ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 07, pp.1648-1650, July, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

INFLUENCE OF TEMPERATURE ON PRODUCTION OF POLYGALACTURONASE, POLYMETHYLGALACTURONASE AND CELLULASE ENZYMES BY *Alternaria solani* IN VITRO

***[1]Amit Kumar Chaurasia, [2]Sushmita Chaurasia, [3]Shubha Chaurasia, [1]Shridha Chaurasia and [1]Chaurasia, S. C.**

[1]Department of Botany, Govt. P.G. College, Tikamgarh 472001 M.P. India
[2]Department of Botany, Govt. College, Jatara, District Tikamgarh 472118 M.P. India
[3]Department of Botany, Govt. College Palera, District Tikamgarh 472221 M.P. India

ARTICLE INFO

Article History:
Received 08th April, 2013
Received in revised form
20th May, 2013
Accepted 25th June, 2013
Published online 18th July, 2013

Key words:
Temperature,
Polygalacturonase,
Polymethylgalacturonase,
Cellulase,
Alternaria solani.

ABSTRACT

An attempt was made to find out the effect of different temperatures ranging from 15^0 C to 45^0 C on production of polygalacturonase (PG), polymethylgalacturonase (PMG) and cellulase (Cx) enzymes by *Alternaria Solani* in vitro. The production of polygalacturonase (PG), polymethylgalacturonase (PMG) and cellulase (Cx) enzymes has been recorded between wide range of temperature, i.e. between 15^0 C to 35^0 C. At 15^0 C temperature, the production of these three cell wall degrading enzymes were found to be very low to which increases gradually with the increase in the temperature upto 28^0 C. The 28^0 C was found to be the best and favourable temperature for maximum production of these enzymes. Above 35^0 C, further higher temperature, i.e. 40^0 C and 45^0 C have been found to detrimental for the production of all the three enzymes as no trace of polygalacturonase (PG), polymethylgalacturonase (PMG) and cellulase (Cx) enzymes has been detected in culture filtrates.

INTRODUCTION

Phytopathogenic microorganisms are known to secrete extra cellular enzymes which degrade the host cell wall. The role of these enzymes in relation to the pathogenicity of organism has been reviewed by Wood (1960) and Bateman and Millar (1966). In soft rot diseases the enzymes play the most important role in pathogenesis (Brown, 1965). In vitro production of pectolytic and cellulolytic enzymes have largely been studied by many investigators because these studies provide information on the capacity of a particular pathogen to produce these enzymes. *Alternaria* species has long been reported for their pectolytic and cellulolytic potential (Logan and Siehr, 1966; Lucas and Sherwood, 1966; Mehta *et al.,* 1974; Agarwal and Hasija, 1978; Sharma, 2000; Sohail *et al*., 2011 and Chaurasia *et al*., 2013). It has also been proved that some of the species of *Alternaria* induce plant invasion by elaborating the pectolytic and cellulolytic enzymes (Eshel *et al*., 2000) and genes encoding endoglucanases from this organism have been characterized (Eshel *et al*., 2002). Recently, the production and activity of polygalacturonase (PG), polymethylgalacturonase (PMG) and cellulase (Cx) enzymes by *Alternaria solani* in culture and in the healthy and diseased tomato fruits have been reported (Chaurasia *et al*., 2013). Prasad *et al*., (1981) indicate that enzymes are important factor for the incidence of the disease and temperature also has important place in various plant diseases. Keeping this in view, the study was undertaken to know the effect of temperature range on production of polygalacturanase (PG), polymethylgalacturonase (PMG) and cellulase (Cx) enzymes in vitro and their relationship with the incident of *Alternaria* fruit rot of tomato in country, if any.

Corresponding author: Dr. Amit Kumar Chaurasia
Near Mau Chungi Naka R.K. Khare House, Chitrans Colony,
Tikamgarh 472001 (M.P.) India

MATERIALS AND METHODS

Organisim

Alternaria solani (Ellis & Mart.) Jones and Grout was isolated from diseased fruits of tomato (Chaurasia and Chaurasia, 2010). The culture was obtained and maintained on potato dextrose agar slants under refrigeration at 4^0 C.

Preparation of crude enzyme

During the investigation the pathogen was grown on the semi ripe tomato fruit media (Chaurasia *et al*., 2013). Twenty five ml. of the medium was poured in each of 150 ml. Erlenmeyer flasks. These flasks were sterilized at 15 lb/sq in pressure for 10 min. After sterilization each flask was inoculated by a 8.0 mm disc taken from the periphery of four day old colony of the pathogen growing on potato dextrose agar medium. The flasks were incubated at eight different temperatures, i.e. 15, 20, 25, 28, 30, 35, 40 and 45^0 C. Three replicates were taken in each case. After six days of incubation the culture filtrate and the mycelial mat were harvested. The culture filtrates were diluted with 35 ml. of distilled water. The filtrate thus obtained was centrifuged for 15 minutes at 10,000 rpm. and the supernatant was used as crude enzyme preparation.

Enzyme assay

Enzyme activity was measured by using standard viscometric method (Hancock and Millar, 1965; Capellini, 1966). Oswald viscometers were clamped in stands which were fixed vertically in water bath, with temperature adjusted to 28^0 C. For the assaying of polygalacturonase (PG), polymethylgalacturonase (PMG) and cellulase (C_X) enzymes, the following freshly prepared substrate components were used:-

Table 1. Production of polygalacturonase (pg), polymethylgalacturonase (pmg) and cellulase (cx) enzymes in "semi ripe tomato fruit medium" at different temperatures

Temperature (^{0}C)	Polygalacturonase (PG) Enzyme activity (%Loss in viscosity) Reaction time (in mts)				REA	Polymethylgalacturonase (PMG) Enzyme activity (%Loss in viscosity) Reaction time (in mts)				REA	Cellulase (CX) Enzyme activity (%Loss in viscosity) Reaction time (in mts)				REA
	20	40	60	80		20	40	60	80		20	40	60	80	
15	17.0	24.2	28.3	30.0	18.86	6.2	10.0	12.3	13.0	0.00	18.3	26.4	30.3	32.2	26.40
20	30.4	38.2	43.2	45.2	60.82	8.1	16.3	20.4	22.2	0.00	27.2	39.5	44.4	47.3	54.40
25	44.3	54.2	60.1	64.2	88.65	10.0	20.3	26.3	30.2	17.53	58.5	69.6	75.2	78.4	117.09
28	56.3	68.2	77.4	80.2	112.61	10.4	22.4	30.2	40.3	20.13	66.6	78.4	84.4	88.3	133.33
30	40.2	50.3	56.3	59.0	80.45	8.5	18.3	24.3	27.2	13.60	52.3	64.2	70.3	72.1	104.60
35	18.4	25.3	30.3	32.1	25.30	7.2	14.1	17.2	18.1	0.00	26.8	34.5	38.2	40.0	53.61
40	0.0	0.0	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.00
45	0.0	0.0	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.00

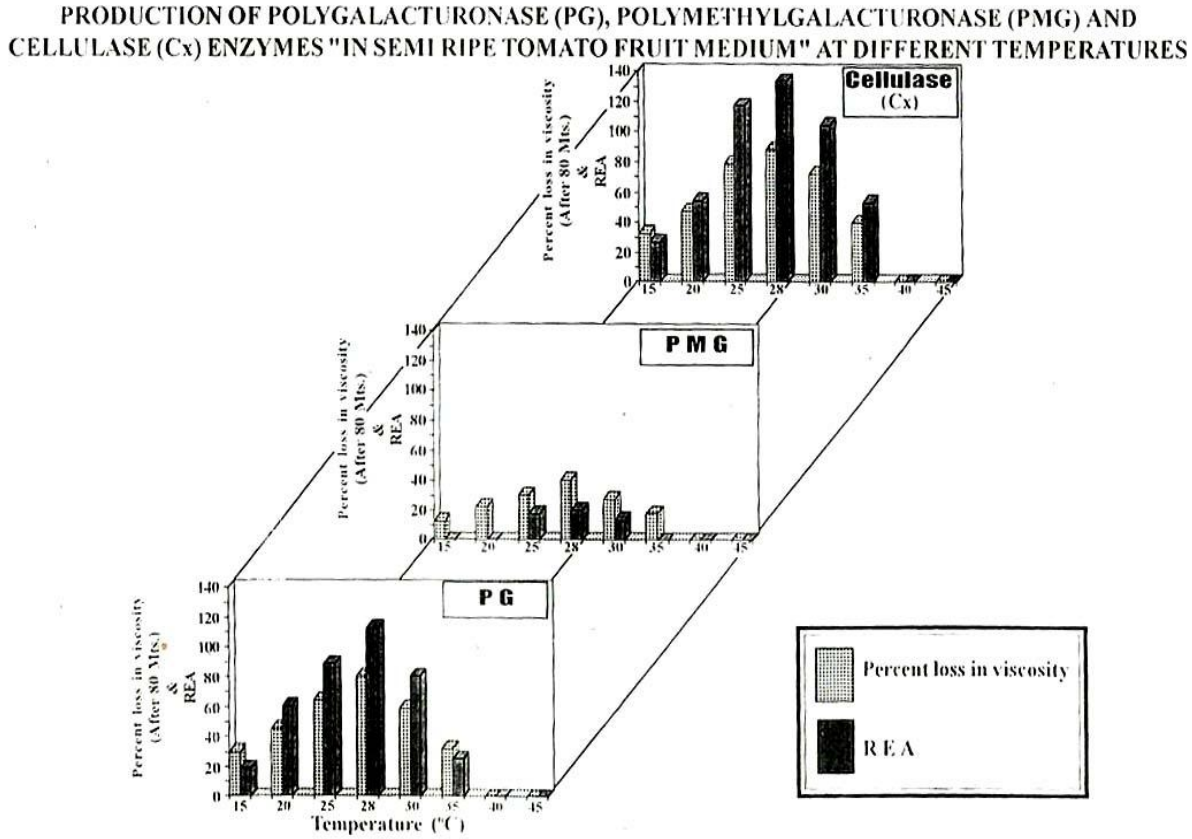

Figure 1.

1-**Polygalacturonase (PG)**

1.2 percent sodium polypectate -3.5 ml
Distilled water -1.5 ml
Citrate phosphate buffer (pH 4.6) -1.5 ml

2-**Polymethylgalacturonase (PMG)**

1.2 percent citrus pectin -3.5 ml
Distilled water -1.5 ml
Citrate phosphate buffer (pH4.6) -1.5 ml

3-**Cellulase (C_X)**

1.2 percent carboxymethyl cellulose -3.5 ml
Distilled water -1.5 ml
Citrate phosphate buffer (pH 5.5) -1.5 ml

At the time of determination of polygalacturonase (PG), polymethylgalacturonase (PMG) and cellulase (C_X) enzyme activity, desired substrate component was taken into the stalk bulb of viscometer. Then, 1.5 ml of freshly prepared enzyme extract was poured into viscometer and soon efflux time of the enzyme reaction mixture was determined at the intervals of 0, 20, 40, 60 and 80 minutes. Efflux time for 8.0 ml of distilled water was also noted in each viscometer.

Determination of per cent loss in viscosity

Per cent loss in viscosity was calculated with the help of the following formula (Capelline, 1966):

$$\text{Percent loss in viscosity} = \frac{ET_0 - ET_t}{ET_0 - ET_W} \times 100$$

where,

ET_0 = Efflux time in seconds at zero time/control.
ET_t = Efflux time in seconds at any specific interval of time.
ET_w = Efflux time in seconds for distilled water.

Determination of relative enzyme activity (REA)

Values for per cent loss in viscosity were determined for 0, 20, 40, 60 and 80 minutes reaction time. These values were then plotted against the reaction time, thus a curve was obtained and from this curve the time to bring a 25 per cent loss in viscosity was determined. Relative enzyme activity (REA) was then calculated by the following formula:

$$REA = \frac{1000}{t}$$

Where,

t = represented the time in minutes to reach 25 per cent loss in viscosity, thus :

$$REA = \frac{1000}{25}$$

RESULTS AND DISCUSSION

It is evident from the results given in Table 1 and Fig. 1 that *Alternaria solani* was able to produce polygalacturonase (PG), polymethylgalacturonase (PMG) and cellulase (Cx) enzymes in a wide range of temperatures i.e. from 15^0 C to 35^0 C. The production of polygalacturonase (PG) and polymethylgalacturonase (PMG) enzymes was increased with increase of temperature up to 28^0 C. In the culture filtrate of 28^0 C, the maximum polygalacturonase (PG) and polymethylgalacturonase (PMG) enzyme production was recorded. Further increase in temperature upto 35^0 C, the production of polygalacturonase (PG) and polymethylgalacturonae (PMG) enzymes was reduced gradually. In culture filtrate of 40^0 C and 45^0 C temperatures, no activity of polygalacturonase (PG) and polymethylgalacturonase (PMG) enzymes were recorded. The production of cellulase (Cx) enzyme has also been recorded between a wide range of temperature i.e. ranging from 15^0 C to 35^0 C. The maximum cellulase (Cx) enzyme production was recorded in the culture filtrate of 28^0 C in which 133.33 relative enzyme activity (REA) was calculated. The temperature ranges between 25^0 C to 30^0 C was found to be more suitable as significantly good amount of cellulase (Cx) has been detected in cultures, which were incubated at 25^0 C, 28^0 C and 30^0 C temperature. The higher temperatures, above 35^0 C have been found to be detrimental for cellulase (Cx) production.

From the above results it is concluded that 28^0 C temperature was found to be the most favourable for the maximum production of polygalacturonase (PG), polymethylgalacturonase (PMG) and cellulase (Cx) enzymes. Higher temperatures above 35^0 C, i.e. 40^0 C and 45^0 C were found to be detrimental for the production of all these three cell wall degrading enzymes. Similar results have also been observed by Mehta (1973) and Chaurasia (2000) in case of *Alternaria solani* and *Sclerotium rolfsii* respectively. The detrimental effect in the production of all these three cell wall degrading enzymes may be due to the lethal effect of pathogen at higher temperatures i.e. 40^0 C and 45^0 C. These findings clearly indicate the important role of temperatures in the enzyme secretion. Variation on production and activity of polygalacturonase (PG), polymethylgalacturonase (PMG) and cellulase (Cx) enzymes can be explained with incidence of *Alternaria* fruit rot of tomato. Disease appeared in tomato orchards during winter season under humid and cloudy environment. Symptoms on fruits appeared suddenly with the rise of temperature ($25\text{-}30^0$ C) in orchards. Now it is concluded that during cloudy environment when the temperature is in the range of $25\text{-}30^0$ C, pathogen produced sufficient polygalacturonase (PG), polymethylgalacturonase (PMG) and cellulase (Cx) enzymes which became more active with increase of environmental temperature and helps in establishment of *Alternaria* and hence rotting of the fruits of tomato.

REFERENCES

Agarwal, G.P. and Hasija, S.K. 1978. In vitro production of pectic enzymes by *Alternaria citri*. Proc. Nat. Acad. Sci., India48:53-61.

Bateman, D.F. and Millar, R.L. 1966. Pectic enzymes in tissue disintegration. Ann. Rev. Phytopath., 4: 119-146

Brown, W. 1965. Toxin and cell wall dissolving enzymes in relation of plant disease. Ann. Rev. Phytopath., 3: 1-18

Capellini, R.A. 1966. Growth and polygalacturonase producing by *Rhizopus stolonifer*. Phytopathology., 56: 734-737

Chaurasia, Shridha 2000. Studies on *Sclerotium rolfsii* Sacc. Causing foot rot disease of brinjal (*solanum melongena* Linn.). M.Sc. Thesis, University of Saugar, Sagar, (M.P.) India., pp-82

Chaurasia, A.K. and Chaurasia, Shridha. 2010. Effect of different inoculation methods and temperatures on tomato fruit rot pathogen *Alternaria solani* and its host range. BIOZONE international journal of life science., 2(1 & 2): 236-243

Chaurasia, Amit kumar., Chaurasia, Shridha., Chaurasia, Shubha and Chaurasia, Sushmita. 2013. Production of polygalacturonase, polymethylgalacturonase and cellulase enzymes by *Alternaria solani*. Accepted for publication in Nationl journal of Life Sciences.

Eshel, D., Ben-Arie., R., Dinoor, A. and Prusky, D. 2000. Resistance of gibberellin-treated persimmon fruit to *Alternaria alternata* arises from the reduced ability of the fungus to produce endo- 1, 4-β- glucanase. Phytopathology., 90: 1256-1262

Eshel, D., Lichter, A., Dinoor, A. and Prusky, D. 2002. Characterization of *Alternaria alternata* glucanase genes expressed during infection of resistant and susceptible persimmon fruits. Molec. Plant Pathol., 3: 347-358

Hancock, J.B. and Millar, R.L. 1965. Association of cellulolytic, Proteolytic and xylolytic enzymes with southern anthracnose, spraying block stem and *Stemphylium* leaf spot of alfalfa. Phytopathology., 55: 356-360

Logan, R.M. and Siehr, D.J. 1966. Solubilization of acid swollen cellulose by an enzyme system from a species of *Alternaria*. Appl. Microbiol., 14: 1015-1018

Lucas, G.B. and Sherwood, R.T. 1966. Role of pectic enzymes of *Alternaria tenuis* in brown spot disease of tobacco. Proc. IVth Inter. Tobacco scientific cong. Athens., pp 410-416

Mehta, P. 1973. Biochemical investigations of fruit rot diseases of tomatoes (with special reference to the pathogenesis caused by *Alternaria solani* (Ellis & Mart) Jones & Grout and *Alternaria tenuis* Auct.). Ph.D. Thesis, Saugar Univ. Sagar (M.P.) India., pp- 413

Mehta, P., Vyas, K.M. and Saxena, S.B. 1974. Production of pectolytic and cellulolytic enzymes by *Alternaria* sp. during pathogenesis of tomato fruits. Hindustan Antibiot. Bull., 16 (4): 210-214

Prasad, G., Mehrotra, R.S. and Shrivastava, S.N. 1981. Influence of temperature on production and activity of pectolytic and cellulolytic enzymes by *Phytophthora nicotianae* var. *parasitica* in vitro. Phytophthora newsletter., 9: 32-33

Sharma, R.L. 2000. "In vivo" and "in vitro" production of cellulolytic enzymes by *Monilinia* sp. causing brown rot of apple. Indian J. Mycol. and Pl. Pathol., 30 (1): 31-33.

Sohail, M., Ahmad, A. and Khan, S.A. 2011. Production of cellulases from *Alternaria* sp. MS28 and their partial characterization. Pak J. Bot., 43 (6): 3001-3006

Wood, R.K.S. 1960. Pectic and cellulolytic enzymes in plant disease. Ann. Rev. Plant Physiol., 11: 299-322.

****