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Hydrolytic enzymes in relation to the fusarial wilt of brinjal

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ABSTRACT

Wilt of eggplant caused by *Fusarium solani* occurs in almost all the fields under cultivation that causes a great loss in yield of the vegetable. *In vitro* and *in vivo* cellulolytic and pectinolytic enzyme activities were assayed during pathogenesis. The β-glucosidase activity, among the all the unit components of cellulolytic enzyme was recorded to be the highest, which was followed in accordance with their activities by endoglucanase and exoglucanase. Pathogen was capable of producing all the unit components of the pectolytic enzymes and showed that the higher activity of both PG and PTE in *in vivo* and *in vitro* conditions than PMG and PGTE which indicated their greater role in pathogenesis and in disease development.

Key words : Brinjal, *Fusarium solani*, cellulase, pectinase.

Eggplant (*Solanum melongena* Linn.) is an important commercial vegetable which enjoys a high demand of premium price through out the year. Wilt caused by *Fusarium solani* (Mart.) Appel and Wollenw is a dreadful disease posing a threat towards the cultivation of brinjal. Cell wall degrading enzymes play a vital role in the development of the disease. Several enzymes are known to degrade pectic and cellulose substances of plant cell walls and as a result of which the pathogen gets an entry into the host tissues (Mesaphy and Levavon, 1992, Chakraborty, 2005). The present paper deals with the studies on the activities of cell wall degrading enzymes like cellulases and pectinases secreted by *Fusarium solani* and their relationship to the development of wilt of brinjal.

MATERIALS AND METHODS

Fusarium solani was isolated from wilted brinjal plants and maintained in pure line on potato dextrose agar (PDA) slants at 4^0C till used. The identification of the pathogen was confirmed by Indian Agricultural Research Institute (IARI), New Delhi (ITCC No.4124.2K).

Cellulolytic enzymes:

In order to obtain the enzyme source, Czapek's medium was used as the basal medium (Mahadevan and Sridhar, 1982) serving as substrate for *in vitro* production of the enzyme.

For *in vivo* study, 25g of tissues from *F. solani* infected plant was cut into 1-2 cm pieces, homogenized with chilled double distilled water. The slurry was passed through two layers of cheese cloth and centrifuged at 2,000 rpm for 30 min, at 4°C. The dialysed supernatants were used as enzyme source. Both *in vitro* and *in vivo* enzyme activities were studied at intervals of after 7, 14, 21 and 28 days of inoculation.

The cellulase enzymes were assayed following mainly the method of Mahadevan and Sridhar (1982). Exoglucanase activity was determined by incubating 0.25 ml of culture filtrate with 0.75 ml of sodium acetate buffer (0.2M) at pH 5.0, along with 50 mg Whatman No.1 filter paper discs at 50^0C for 60 minutes.

Endoglucanase or carboxymethyl cellulase (CMCase) activity was determined by incubating 0.5 ml of culture filtrate with 0.5 ml of sodium acetate buffer (0.2M) at pH 5.2 and 1 ml of carboxymethyl cellulose (1%) dissolved in acetate buffer at pH 5.2. The reaction mixture was then incubated at 50^0C for 30 minutes.

For determination of? b-glucosidase activity, 0.5 ml of sodium acetate buffer (0.2M) at pH 5.0 were incubated with 1.0 ml of 0.01% cellobiose (Sigma) in sodium acetate buffer (pH 5.0) at 50^0C for 30 minutes.

In all the cases after a specific period of incubation, reactions were terminated by adding 3 ml of dinitrosalicylic acid (DNS) reagent, boiled for 5 minutes, cooled at room temperature and the absorbance read at 540 nm in a Spectrophotometer (Sicospec 100). Enzyme activity was expressed in terms of unit (U) defined as the amount of enzyme required to liberate 1.0 m mole of reducing sugar (glucose) per minute under conditions of assay.

Pectolytic enzyme:

For studying the *in vivo* enzyme activity, the same method was followed as done for cellulolytic enzyme activity.

To study *in vivo* enzyme activity 20g of tissue was cut into pieces of 1 to 2 cm, homogenized with 0.15M-chilled sodium chloride at a rate of 3 ml per gm. The slurry was passed through two layers of cheese cloth and centrifuged at 10,000 rpm for 2 min at 4°C. The