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In vitro screening of rice germplasm for resistance to brown spot disease using phytotoxin *

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Summary. Two R_1 IR_8 plants derived from somatic cells cultured in 25% *Helminthosporium oryzae* toxin-medium, and one IR_{54} plant derived from a control (toxin free medium) were found to have mutated resistance to brown spot disease of rice. In the second generation (R_2, the offspring of the R_1 generation) of the three resistant populations, the segregation of resistance and susceptibility to the disease was observed. This is the first report about a disease resistant mutation obtained successfully in rice by tissue culture and in vitro screening with phytotoxin.

Key words: In vitro screening – Disease resistance – *Helminthosporium oryzae* – Phytotoxin – Indica rice

Introduction

Carlson's pioneer research (1973) showing in vitro screening using phytotoxin has enabled great advances in breeding for disease resistance. Since then, several successful attempts have been reported: in corn with *Helminthosporium maydis* toxin (Gengenbach and Green 1975, 1977), sugarcane with *H. sacchari* toxin (Larkin and Scowcroft 1983), rape with *Phoma lingam* toxin (Sacristan and Hoffmann 1979) and potato with *Phytopathora infestans* toxin (Behnke 1979, 1980). However, there is no report available on screening for disease resistance in rice with phytotoxin. Previously we were able to demonstrate that under culture conditions rice calli react to both pathogen and toxin as does the intact plant (Vidhyaseharan et al. 1984). In this paper we report on mutations for disease resistance obtained by in vitro screening with phytotoxin.

Materials and methods

Mature seeds of IR_8, IR_{36} and IR_{54} and the pathogen *H. oryzae*, Race 46 were used in this experiment. All of the IR varieties were sensitive to Race 46 pathogen.

The crude production of toxin was extracted by the methonal-chloroform method (Vidhyasehara et al. 1984) from Race 46. The toxin medium was calculated by volume (V/V). The media for induction, subculture and regeneration were those used by Ling et al. (1985). Only first or second passage calli were used in the toxin treatment. In order to being as many cells as possible into contact with the toxin, the calli were crushed into as small pieces as possible (about 1 mm in size) with an inoculating spoon. The small pieces of callus were shaken in different concentrations of toxin media (0% (control), 1%, 5%, 10%, 25% and 50%) for 1–5 or more days. After the treatment by toxin, the calli were transferred to toxin free medium for subculture and regeneration.

R_1 plant leaves were cut out and tested in a bioassay for their response to Race 46. The 20-day-old seedlings (3 tiller stage) of some R_2 systems which were resistant to the pathogen in the R_1 generation were inoculated with the spores of Race 46 and cultured in a culture-chamber (100% R.H., 26°–28 °C, 3 days). Those plants in which the number of leisons was less than 6 (0–5) were classified as resistant; those with more than 13, as susceptible.

Results and discussion

The effect of H-toxin on callus growth

After being treated by toxin and transferred to toxin free medium, all of the calli lost their ability to grow and died if the shaking duration in the toxin medium (for all 5 concentrations) was longer than 2 days. In the 2 day shaking treatment, the percentages of dead callus

* This research was conducted at the IRRI, Manila

Table 1. H-toxin treatment and the response of the regenerated plant to bioassay in IR_8

Toxin concentration (%)	0	2.5	5.0	10.0	25.0	50.0	Total
No. of calli subcultured after treatment	64	64	64	64	360	360	976
No. of surviving calli [a]	28	20	24	8	24	11	115
No. of plants regenerated	3	1	2	1	4	1	12
Response to bioassay	3s	1s	2s	1s	2R 2s	1s	

[a] Survival is measured as those calli surviving 2 days after shaking in the toxin mediun, followed by 6–8 weeks subculture in the toxin-free mediun
[b] R = resistance; S = susceptible

Table 2. Response of R_1 plants to spores of *H. oryzae* in IR lines of rice [a]

Treatment	Response to the parasite	Varieties				Total
		IR_8	IR_{36}	IR_{54}	$IR_{6023\text{-}10\text{-}1\text{-}1}$	
Toxin medium	Resistant	2[b]	0	0	0	2
	Susceptible	7	2	23	0	32
Toxin-free medium	Resistant	0	0	1	0	1
	Susceptible	3	12	42	6	63
Total		12	14	66	6	98

[a] Cut leaf bioassay: made 2 days after inoculation of the spores; repeated two times
[b] In 25% H-toxin medium for 2 days

in the 50%, 25%, 10%, 5%, 2.5% and 0% toxin medium were 98%, 93%, 88.5%, 63%, 68.7% and 57%, respectively (Table 1). H-toxin could also inhibit the callus growth even if they survived in the toxin free medium. The inhibition degree was increased by the toxin concentration. The surviving callus kept the ability of regeneration (Table 1).

The response of regenerated (R_1) plants and their offspring to pathogen spores in the bioassay

Ninety-eight first regeneration (R_1) plants were subjected twice to a bioassay: three resistant plants were found (Table 2). They were derived from two calli of IR_8 and one of IR_{54}. Two days after inoculation, no symptom was present on the cut leaves (Fig. 1) while, most of the R_1 plants showed typical symptoms of the disease on the cut leaves, even after only 24 h (Fig. 1).

Table 1 shows the results of H-toxin treatment and the response of IR_8 regenerated plants to the bioassay. Of 976 pieces of callus, 12 regenerated to plants and matured. Of these, two plants derived from treatment in 25% toxin medium showed resistant to the spores of the pathogen in the bioassay, while the other 10 plants, from varying treatments, were susceptible. It seems that higher concentrations of toxin might be more effective in screening for disease resistance.

On the other hand, of the more than 1,000 original calli of IR_{54} treated in toxin media, 23 regenerated into mature plants. No plant derived from toxin treatment was shown to be resistant. However, one plant of the 43 derived from the control treatment was found to be resistant to the pathogen in the bioassay (Table 2). This result shows that mutation for disease resistance can arise through somavariation. It could be possible that resistance or tolerance in in vitro screening might obtained by somavariation and selection through selective pressure. It could be expected that various mutants (disease resistance, insect or salt tolerance and other stress conditions) might be found if the corresponding selective pressure was given to the R_1 plants.

All three resistant systems derived from R_1 resistant plants segregated into resistant and susceptible plants in the R_2 generation. The number of resistant and susceptible plants in a resistant system of R_2 was 9 and 1; 5 and 1 (from IR_8; derived from 25% toxin treatment) as well as 4 and 5 (from IR_{54}; toxin-free treatment). All of the control seedlings of IR_8 and IR_{54} (in-

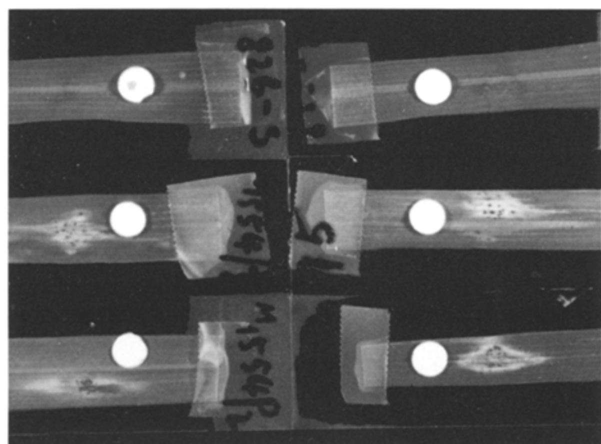

Fig. 1. The top row shows R_1 plants (IR_8 and IR_{54}) resistant to the pathogen (no symptom on the cut leaves); the bottom two rows, the susceptible plant (symptoms on the cut leaves)

cluding the susceptible R_1 plants and the normal plants without tissue culture) were susceptible. The results revealed that resistance to brown spot disease in rice, selected by H-toxin in vitro, was a dominant mutation. The R_1 resistant plants were heterozygotes and so the resistance could be expressed in the R_1 generation; they then segregated into resistant and susceptible plants in the R_2 generation. Atkins and Jodon (1963) demonstrated that resistance to the disease is controlled by dominant genes. The inheritance of the mutant isolated by toxin in vitro will be reported elsewhere.

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