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Production of pectic and cellulolytic enzymes by three soil borne fungal pathogens of chickpea

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A comparative assessment of the extracellular production of pectinolytic (polygalacturonase and pectin methyl esterase) and cellulolytic (Cx)enzymes of three soil borne fungal pathogens of chickpea, namely *Fusarium oxysporum* f.sp. *ciceri*, *Sclerotium rolfsii* and *Macrophomina phaseolina*, causing wilt, wet foot rot and dry root rot respectively, was made. Enzyme production was highest by *S. rolfsii* followed by *M. phaseolina*. The role of these enzymes in the pathogenesis of the respective diseases are discuseed.

Key words: Pectinolytic and Cellulolytic enzymes, *Fusarium oxysporum* f.sp. *ciceri*, *Sclerotium rolfsii*, *Macrophomina phaseolina*

INTRODUCTION

A major constraint to chickpea (*Cicer arietinum* L.) cultivation is some soil borne diseases. In West Bengal the most important fungal pathogens of chickpea are *Fusarium oxysporum* Snyder and Hansen f.sp. *ciceri*, Matuo and Sato, *Sclerotium rolfsii* Sacc. and *Macrophomina phaseolina* (Tassi) Goid, causing wilt, wet foot rot and dry root rot respectively (Biswas and Sen Gupta, 1981). These fungi produce an arrey of enzymes, of which pectic and callulolytic enzymes play very important role in pathogenesis. Production of pectic and cellulolytic enzymes in culture by the chickpea wilt pathogens, *F. oxysporum* f. sp. *ciceri* (Gupta and Kohli, 1967; Perez and Aldave, 1990) and *M. phaseolina* (Ali *et al*, 1969; Chan and Sackston, 1970; 1972) and *S.rolfsii* (Hussain, 1958; Bateman, 1969; Jones and Bateman, 1972) causing diseases of some plants has been reported. A comparative assessment of the extracelluer production of pectic and cellulolytic enzymes by these three fungal pathogens has been made the results of which are presented in this paper.

MATERIALS AND METHODS

The fungal isolates were isolated from infected chickpea plants collected from the field. Confirmation of the pathogenicity was established by growing seedlings of a susceptible cultivar of chickpea (JG 62) in potted soil inoculated with the inoculum (2% inoculum, W:W) of the respective fungi grown in sand maize meal medium in Erlenmeyer flasks for 10 days at 28°C.

For production of the enzymes Czapes Dox medium was used for *F. oxysporum* f. sp. ciceri and *S. rolfsii* and soybean seed extract both for *M. phaseolina*. The pH of the media was adjusted to 5.5.Carboxy methyl cellulose (0.5%) (in medium for estimating cellulolytic (C_x) enzyme activity) and citrus pectin (0.5%) (in medium for estimating pectic enzymes) were added separately in each of the 250 ml Erlenmeyer flasks containing 50 ml of the media. The sterilized media were inoculated separately with a 6 mm disc of 4 day-old-test fungi grown on PDA plates at 28°C for 7 days. The culture filtrates were collected by filtering through folds of cheese cloth. The filtrates were cold centrifuged at 5000 rpm for 15 min and the supernatants dialysed against deionized water for 24 h at 4°C. The test filtrates (enzyme preparations) were stored at 4°C. The cellulase (Cx) activity was measured colorimetrically following DNS test for reducing sugars (Gascoigne and Gascoigne, 1960). D-glucose (1 mg/ml) was used as the standard. Activity of the pectic enzyme, pectin methyl esterase (PME), was measured by continuous titration method (Hancock *et al*., 1964) and that of polygalacturonase (PG) activity colorimetrically by measuring the reducing groups by

DNS method similar to that used for enzymatic hydrolysis of CMC. D-galacturonic acid (1 mg/ml) was used as standard.

RESULTS AND DISCUSSION

Earlier studies in this laboratory showed that *S. rolfsii* is an early invador of young chickpea seedlings and within 5 days of inoculation 100% mortality of all the 7-days-old seedlings was recorded. In *M.phaseolina* and *F. oxysporum* f.sp. *ciceri* inoculated seedlings symptoms appeared at much later stage (Chattopadhyay, 1994). Very high enzymic activity, both cellulolytic (Cx) and pectic (polygalacturonase and pectin methyl estarase), was demonatrated by *S. ralfsii* in the present studies (Tables 1 and 2) followed by *M. phaseolena*. Comparatively lower amount of enzymes was produced by *F. oxysporum* f. sp. *ciceri*.

Although all the three test fungi extracellularly produced the pectic and cellulolytic enzymes studied in variable amounts and the role of these enzymes in pathogenesis, particularly for rot and wilt producing fungi, is well known, the nature of pathogenesis is, however, different.

Table 1. Cellulase (C_x) activity in the culture filtrates of the three test fungi

Fungus	Amount of D-glucose unit (released µg/ml/hr)
M. phaseolina	180
F. oxysporum f.sp. *ciceri*	120
Sclerotium rolfsii	210

Table 2. Pectic enzyme activity in the culture filtrates of the three test fungi

Fungus	PG activity (amount of D-galacturonic acid released µg/ml/hr)	PME activity (amount of 0.1(N) NaoH (ml) required at different time intervals (min))			
		20	40	60	80
M. phaseolina	500	0.24	0.44	0.76	0.90
F. oxysporum f. sp. *ciceri*	270	0.20	0.30	0.30	0.40
S. rolfsii	540	0.30	0.50	0.80	1.00

Harter (1916) suggested that pathogenic effects of *S. rolfsii* were the result of enzymic action. Hussain (1958) and Bateman and Beer (1965) concluded the involvment of cellulolytic and pectic enzymes of *S. rolfsii* in the rapid destruction of pectic and native cellulose substances within the hosts. As the enzymic action starts very early on the host seedling, total rotting of young host tissues results in wet rot.

Chan and Sackston (1972) on the otherhand, showed that the activity of PGTE and cellulase increased with time after plant inoculation with *M. phaseolina*. These enzymes were not important in initial penetration by the pathogen, but significant in further development of the disease. This may explain the destructiveness of *M. phaseolina* at a later stage of growth of chickpea as observed in the present studies.

F. oxysporum f. sp. *ciceri* is a vascular pathogen. Pectic and cellulolytic enzymes of this fungus act on the vascular components of the host degrading the pectic and cellulose substances of the cell walls resulting in vascular browning and production of gummy substances in the vascular lumen interferring flow of fluids (Diamond, 1955).

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