

## Production of pectic enzymes by *Corynespora cassiicola* (Berk. and Curt.) Wei. causing leaf spot disease on blackgram

G. BINDU MADHAVI AND K.V.M. KRISHNA MURTHY

Dept. of Plant Pathology, Agriculture College Bapatla, Andhra Pradesh

**Abstract :** Production of pectic enzymes by *Corynespora cassiicola* (Berk. and Curt.) wei. causing leaf spot disease on blackgram was studied both in vitro and in vivo. The principal enzymes involved in disease production are Pectin methyl esterase (PME), Pectin Methyl Transeliminase (PMT), Polygalacturonase (PG). The activity of PG was more in vivo (35.31%) than in vitro (17.86%) where as the activity of PME & PMT were more in vitro than in vivo. None of the enzymes could detected in healthy tissue. (**Key words :** Pectic enzymes, *Corynespora cassiicola*, Leaf spot disease Blackgram, Parasitism).

The pectic enzymes produced by pathogenic fungi have received special emphasis in Studies on parasitism in plants. The importance of these enzymes in leaf spot disease (Hancock, *et al.* 1964) has been demonstrated. The present study reports the production of pectic enzymes by *C. cassiicola*, in vitro and in vivo.

### Materials and Methods

The enzyme preparations were assayed for the presence and activity of pectic enzymes (PME, PMT & PG). One per cent pectin solution was prepared by adding pectin to distilled water and the solution was stirred well. The pH of pectin solution was adjusted with 0.1 N HCl or 0.1 IN NaOH.

#### Preparation of enzyme extract in vitro

The fungus was grown on 50 ml sterilized potato dextrose extract in 250 ml Erlenmeyer flasks. Culture filtrates were prepared from 10 day old cultures by filtering the contents through Whatman No.1 filter paper and the filtrates were collected. The filtrates were centrifuged at 4000 rpm for 20 min and clear supernatant was collected in a cellophane pouch for the dialysis. Twenty ml of the enzyme extract was dialysed against 500 ml of distilled water for half an hour at 4°C in refrigerator. The dialysed enzyme extract was stored after adding a few drops of toluene in the deep freezer of the refrigerator for further use.

#### Preparation of enzyme extract in vivo

The enzyme extract was prepared by grinding 5 g of diseased and healthy leaf tissue separately in 15 ml of distilled water with 15 ml of 0.5 N NaCl using pestle and mortar. The ground tissue extract was strained through several layers of muslin cloth and squeezed. The filtrate was centrifuged at 4000 rpm for 20 min. The clear supernatant was collected and dialysed as explained earlier. The enzyme

preparations were assayed for the activities of pectic enzymes.

The activity of PME was measured in terms of increase in the activity of hydrolysis of pectin and expressed as microequivalent of methoxyl group removed by the enzyme sample (Kertesz, 1955). The activity other enzymes (PG, PMT) was assayed by determining the loss in viscosity of the reaction mixture immediately and after 2 h at 30°C following the method of Bell *et al.* 1955. The pre cent enzyme activity was calculated by using Uritani and Stahman equation.

$$= \frac{T \text{ control} - T}{T \text{ control} - T_{H_2O}} \times 100$$

- T control - Flow time in seconds of the reaction mixture with inactivated filtrate or enzyme extract
- T - Flow time in seconds of the reaction mixture at a definite interval (reaction time)
- T H<sub>2</sub>O - Flow time in seconds of the reaction mixture in which water was substituted for substrate pectin solution

The composition of the reaction mixture for the assay of different enzymes was as follows.

- PME 20 ml of pectin solution containing 0.1 N NaOH, 3 ml of enzyme extract
- PG 5 ml of sodium poly pectate (pH 4.5), 1.5 ml of phosphate citrate buffer (pH 4.5), 1 ml of distilled water
- PMT 5 ml of 1 per cent pectin (pH 5.5), 1.5 ml of Tris HCl buffer (pH 8.5), 1 ml of distilled water



**Table 1.** Production of Pectin Methyl Esterase (PME) by *Corynespora cassiicola* *in vitro* and *in vivo*.

Treatment	PME activity expressed in mg methoxyl group removed by 1 ml of enzyme extract at 5 h	PME activity expressed in mg methoxyl group removed by 1 ml of enzyme extract at 24 h
<i>In vitro</i>	0.0931	0.499
<i>In vivo</i>		
Diseased tissue	0.055	0.379
Healthy tissue	0.000	0.000

**Table 2.** Percentage activity of polygalacturonase enzyme produced by *Corynespora cassiicola* *in vitro* and *in vivo*

Treatment	Per cent reduction in viscosity after the time interval (min)			
	10	30	60	90
<i>In vitro</i>	7.08	9.50	12.08	17.86
<i>In vivo</i>				
Diseased tissue	9.92	24.20	31.34	35.31
Healthy tissue	0.00	0.00	0.00	0.00

**Table 3.** Percentage activity of Pectin Methyl Transeliminase (PMT) enzyme produced by *Corynespora cassiicola* *in vitro* and *in vivo*

Treatment	Per cent reduction in viscosity after the time interval (min)			
	10	30	60	90
<i>In vitro</i>	10.03	23.36	35.47	42.45
<i>In vivo</i>				
Diseased tissue	6.83	22.61	31.34	36.04
Healthy tissue	0.00	0.00	0.00	0.00

This mixture was placed in Ostwald viscometer immersed in water bath which maintained a temperature of 30°C. The flow time was recorded at each reaction time interval. A control was run with a sample of enzyme extract heated for 1 min at 100°C. Another check was run by using water as substrate in the place of pectin solution and the flow time was recorded.

### Results and Discussion

*In vitro* : By keeping the reaction mixture for 5 h, (Table1) the PME activity was 0.0931, but there was a perceptible increase in the activity of the enzyme activity (0.499) when the reaction mixture was incubated for 24 h.

*In vivo* : The activity of PME was 0.055 in the diseases tissue after 5 h but increased to 0.379 after 24 h incubation of the reaction mixture. But no enzyme activity was seen in healthy tissue even after

24 h of incubation. The activity of PG (Table2) was 7.08 after the reaction time of 10 min and the activity increased to 17.80 per cent *in vitro* and 35.31 per cent *in vivo* after 90 min of reaction time.

The PMT activity (Table3) *in vitro* was 10.03 per cent after the reaction time of 10 min and it increased to 42.45 per cent after 90 min. The activity was 6.83 per cent after the reaction time of 10 min and increased to 36.04 per cent after 90 min *in vivo*.

It may be assumed that all the enzymes detected *in vivo* during pathogenesis were of fungal origin since non could detected in healthy tissue. The results suggests the role of PME, PG and PMT produced by *Corynespora cassiicola* in causing the leaf spot disease on blackgram. The involvement of pectic enzymes in producing leaf spot disease was well established (Hancock *et al.* 1964; Vidhyasekaran *et al.* 1973). Agarwal and Gupta (1978) demonstrated the production of PG and PMT by *C.cassiicola* in



the diseased plant and the involvement of these enzymes in the fruit rot a papaya was described. The pectic enzymes were not detected in healthy plant. This observation was in accordance with the finding of the present study.

#### Acknowledgements

The authors are grateful to Acharya N.G. Ranga Agricultural University for providing necessary facilities for conducting the experiment.

#### References

- Agarwal, G.P. and Gupta, S. (1978). Production of pectic enzymes by *Corynespora cassicola* causing fruit rot of papaya. *Current Science* 47 : 161-163.
- Bell, T.A., Etchells, J.L. and Jones, I.D. (1955). United States Department of Agricultural Research Services (Rep.), p. 72.
- Hancock, J.G., Miller, R.L. and Lorbeer, J.W. (1964). Role of pectolytic and cellulolytic enzymes. In *Methods in Enzymology Volume 1* (Ed.) Colowick, S.P. and Kalpan N O Academic Press Inc. New York. p. 763.
- Kertesz, Z.I. (1955). Pectic enzymes. In : *Methods in Enzymology Volume 1*. (Colowick, S.P. and Kalpan, N.O. eds.) Academic Press Inc. New York. p. 763.
- Vidhyasekaran, P., Parambaramani and Govindaswamy, C.V. (1973). Role of pectinolytic enzymes in pathogenesis of obligate and facultative parasites causing disease. *Indian Phytopath.* 26 : 197-204.

(Received : December 1999 ; Revised : March 2001)

Madras Agric. J., 87(10-12): 577 - 579 October - December 2000

## Factors influencing the extent of participation of milch animal rearing beneficiaries in poverty alleviation programmes

R. NETAJI SEETHARAMAN AND R. VELUSAMY

Department of Agrl. Extension, Tamil Nadu Agricultural University, Coimbatore - 641003, Tamil Nadu

**Abstract :** The study was conducted to know the factors influencing the extent of participation among 90 milch animal rearing beneficiaries in poverty alleviation programmes. The study revealed that social participation and farm power status were the important factors influencing the extent of participation. (*Key words :* Extent of participation, Milch animal rearing, Poverty alleviation programmes)

It has been long argued that people's participation is essential for the success of poverty alleviation programmes. Identifying the factors influencing the extent of participation will be useful in changing the existing conditions, procedures and other requisites so as to make them convenient for the participation by the beneficiaries. Kareem and Jaramaiah (1998) reported that the characteristics like education and occupation and significant influence in extent of participation of IRDP, milch animal rearing is important and leading trade. Keeping this in view the study was undertaken with the following objective.

To identify the factors influencing the extent of participation in poverty alleviation programmes.

#### Materials and Methods

The study was conducted among 90 milch

animal rearing beneficiaries in Namakkal and Sivaganga districts. The socio-economic variables developed by Mansingh (1993) was used as important factors for participation namely, educational status, occupational status, family status, farm status, farm power status, communication status, social participation status and material status. For identifying the factors influencing the extent of participation correlation, multiple regression and factor analysis were used.

#### Results and Discussion

Correlation analysis was carried out to find out the relationship between socio-economic variables and extent of participation in poverty alleviation programmes. The results are presented in Table 1.

It could be understood from the Table that out of 8 variables, 6 variables, viz., family status,