Short communication

Protoplast fusion enhances mycoherbicidal efficiency of *Fusarium pallidoroseum* (Cooke) Sacc. – a pathogen of water hyacinth

A. Naseema* and P. Manju Elizabeth

Department of Plant Pathology, College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram 695522, Kerala, India.

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Abstract

Protoplast fusion between *Fusarium pallidoroseum* and *F. oxysporum* was carried out to develop an efficient mycoherbicide against water hyacinth. The fusants were selected by growing in PDA media amended with lead acetate (4500 ppm) + Nizral (2000 ppm). Six fusants (F1, F2, F3, F4, F5, and F6) grew well in the media – of which two (F5 and F6) exhibited high infectivity of *F. oxysporum* and had limited host range as in the case of *F. pallidoroseum* suggestive of the potential of these interspecific hybrids to be used as biocontrol agents against water hyacinth.

Keywords: Biocontrol agents, *Eichhornia crassipes*, Host range, Interspecific hybrids.

Fusarium pallidoroseum (Cooke) Sacc. is a pathogen with good mycoherbicidial potential against water hyacinth [*Eichhornia crassipes* (Mart.) Solms] (Praveena et al., 2007) and has a narrow host range among the cultivated and other plants (Naseema et al., 2001). *F. oxysporum* (Schlecht.) Snyder and Hansen, although is highly infectious to water hyacinth, has a wider host range, infecting some of the cultivated plants (Ancy, 2003). Protoplast fusion has been frequently used to develop intraspecific, interspecific, and intrageneric suprahybrids of fungi with higher potentiality than their parents (Madhosingh, 1994). An attempt was made to enhance the mycoherbicide efficiency of *F. pallidoroseum* for biological control of water hyacinth by protoplast fusion. Although various biotechnological methods are available for the development of superior strains with enhanced biocontrol efficiency, the advantage of protoplast fusion is that the higher probability to obtain recombinants, which allows testing a large number of recombinants over short periods.

Protoplasts of *F. pallidoroseum* and *F. oxysporum* were isolated from mycelia grown in potato dextrose broth for 18 h at room temperature (28±2°C). The mycelia were aseptically transferred to sterile petri plates and washed repeatedly with sterile distilled water. It was then incubated in a 3 ml mixture of EDTA (100 mM), MES (100 mM), and DTT (5 mM) at pH 6 for 60 min (30°C) with constant stirring at 100 rpm. The mycelia were separated from the incubation mixture by centrifugation at 10,000 g for 10 min. The pellet was then mixed with 80 mg lytic enzyme from *Trichoderma harzianum* (Sigma Aldrich, USA) in 4 ml 0.01M citrate buffer (pH 5.85) containing 0.6 M KCl and 5 mM DTT. This mixture was incubated for 5 h at 30°C and stirred slowly at 30 rpm in a 125 ml conical flask. The viability of released protoplast was checked by staining with Evan's blue.

After the release of maximum number of protoplasts, the contents were filtered through sintered glass filter (Jena, porosity D_2). The filtrate was freed of enzymes

*Authorfor correspondence : Phone +91 471 2740810; Email <naseemadr@yahoo.com>.

by centrifugation with osmotic stabilizer (0.6M KCl) at 100 g for 6 min and the pelleted protoplasts were re-suspended in a known amount of the osmotic stabilizer. Purity of the protoplast from mycelia/debris was checked under phase contrast microscope. The protoplast was concentrated by sucrose gradient centrifugation and enumerated using a haemocytometer (Fig. 1). The protoplasts of *F. oxysporum* and *F. pallidoroseum* along with 100 µl polyethylene glycol each were poured into

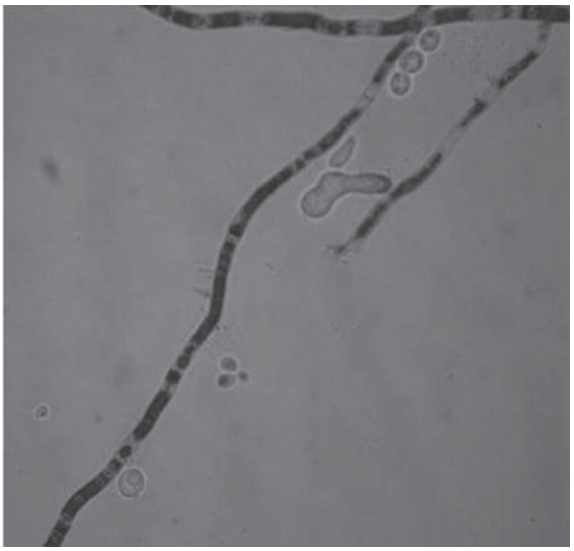

Figure 1. Protoplast getting released from mycelium.

sterile petri plates. Over the fusion mixture, 15 ml of sterile molten regeneration media (200 g potato, 20 g D-glucose, 0.6 M KCl, 20 g agar-agar, and 1 L water), cooled to bearable temperature, was added and rotated to ensure uniform distribution of the protoplasts. The plates were incubated at room temperature for 24 h and the individual colonies at random were aseptically transferred to potato dextrose agar (PDA) medium. Altogether seven fusants viz., F1, F2, F3, F4, F5, F6, and F7 were selected. Antibiotics *viz.*, amphotericin B (Fungizone), fluconazole (Diflucan), ketoconazole (Nizral), miconazole (Micatin) and natamycin (Natacyn) at 100 to 2000 ppm, and salts of heavy metals *viz.*, cadmium nitrate, copper sulphate, ferrous sulphate, lead acetate, lithium chloride, manganese sulphate, nickel nitrate and Zinc sulphate at 100 to 4500 ppm were tried, to obtain a marker for identifying the fusants (Lalithakumari, 2000). As *F. oxysporum* and *F. pallidoroseum* are resistant to lead acetate (4500 ppm) and Nizral (2000 ppm) respectively, the fusants were selected by growing in PDA media amended with lead acetate (4500 ppm) + Nizral (2000 ppm).

All fusants, except F7, grew well in the media (Fig. 2). The extent of infection produced by these fusants was tested along with the parents under *in vitro* conditions. Host specificity of the six selected fusants was also evaluated, on plants susceptible to the parents. The fusants F5 and F6 exhibited high infectivity similar to *F. oxysporum* (Fig. 3). However, these fusants had narrow host specificity, similar to F. *pallidoroseum.* The fusants were identified as interspecific hybrids with superior biocontrol efficiency than their parents (*F. oxysporum* and *F. pallidoroseum*) in terms of pathogenicity and host specificity.

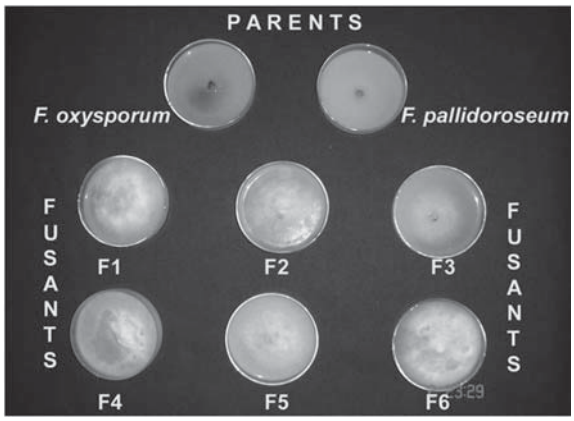

Figure 2. Growth of fusants and parents in selection medium.

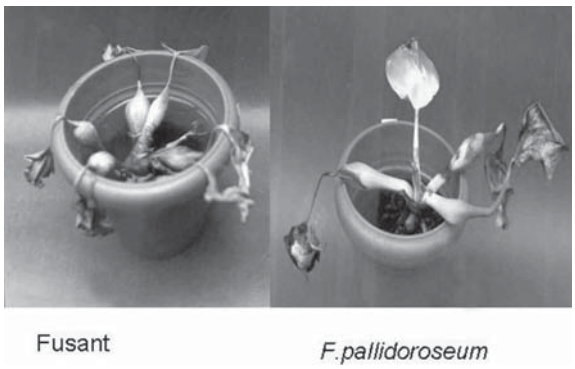

Figure 3. Effect of fusant and parent on water hyacinth.

Acknowledgements

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