

Production of pectolytic and cellulolytic enzymes by *Macrophomina phaseolina*

R.D.N. SINGH and S.A.K.M. KAISER

Department of Plant Pathology, Agriculture Research Complex,
Bidhan Chandra Krishi Viswavidyalaya, Kalyani 741235, West Bengal

By viscometric assay method it was observed that the pathogen *Macrophomina phaseolina* inciting charcoal rot in maize (*Zea mays*) showed activity of both cellulase and polymethylgalacturonase in the culture filtrate of potato dextrose broth medium after 10 days growth, but absolutely no activity of polygalacturonase. Extract from the maize stalk 12 days after inoculation showed activity of only cellulase and it was much greater than that in the culture filtrate. However, there was absolutely no activity of either polymethylgalacturonase or polygalacturonase.

Key words : Charcoal rot, enzymes, *Macrophomina phaseolina*, maize

INTRODUCTION

Several workers have reported production of both pectolytic and cellulolytic enzymes by different isolates of *Macrophomina phaseolina* (Tassi) Goid. [= *Rhizoctonia bataticola* (Taub.) Butl.] in their respective host plants (Chan and Sackston, 1970; Dhingra *et al.*, 1974; Rai and Srivastava, 1976; Goyal and Mehrotra, 1980). But knowledge on this aspect is scanty in respect of this pathogen causing charcoal rot of maize (*Zea mays* Linn.).

MATERIALS AND METHODS

A virulent strain of *M. phaseolina* isolated from the sclerotia occurring inside the charcoal rot affected stalks of a susceptible maize hybrid VL-54 was used. The different enzymes on which the present study was undertaken were : (i) carboxymethylcellulase (CMS or C_x), (ii) polymethylgalacturonase (PMG) and (iii) polygalacturonase (PG). The different substrates used were : (i) 1.2% carboxymethylcellulose for C_x, (ii) 1.2% pectin for PMG and (iii) 1.2% sodium polypectate for PG. The following methods were adopted for the assay of enzymes.

Preparation of extracellular enzymes from the culture filtrate (in vitro)

For this purpose the pathogen *M. phaseolina* was grown at 29±1°C for 10 days inside 250 ml Erlenmeyer flasks each of which contained 50 ml potato dextrose broth medium fortified with 1.2% pectin pure NF/1 for pectic enzymes and carboxymethyl cellulose

sodium salt for cellulase. After this period, the clear culture filtrate was stored in dialysing tube and was dialysed against sterile glass distilled water for 24 h at 70°C. The dialysate was used as enzyme source immediately after dialysis.

Preparation of enzyme from the inoculated maize stalk (in vivo)

A susceptible maize hybrid Deccan 101 was grown during *rabi* season (December-April) in large (31 cm diam) earthen pots filled with sterilized garden soil and compost (5:1 ratio). Before planting N, P and K fertilizers in the form of ammonium sulphate, superphosphate and muriate of potash were added to the soil in pots @ 400 kg, 250 kg and 120 kg/ha. After germination a single plant was maintained in each pot using near optimum irrigation with sterile tap water at regular interval. The toothpick method of inoculation (Young, 1943) was followed to inoculate the basal internodes of the test plants with the pathogen at flowering. Inoculated internodes were collected after 12 days, washed thoroughly with sterile distilled water and aseptically cut into small pieces. Fresh cut pieces (250 g) was then crushed at 4°C in a prechilled mortar by adding a little sterile neutral sand. The crushed material was blended for 2 min at 4°C in a prechilled Waring Blender by adding 50 ml chilled sterile glass distilled water. The blended material was strained and the extract thus collected was treated in a similar manner as in the case of culture filtrate prior to assay of enzymes.

Viscometric assay of different enzymes

Viscometric assay of CMS (C_x), PMG and PG in the culture filtrate as well as in the diseased stalks of maize was carried out by using 20 ml Fenske-Ostwald Viscometers at 28°C following the method described by Bell *et al.* (1955) with some modification suggested by Hancock *et al.* (1964). The activity of different enzymes was determined by measuring the reduction in viscosity of the respective substrates. Unless otherwise stated, all viscometric measurements were made in viscometers containing 1 ml of enzyme preparation and 9 ml of substrate for the study *in vitro* and 2 ml of enzyme preparation, 1 ml of 0.001 M calcium chloride and 8 ml substrate for the study *in vivo*, all buffered at pH 5.2 with 0.02 M citrate buffer (Gomori, 1955).

RESULTS AND DISCUSSION

Both pectolytic enzymes (PME, PMG and PG) and cellulolytic enzyme or cellulase (CMC or C_x) play important role in plant pathogenesis. But in comparison to 'soft rot inducing disease', C_x plays more important role in pathogenesis in case of 'dry rot inducing diseases' as reported by Bateman (1964) in the hypocotyl tissue of bean infected by *Rhizoctonia solani* which penetrated the cell wall of the host tissue and then by destroying the native cellulose it caused collapse of the invaded cells.

In the present study the charcoal rot pathogen *M. phaseolina* also showed activity of both PMG and C_x in the culture filtrate after 10 days growth. Activity of C_x was comparatively much greater and there was absolutely no activity of PG (Table 1). Extract from the maize stalk 12 days after inoculation with the pathogen showed activity of C_x only. It was much greater than that in the culture filtrate but there was no activity of either PMG or PG (Table 2).

Production of both pectolytic and cellulolytic enzymes by different isolates of *M.*

Table 1. Activity of different enzymes in culture produced by the charcoal rot pathogen *M. phaseolina* (after 10 days)

Different enzymes	Reduction (%) in viscosity in different minutes after mixing with the substrate								
	O min								
	Immediate after mixing	10 min	20 min	30 min	40 min	60 min	80 min	100 min	120 min
CMC (Cx)	5.46	19.12	24.00	35.27	40.23	45.10	51.41	52.55	55.00
PMG	3.10	10.31	11.64	15.67	16.33	17.51	21.26	21.26	21.26
PG	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 2. Activity of different enzymes in the extract of maize stalks inoculated with the charcoal rot pathogen *M. phaseolina* (after 12 days)

Different enzyme	Reduction (%) in viscosity in different minutes after mixing with the substrate								
	O min								
	Immediate after mixing	5 min	10 min	20 min	30 min	40 min	60 min	75 min	90 min
CMC (Cx)	0.00	20.38	74.19	83.35	86.69	89.70	91.91	92.91	92.91
PMG	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PG	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

phaseolina in their respective host plants was amply demonstrated by different workers. Ramasamy and Shanmugam (1977) recorded maximum activity of endopolygalacturonase (endoPG) *in vitro* by *R. bataticola* (*M. phaseolina*) infecting cotton on the 8th and 12th day. Activity of C_x was maximum on the 12th day of incubation *in vitro* or 15 days after inoculation *in vivo*. Abdelal *et al.* (1983) reported maximum activity of PG in the culture filtrate of *M. phaseolina* after 3-6 days of incubation which decreased after 6-15 days. Activity of C_x increased with the increase in seedling age and was much higher in extract of diseased soybean plants than healthy ones.

The present observation confirms Ramasamy and Shanmugam (1977) and Abdelal *et al.* (1983) who reported maximum C_x activity in the extract of the inoculated maize stalk after 12 days. But the activities of both PMG and PG were not detectable *in vivo* in the present case probably the pathogen ceased to produce those enzymes at that stage of infection. The present study indicates that the activity of C_x is predominant in pathogenesis by *M. phaseolina* inciting charcoal rot in maize.

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