of contamination of rice seedlings by *Rice yellow mottle virus* (RYMV) in seedbeds on the onset and spread of rice yellow mottle in the field was investigated. Rice seedlings were artificially contaminated in seedbeds at different rates (0.1, 0.5, and 2.5%) and pooled in bundles before transplantation, as done by farmers. RYMV was successfully transmitted through contaminated hands and bundling healthy and diseased seedlings together. Hand contamination was responsible for 4.5% infection. Disease incidence in the field after secondary spread reached 32% for 2.5% seedbed contamination rate but remained limited (less than 10%) for all other rates. Eradicating infected plants from seedbeds lessened disease incidence in the field. This technique may be used in conjunction with other prophylactic measures to efficiently control rice yellow mottle disease.

Introduction

Rice (*Oryza sativa*) is a principal staple food for many people in the world. The world rice production in 2004 was around 600 million tons (Mt) with an average yield of 4 t ha^{-1} (FAO, published online: www.Fao.org). Up to 90% of the rice produced was from the Asian continent. The contribution of the African continent was evaluated at only 4%. Although rice has been cultivated for a long time in Africa (Porteres, 1950), average yields remain among the lowest in the world (i.e. 1–3 tonnes ha^{-1}).

Rice yellow mottle virus (RYMV) is the main virus of rice in Africa and is also one of the major factors of yield losses. The virus was first reported in Kenya (East Africa) along the shore of lake Victoria (Bakker, 1970). It now occurs in most rice-growing African countries including Madagascar (Abo et al., 1998; Traoré et al., 2001), but not outside the African continent. Thus, it was speculated that RYMV is an indigenous virus which gained importance with the intensification of rice production and the introduction of a diversity of exotic varieties mainly from Asia (Awoderu, 1991; Thresh, 1998).

RYMV is a member of the *Sobemovirus* genus. It is naturally transmitted mainly by beetles, and also readily transmitted by mechanical inoculation (Bakker, 1970). Transmissions by rats, cows and donkeys or by wind were recently reported (Sarra and Peters, 2003; Sarra et al., 2004). Although RYMV can invade rice seeds, it has been shown to be non seed-transmitted (Bakker, 1974, Fauquet and Thouvenel, 1977; Konaté et al., 2001). RYMV-infected plants are characterized by yellow discolouration of the leaves, delayed flowering, poor emergence of panicles, spikelet sterility, and stunting (Bakker, 1974). Yield losses depend on rice variety and plant age at the time of infection. Most rice varieties grown with high yielding potential are susceptible to RYMV and may be subjected to losses of up to 100% (Konaté et al., 1997).

The introduction of exotic rice varieties into Africa was accompanied by some changes in rice cropping practices, especially in the irrigated areas of West Africa. The most significant changes included growing two crops per year and shifting from a wide use of direct seeding of rice fields to an exclusive use of the seedbed system (Coulibaly et al., 2001). In this system, rice seeds are first germinated in seedbeds for three to four weeks. Then, seedlings are uprooted from the seedbeds, pooled into bundles, and carried to the fields. Bundles of smaller sizes are made and scattered all over the field. Seedlings are taken from these bundles and transplanted in straight lines. In some cases, farmers under- or over-estimate their seedbeds sizes. In these cases, extra seedlings are taken from or given to neighbours. Since RYMV is highly infectious (Bakker, 1974; Fauquet and Thouvenel, 1977), handling seedlings during transplantation from seedbeds to rice fields may lead to primary spread of the virus by any mechanical means.

In this paper, we assessed RYMV contamination rates in seedbeds under field conditions and investigated the spread of the virus from seedbeds. The epidemiological significance of the findings is discussed.

Materials and methods

Surveys for RYMV infection in seedbeds

Seedbeds were surveyed for contamination of rice seedlings by RYMV in Sélingué and the 'Office du Niger' zones which are two important rice-growing areas in Mali. Surveys were done in seedbeds set for both rainy and dry season crops. Seedbeds were chosen at random but only those with seedlings of 3–4 weeks of age were included in this study. Altogether, 127 and 195 seedbeds were visited in July-September (rainy season) and February–March (dry season), respectively. Plantlets were scrutinized for rice yellow mottle symptoms in the whole seedbed if its size was less than 20 m^2 or in 2 m wide-strips all around the seedbed when the size was larger. All infected seedlings in the strips were counted whereas the total number of seedlings was calculated from the average plant density determined from ten random squares of 0.5×0.5 m. When identification of infected seedlings based on symptoms was doubtful, leaves were sampled and assayed by ELISA as described in Konaté et al. (1997).

Testing RYMV transmission by contaminated hands

RYMV was first propagated in the susceptible rice variety BG 90-2 (15 plants per pot). Infected plants were used for transmission through contaminated hands. A disposable latex glove was worn and leaves of all fifteen infected plants in a pot were caught between fingers which were subsequently used to catch leaves of a healthy 2-week old plant. Then, the glove was discarded and a new one was worn before touching the infected plants anew. Virus transmission to 100 plants was tested in this way and the whole experiment was done in three repeats with a total of 300 plants tested.

Testing RYMV transmission through bundling

Rice seeds were sown in two separate seedbeds. At 14 days post-germination, all plants in one seedbed were mechanically inoculated whereas those in the second seedbed were kept healthy. Two weeks later, non-inoculated plants were uprooted and pulled in bundles with diseased plants. Each bundle was made of 1000 seedlings and proportions of infected plants in the bundles were 0.1, 0.5, and 2.5%, respectively. Seedlings from bundles were subsequently transplanted in the field while discarding diseased plantlets as one came across them. Transplantation was done in three replicates for each contamination level and plots were arranged in a complete randomized block design.

Effect of seedling contamination rates in seedbeds on disease development in the field

Seeds of the susceptible rice cultivar BG90-2 were sown in two sets of three seedbeds and two weeks later, seedlings were mechanically inoculated at random with *Rice yellow mottle virus*. Proportions of inoculated seedlings in seedbeds from each set were 0.1, 0.5 and 2.5%, respectively. Inoculated plants were immediately tagged by attaching a coloured thread. Rice yellow mottle symptoms developed fully 2 weeks later. All diseased plants were removed from seedbeds in one set and the rest of the seedlings were uprooted, put into bundles and subsequently transplanted in the field. Accordingly, seedbeds of this set were named S1*, S5* and S25* with respective contamination rates of 0.1%, 0.5% and 2.5%. For the second set of seedbeds (S1, S5 and S25), diseased and healthy seedlings were uprooted together and put into bundles. However, infected plants were discarded during transplantation and only healthy plants were transplanted in the field. All bundles were made of 1000 seedlings and field plots were arranged as indicated above.

Results

Levels of seedbed contamination by RYMV in field conditions

Rice seedlings in seedbeds showed two types of yellowing symptoms. In the first type, leaves were uniformly yellow. Such symptoms which were sometimes observed on wild rice *Oryza longistaminata* were found to be unrelated to infection by RYMV (O. Traoré and M.D. Traoré, unpublished data). Similar misleading yellowing symptoms may be induced by iron or nitrogen deficiency (Thottappilly and Rossel, 1993). Contrasting with the first type, mottling was always present along with the second type of yellowing symptoms. Therefore, the second type was more characteristic of rice yellow mottle (Bakker, 1974) and was used to identify RYMV-infected seedlings in seedbeds. This choice was further shown to be adequate as only plant samples bearing mottle-associated symptoms were ELISA positive (data not shown). In addition to rice seedlings germinated from sown seeds, infected plants in seedbeds also included stubble of infected rice from previously harvested crops and the perennial wild rice *Oryza longistaminata*. Such situations were particularly observed at Sélingué where the immediate surroundings of seedbeds were often occupied by numerous grasses and infected rice stubble.

Proportions of contaminated seedbeds during the rainy season varied between 0% mainly in the 'Office du Niger' zone and 23% at Sélingué (Table 1). No infection was observed during the dry season in any zone in spite of the much greater number of seedbeds examined, especially at Sélingué. This was probably responsible for low disease incidences (often less than 15%) and the late onset of the disease in the field (4 to even 6 weeks after transplantation).

Table 1. Seedbed contamination by *Rice yellow mottle virus* in some locations of Mali during the 2001 rainy season and the 2002 dry season

Location[1]	Time of the survey[2]	
	July–September	February–March
Niono	0/20[3]	0/49
Molodo	0/25	–
N'Débougou	1/21	0/12
Sélingué	14/61	0/134
Total	15/127	0/195

[1]Localities of Niono, Molodo and N'Débougou are part of the 'Office du Niger' zone.
[2]July–September: rainy season; February–March: dry season.
[3]Number of contaminated seedbeds/total number of seedbeds examined.

On the whole, proportions of infected rice seedlings in contaminated seedbeds were low but varied noticeably between 0.01% and 0.17% from one seedbed to another. Most of the time, infected seedlings were scattered in seedbeds and clusters of diseased plantlets were rarely observed probably because of low disease incidence. There was also a great variation in seedbed size (3–1250 m^2) but this was not related to disease incidence.

RYMV transmission through contaminated hands and bundling

Successive handling of infected and healthy plants resulted in the infection of healthy ones indicating a successful transmission of RYMV through contaminated hands. Five plants out of 100 were infected in each of two repeats of the experiment whereas in the third repeat, only 3 out of 100 plants were infected. Hence, a total of 13 out of 300 handled plants were infected through contaminated hands.

Pooling healthy and infected seedlings into bundles also resulted in contamination of healthy ones (Figure 1). First symptoms appeared 2 weeks after transplantation. At that time, symptoms were not observed on plants in controls plots, indicating that bundling was responsible for primary spread of RYMV in the field. A few plants in control plots

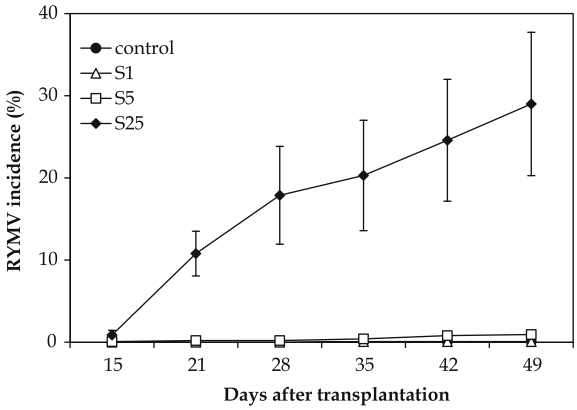

Figure 1. Effect of bundling diseased rice seedlings together with healthy ones on the spread of RYMV in the field. Diseased seedlings were put into bundles at different rates (0.1, 0.5, and 2.5% respectively) with healthy ones. Then, only healthy seedlings were further transplanted into the field. Diseased plants were discarded during transplantation. Error bars represent the standard errors of the means.

developed symptoms only after 28 days post-transplantation, an indication that secondary spread from first contaminated plots started at that time. Disease incidence was further assessed over six weeks starting from the appearance of first symptoms in the field. Two-way analysis of variance revealed significant effects of bundle contamination level ($F = 146.4$, $df = 3$; $P < 0.001$), date ($F = 7.79$, $df = 5$, $P < 0.001$) and contamination level × date ($F = 4.47$, $df = 15$, $P < 0.001$). In particular, disease development was significantly higher with 2.5% contamination between 21 and 49 days after transplantation. Little spread occurred in the control plots as well as in plots where bundles contained 0.1% or 0.5% of infected plants. Disease incidence was similar in all these treatments and did not exceed 10% even at 49 days post-transplantation (Figure 1).

Effect of seedbed contamination on RYMV incidence in the field

Two weeks after transplantation, rice mottle symptoms were observed in 15 out of 21 plots. Infected plants were identified in all nine plots where seedlings from seedbeds S25, S25* and S1 were transplanted. Two out of three plots corresponding to each of seedbeds S1*, S5 and S5* were also contaminated. No disease was observed in any of the three control plots, indicating that seedbeds were the source of primary spread of RYMV in the contaminated plots. As expected, symptoms appeared on transplanted seedlings that came from seedbeds from which diseased plants were bundled together with healthy ones prior to transplantation. Surprisingly, infection occurred also in field plots where seedlings from disease-eradicated seedbeds were transplanted. Therefore, removing diseased seedlings from seedbeds before bundling did not prevent primary spread of RYMV in the field. As in some instances a few non-inoculated seedlings were found infected afterwards, this result indicated that some contamination occurred inside the seedbeds but infection remained latent before transplantation.

Seedling contamination rates in seedbeds had a marked effect on disease spread (Figure 2). Contamination rate ($F = 170.08$, $df = 6$, $P < 0.001$) and date ($F = 38.24$, $df = 5$, $P < 0.001$) effects as well as the contamination rate × date interactions were significant in two-way analysis of variance. Most significant spread occurred in plots in which seedlings from seedbed S25 were transplanted. Disease incidence exceeded 30% at 49 days post-transplantation. Disease spread in plots that received seedlings from seedbed S25* was much less (not more than 10%), indicating a substantial effect of the eradication procedure used in the seedbed. RYMV incidence in all other plots including the

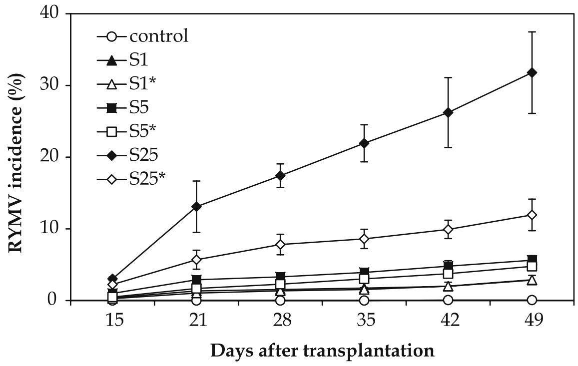

Figure 2. Rice yellow mottle incidence in a field where rice seedlings from contaminated seedbeds were transplanted. Contamination levels were 0.1% for seedbeds S1 and S1*, 0.5% for seedbeds S5 and S5*, and 2.5% for seedbeds S25 and S25* respectively. Infected seedlings were removed from seedbeds S1*, S5* and S25* and remaining plants in each case were pooled into bundles for transplantation. For seedbeds S1, S5 and S25, all seedlings were uprooted and put into bundles. Then, tagged inoculated seedlings were discarded during transplantation. Error bars represent the standard errors of the means.

control did not exceed 5% so that the eradication effect that was expected from seedbeds S1* and S5* was not apparent.

Discussion

Natural RYMV infection rates in seedbeds were quite low. But, rice seedlings are usually transplanted in the field regardless of their sanitary status. Therefore, usage of such seedbeds is similar to the situation of viruses transmitted at low rates by seeds. If secondary spread in the field is efficient, disease epidemics may occur (Dinant and Lot, 1992; Johansen et al., 1994). Infected plants in seedbeds included rice stubble and wild rice *O. longistaminata*, indicating that not much care was taken in setting and managing seedbeds. Although infected rice stubble and *O. longistaminata* plants are not further transplanted in the field they are pooled into bundles with rice seedlings and discarded only during transplantation.

RYMV has been shown to be highly infectious (Bakker, 1974; Fauquet and Thouvenel, 1977). This was also confirmed here, as transmission occurred through contaminated hands. Mechanical sap inoculation of RYMV to susceptible rice cultivars is usually associated with incubation times of 5–8 days (Bakker, 1970; Ndjiondjop et al., 2001). In our experiments, two weeks were needed for symptom expression possibly because of the smaller amount of viral particles inducing the disease when seedlings were handled and pooled into bundles (Hull, 2002). Bundling diseased and healthy rice seedlings together resulted in primary spread of RYMV in the field. Contamination of healthy seedlings in the bundles may have occurred through contaminated hands while handling plants or may have also resulted from virus transmission by direct contact between healthy and diseased seedlings (Abo et al., 2000). Hence, both means of transmission are complementary.

Primary spread even occurred from seedbeds from which infected seedlings were removed before bundling and transplantation. Possibly, contamination of healthy seedlings in seedbeds occurred by repeated contact with diseased plants due to high plant density and wind (Abo et al., 2000; Sarra et al., 2004). In spite of the occurrence of such contamination, removing infected plants from seedbeds led to a substantial decrease in disease development in the field. Eradication has been found effective for controlling several economically important plant virus diseases like peach mosaic or citrus tristeza (Thresh, 1988). This technique can also be used in seedbeds as a control measure against rice yellow mottle. Because of serious damage caused by rice yellow mottle, particularly in West Africa, an increasing number of farmers are aware of symptoms induced by RYMV. This is an important prerequisite for using eradication to control the disease efficiently.

We have shown that for seedbed contamination levels lower than 2.5%, little spread of the disease occurred in the field. In our experiments, diseased seedlings from seedbeds were not transplanted. Because in field conditions, seedlings from seedbeds are transplanted by farmers regardless of their sanitary status, more spread is likely to be expected even from low seedbed contamination levels. Consequently, in addition to disease eradication, special care should be taken to prevent contamination in seedbeds. Apart from possible internal virus sources (infected rice stubble or *O. longistaminata* plants), potential vectors bringing RYMV to seedbeds from outside sources are insects (Bakker, 1974) and mammals like cattle, donkeys and rats (Sarra and Peters, 2003). Setting seedbeds in clean environments and protecting them from grazing by cattle should help to limit contamination of seedlings and further spread of the disease in the field.

Overall our results indicated that the occurrence of infected seedlings in seedbeds and their handling may be of serious concern for rice mottle disease management. Although the effect of naturally-contaminated seedbeds on the spread of the disease in the field has not been dealt with in this study, high incidence of rice yellow mottle reaching 100% was reported in Mali and was tentatively connected to infection from seedbeds (Sy, 1994). As reported for some plant viruses (Thresh, 1982), it is likely that man himself plays the major role in the primary spread of RYMV in fields through some cropping practices. These include setting seedbeds in places where infection sources occur and handling infected and healthy seedlings together through bundling and transplantation. As transplantation is an important agronomic practice for improving rice yields, handling rice seedlings from seedbeds cannot be avoided. Therefore, the use of prophylactic control measures aimed at reducing as much contamination as possible in seedbeds is of prime importance.

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