Short Communication

Necrosis Caused by Rice Tungro Viruses in *Oryza glaberrima* and *O. barthii*

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We have been screening wild relatives of rice and African cultivated rice, *O. glaberrima* for resistance to tungro. When 4 accessions of *O. glaberrima* were tested for tungro resistance, they showed severe systemic necrosis within 4 weeks after inoculation. This necrosis was also observed in several accessions of *O. barthii*, a close wild relative of *O. glaberrima*. To determine if systemic necrosis caused by tungro viruses is a common reaction in these 2 species, 99 accessions of *O. glaberrima* and 53 of *O. barthii* collected widely from various African countries were evaluated. Of these, 96 *O. glaberrima* accessions and 51 *O. barthii* accessions showed necrosis. Systemic necrosis is therefore considered to be specific to these two species.

KEY WORDS: *Oryza glaberrima*, *Oryza barthii*, necrosis, rice tungro disease.

Introduction

Tungro is one of the most serious diseases of rice in South and Southeast Asia. It is a composite disease caused by rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). These viruses are transmitted by leafhoppers (HIBINO 1989). The major symptoms of tungro-affected rice plants are stunting and discoloration of the leaves, with colors ranging from various shades of yellow to orange. RTBV causes the tungro symptoms and RTSV intensifies them (OU 1985).

We have been evaluating *Oryza* spp. for sources of tungro resistance for use in our breeding programs. Among species in this genus, West African cultivated rice *O. glaberrima* STEUD and its close wild relative *O. barthii* CHEV showed severe systemic necrosis after inoculation of rice tungro viruses (RTVs). Although interveinal necrosis had been reported in *O. glaberrima* and *O. barthii* as a symptom of tungro (RAO *et al.* 1978), the systemic necrosis we have observed is the most severe reaction to tungro, and it has never been observed in any other species tested in the genus *Oryza*. Since the geographic distribution of *O. glaberrima* and *O. barthii* is restricted to Africa, where neither RTVs nor vector insects have been reported, such a severe reaction may be specific to these species. These two species collected from various countries in Africa were examined to determine whether necrosis is a common reaction to infection of RTVs.

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Materials and Methods

Ninety-nine accessions of *O. glaberrima* and 53 of *O. barthii* were randomly selected from the germplasm collection of the International Rice Germplasm Center (IRGC) and were tested for inoculation of RTVs. Seedlings were transplanted into clay pots (13-cm-diameter) at 5 seedlings per pot 8 days after seeding. Two days after transplanting, the seedlings were inoculated with green leafhopper (GLH) (*Nephotettix virescens* DISTANT) adults, which had been fed on TN1 infected with both RTBV and RTSV for 4 days, at 10 insects per seedling for 4 hours. Twenty seedlings were tested in each accession of *O. glaberrima*. Three to 20 plants were tested in each accession of *O. barthii*. TN1 (*O. sativa* L.) was tested for inoculation of RTVs as a susceptible check variety.

The inoculated plants were observed every 3 days after inoculation. Some plants wilted and died as a result of sucking by GLH. The plants that died due to factors other than necrosis were eliminated. At 30 days after inoculation, the leaves of the surviving plants were individually sampled to diagnose RTBV and RTSV using enzyme-linked immunosorbent assay (ELISA) (BAJET *et al.* 1985).

Results and Discussion

In a preliminary observation, when 4 accessions of *O. glaberrima* had been inoculated with RTVs, few plants survived beyond 3 weeks after the inoculation (Fig. 1~3). All plants infected with both RTBV and RTSV showed necrosis and died within 4 weeks after inoculation. In these accession, when the seedlings were caged with virus-free GLH, they did not show any necrosis even when they were sucked by GLH (Fig. 4). Therefore, it was considered that the necrosis was caused by infection of RTVs.

In the current study, of 99 accessions tested in *O. glaberrima*, 96 showed systemic necrosis (Table 1). Of the 96 accessions, some plants of 95 accessions survived beyond 30 days after inoculation. Most of these plants were infected with RTBV alone. No surviving plant in these accessions was doubly infected with RTBV and RTSV. It may be assumed that in the 95 accessions, all plants infected with both viruses showed systemic necrosis and then died before 30 days after inoculation. In the other accession of *O. glaberrima* (IRGC 101438, Guinea), 8 plants showed necrosis and died; 12 plants did not show any necrosis and survived; while 7 of them were infected with both viruses. The plants of this accession showed various reactions to infection of RTVs.

The remaining 3 accessions of *O. glaberrima*, IRGC 101049 (Africa), 101855 (Burkina Faso) and 103616 (Mali), showed yellow-orange discoloration of leaves but no necrosis, although they were infected with both RTBV and RTSV 30 days after inoculation.

It has been suggested that the origin of *O. glaberrima* is in the Niger River Delta and that the primary center of diversity is in the swampy basin of the upper Niger River in Mali (CHANG 1976). Burkina Faso is located close to this area. Two of the 3 accessions which did not show necrosis were derived from these countries. The accessions from this area may be rich in genetic diversity.

Systemic necrosis was also observed in 51 out of 53 accessions of *O. barthii* (Table 1).

Fig. 1-3. Reaction to infection of rice tungro viruses at 3 weeks after infection.

1: Leaves of *O. glaberrima* (IRGC acc. no. 100153).

2: Plants of *O. glaberrima* (100139).

3: Whole plants of TN1 (left) infected and not infected, and *O. glaberrima* (100139) (right) infected and not infected.

Fig. 4. *O. glaberrima* (100139) 3 weeks after being caged with viruliferous green leafhopper (GLH) (left), with virusfree GLH (middle) and without GLH (right).

Table 1. Distribution of systemic necrosis caused by infection of rice tungro viruses on *O. glaberrima* and *O. barthii*

Origin	<i>O. glaberrima</i>		<i>O. barthii</i>	
	No. of accessions		No. of accessions	
	Tested	With necrosis	Tested	With necrosis
West Africa				
Burkina Faso	8	7	1	1
Gambia	1	1	2	2
Guinea	10	10	3	3
Guinea-Bissau	10	10		
Ivory Coast	5	5		
Liberia	14	14		
Mali	11	10	8	8
Nigeria	7	7	2	2
Senegal	12	12	3	3
Sierra Leone	2	2	2	2
Central West Africa				
Cameroon	4	4	5	5
Chad	11	11	16	15
Congo	1	1		
East Africa				
Sudan	1	1	5	4
Tanzania			2	2
South Africa				
Botswana			1	1
Zambia			1	1
Africa ¹⁾	1	0	1	1
via CRRI ²⁾	1	1	1	1
Total	99	96	53	51

¹⁾ Country is not specified.²⁾ Central Rice Research Institute, India.

In 27 of 51 necrosis-affected accessions, some surviving plants were infected with both RTBV and RTSV. Only 2 accessions, IRGC 100933 (Sudan) and 104112 (Chad), did not show any necrosis although they were doubly infected with RTBV and RTSV.

As mentioned above, it was observed that in only 1 of 99 accessions of *O. glaberrima* (IRGC 101438), some plants showed the necrosis, while others did not show any necrosis although they were infected with both RTBV and RTSV. In *O. barthii*, however, similar reactions were observed in more than half of the accessions tested. It has been suggested that *O. glaberrima* is derived from *O. barthii* (MORISHIMA *et al.* 1963). It is possible that the wild relative is more heterogeneous than the cultivated species, and that some of the heterogeneity was lost during the process of domestication.

The systemic necrosis caused by infection of RTVs was observed in *O. glaberrima* and *O.*

barthii, but it has never been reported in any other species. Although the accessions tested were collected widely from various countries in Africa, most of them showed similar systemic necrosis in both species. Therefore, necrosis is considered to be specific to species *O. glaberrima* and *O. barthii*.

These species provide a useful material for studying the mechanism of pathogenicity of RTVs, and are of help to understand the tolerance to tungro. As individual plants showing these symptoms are killed rapidly and eliminated as disease sources in fields, these accessions can be utilized breeding as a kind of resistance source.

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*Oryza glaberrima*および*O. barthii*におけるイネツングロウイルスによる壊死

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ツングロ病は、南アジアおよび東南アジアにおける、きわめて重要なイネのウイルス病である。本病の病徴として、矮化、葉身の黄化、分けつの減少などが報告されている。これまで、アジアの栽培イネ *O. sativa* には見いだされていない高度の抵抗性を求めて、西アフリカの栽培イネ *O. glaberrima* を含め、野生イネ約 20 種に対して、ツングロ病抵抗性系統の検索を行ってきた。その過程で、*O. glaberrima* およびその近縁野生種 *O. barthii* がウイルス感染によって全身に壊死を起し、ウイルス接種後数週間て枯死することを発見した (Fig.1-4)。これは、現在までにみられた最も激しいツングロの病徴で、他の種には未だみられていない。

そこで、この病徴がこれらの種に普遍的であるかどうかを確認するため、広くアフリカ各国から採集された *O. glaberrima* 99 系統および *O. barthii* 53 系統を用いて接種・検定を行った。あらかじめウイルスを獲得吸汁させておいた媒介虫タイワンツマグロヨコバイ成虫を、播種後 10 日の幼苗に、苗当たり 10 頭 4 時間接種した。その結果、*O. glaberrima* 96 系統および *O. barthii* 51 系統に壊死がみられた (Table 1)。この壊死は、系統の起源国に関わらず普遍的に観察され、これら 2 種に特異的であると考えられた。