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in

Chataigner J. (ed.).
Maladies du riz en région méditerranéenne et les possibilités d'amélioration de sa résistance

Montpellier : CIHEAM
Cahiers Options Méditerranéennes; n. 15(3)

1997
pages 51-53

In vitro screening for rice resistance to *Pseudomonas syringae*

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Abstract. In our study we investigated:
1. The influence of different concentrations of syringomycin, isolated from *Pseudomonas syringae* on the regeneration of calli, obtained by anther culture from 3 rice cultivars.
2. Field testing for resistance of the obtained doubled haploid lines. Anthers from 3 rice cultivars, Mariana, Krasnodarski 424, Belozem, were placed on N_6 media for callus induction. After 30 days, 1348 calli were translocated in MS media for regeneration with 3 levels of syringomycin: 10, 20 and and 30 active units in ml active ingredients and control without toxin. After 20 days at 26°-28°C, 262 calli with rhizogenesis, 64 green regenerants and 165 albino were recovered. Syringomycin increases about 3 times rhizogenesis and regeneration. From 52 green regenerants tested in the field, only 2 from the donor variety Mariana show field resistance to *Pseudomonas syrin - gae*.

I – Introduction

A perspective trend in the plant biotechnology has been to develop *in vitro* breeding methods for resistance to fungus, bacterial and other plant pathogens, or resistance to toxins, released by them (Daub, 1986, Pauly et al., 1987, Boyadjiev et al., 1990, Yin Daochuan et al., 1982). Screening explants *in vitro* has been successfully used for callus from young inflorescence and immature embryos, somatic tissue culture (Gao Ningwei, 1981) or anther culture callus (Fraser, 1985). The screening on a haploid level will be more effective for oligogenic characters like a bacterial resistance genes of rice (Xu Janlong et al., 1994). For the present, the question of resistance screening *in vitro* and the field reaction is still open (Li et al., 1990).

As a model system in our study, we used the toxin syringomycin, separated from pathogenic bacteria *Pseudomonas syringae p. v. atrofaciens*, and anther culture calli, obtained from 3 rice cultivars : Mariana, Krasnodarski 424, Belozem (Var. Japonica). The aim of this study was to establish the influence of the toxin syringomycin in the regeneration process in an anther calli system, and a field testing for resistance of the obtained doubled haploid (DH) lines.

II – Materials and methods

1. Donor cultivars and anther culture

For callus induction, anthers from 3 Bulgarian cultivars Mariana (DH cultivar, obtained with the method of anther culture from F1 hybrid combination Belozem x Plovdiv 22), Krasnodarski 424 (standard in Bulgaria) and Belozem were used. All cultivars are susceptible to *P. syringae*.

The donor cultivars were grown in normal field conditions. Anthers in midunonuclear stage were excised and placed on N_6 solid media for callus induction. All manipulations were described before (Boyadjiev et al., 1988). After 30 days from the callus induction, 1348 calli were translocated in MS media for regeneration with 3 levels of syringomycin: 10, 20, 30 units/ml and control without toxin. All green regenerants were grown in a greenhouse until maturation, and normal euploid seeds were sowed in the field and tested for resistant.

2. Pathogen, method for inoculation and estimation in the field

The inoculum was made by 24-48 h *P. syringae* bacterial culture. Donors cultivars (control) and regenerated lines were inoculated at 4 concentrations: 0-steril distilled water, 10^5 cfu/ml, 10^7 cfu/ml and 10^9 cfu/ml. From each variant, simultaneously in control and trials, 5 plants were marked with different color cottons and inoculated at the optimal growth stages (before heading). The assessment was carried out 10-14 days after infection when the symptoms are clearly seen. We formed 3 resistance groups according to the degree of infection:

0.0-37.5: resistant (R)
37.5-62.5: moderately susceptible (MS)
62.5-100.0: susceptible (S)

III – Results and discussion

Anthers from 3 rice cultivars were placed on N_6 media with 2 mg/l 2.4 D for callus induction. After 30 days, 1348 calli were translocated on NS media for regeneration (without 2.4 D) supplemented with 3 levels of syringomycin: 10, 20, 30 active units per ml ingredient and control, without toxin. The results from the rhizogenesis and the regeneration are showed in Table 1. By comparing the results between control and variants with 3 levels of syringomycin, a significant increase of rhizogenesis and regeneration for both albino and green plants is observed for the 30 ppm. syringomycin treatment.

Table 1. Rhizogenesis and regeneration of rice anther calli with 3 levels of syringomycin from 3 cultivars, Mariana (M), Krasnodarski 424 (K) and Belozem (B)

N	Parameter	Control without syringomycin			10 ppm syringomycin		
	Cultivars	M	K	B	M	K	B
1	Calli set	108	120	105	111	110	106
2	Calli with rhizogenesis	12	10	8	18	20	10
3	Green regenerants	1	5	2	2	6	3
4	Albino regenerants	1	4	12	2	4	7
N	Parameter	20 ppm syringomycin			30 ppm syringomycin		
	Cultivars	M	K	B	M	K	B
1	Calli set	113	115	121	114	108	117
2	Calli with rhizogenesis	17	25	20	46	50	26
3	Green regenerants	2	4	5	13	10	11
4	Albino regenerants	3	8	14	20	56	44

232 calli with rhizogenesis were obtained on syringomycin and 30 on control. 56 green and 158 albino regenerants were recovered from syringomycin screening and 52 green regenerants from the toxin screening were tested for resistance in the field with donor cultivars as control. Only 2 lines from Mariana showed resistance; all the other testing lines and control were susceptible. Mariana is a doubled haploid cultivar but in this case homozigote condition is probably not crucial.

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