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Production of Cell Wall Degrading Enzymes by *Colletotrichum loeosporioides* Causing Mango Anthracnose

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ABSTRACT

Mango (*Mangifera indica* L.), is infected by different fungal pathogens, among them anthracnose is the most serious fungal disease and is caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. It produces cell wall degrading enzymes *viz*., polygalacturonase and pectin methyl esterase. The activity of these cell wall degrading enzyme was assayed. The virulent isolates of *Colletotrichum gloeosporioides* produced more pectinolytic enzymes and their activity increased with the increase in age of the culture.

Key Words: Colletotrichum gloeosporioides, Pectin methyl esterase, Polygalacturonase.

INTRODUCTION

Mango (*Mangifera indica* L.) is grown over an area of 25.16 lakh ha with total production of 1.83 lakh ton (Anon, 2014). Among various diseases, anthracnose is caused by *Colletotrichum gloeosporioides* Penz. and Sacc. (teleomorph Glomerella cingulata) is a major constraint in successful cultivation of mango. It produces cell wall degrading pectinolytic enzymes such as pectin methyl esterase (PME) and poly galacturonase (PG) that may have important roles in the infection process and in the development of disease symptoms (Bateman and Basham,1976; Walton, 1994). These enzymes completely degrade the pectic polysaccharides in the cell walls of plant and fruits. The importance of such enzymes in pathogenicity was supported by the ability of purified enzymes to reproduce disease symptoms (Barash *et al*, 1984; Holtz and Knox-Davies, 1985).

Thus, present paper describes a study of the *Colletotrichum gloeosporioides* to produce pectolytic enzymes in culture to determine the pathogenicity. The resultant information can confidently lead to new strategies for management of the disease.

MATERIAL AND METHODS

In the presence of pectic substances Colletotrichum produces enzymes namely poly galacturonase (PG) and pectin methyl esterase (PME) in culture filtrate and their level was determined in different fifteen isolates, collected from various hosts from different locations of Himachal Pradesh by adopting the method described by Mahadevan and Shridhar (1986).

Preparation of pectic enzymes of fungus

100 ml of Czapek's medium was prepared containing 2-4 per cent pectin (pH 6.5) in 500 ml of Erlenmeyer flask. It was then inoculated with 1ml of spore suspension (1.2 × 104 cfu/ml) of one isolates of Colletotrichum. Similarly, other fourteen isolates were inoculated and incubated at 28 ± 20C for 10 d. After that each isolate culture was centrifuged at 2,000 rpm for 30 min to remove the spores and supernatant was used as enzyme source. The PG enzyme activity was measured by estimating the loss in viscosity of sodium polypectate in sodium acetate-acetic acid buffer at pH 5.2. To 1 ml of buffer, 2 ml of enzyme source was added and immediately transferred to Ostwald-Fenske Viscometer (size 150) and placed in a water bath at 30±1oC. These

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contents were gently mixed by drawing air rapidly through the large arm of the viscometer by suction. Viscosity losses were measured at regular time intervals of 5, 10, 15, 30, 50 and 120 min and expressed in per cent loss in viscosity calculated by the following formula

Per cent loss in viscosity: T0 - T1/ T0 - TH20 ×100

where, T0 = flow time in seconds at zero time, T1= flow time of the reaction mixture at time T, TH20 = flow time of distilled water

Similarly, the enzyme activity (pectin methyl esterase) was determined by measuring the change in pH of the reaction mixture. 10 ml of 1 per cent pectin was added to 10 ml of enzyme source. After that one ml of methyl red solution was added to it and pH 7 was adjusted with 0.1 M NaOH until the mixture became faint yellow. The reaction mixture was incubated in 50 ml Erlenmeyer flasks in a water bath at 300C. At 20 min intervals, the redness which had appeared in the reaction mixture was titrated back to yellow with 0.1 M NaOH. The enzyme activity was expressed as the amount (number) of ml 0.1 M NaOH necessary to titrate the reaction mixture back to yellow.

RESULTS AND DISCUSSION

Activity of Poly galacturonase (PG)

The perusal of data (Table 1) indicated that all the fifteen isolates exhibited significant variation in the activity of PG enzyme. Its activity in the culture filtrate of all the isolates increased with faster rate up to 14 d of incubation and thereafter the activity though increased but at a lower speed. Maximum activity of this enzyme was found in isolate I4 (mango) with 69.57 per cent loss in viscosity at 21st day of inoculation and it was closely followed by isolate I15 (69.03%), I6 (68.51), I7 (68.47), I8 (68.64) and I12 (68.46). Minimum activity was recorded in isolate I13 (mango) with 10.26, 23.74, 55.61 and 60.43 per cent loss in viscosity at1st, 7th, 14th and 21st d of inoculation, respectively.

Table 1. Production and activity of PG produced by Colletotrichum isolates.

Isolate No.	Host, Place	Per cent loss in viscosity (day)			
		1st	7th	14th	21st
I1	Mango, Solan	14.77(22.56)	28.83(32.46)	52.82(46.60)	61.81(55.81)
I2	Mango, Solan	13.71(21.69)	27.86(31.83)	52.10(46.19)	66.76(54.78)
I3	Capsicum,Solan	13.84(21.80)	26.69(31.08)	50.63(45.34)	65.46(53.99)
I4	Mango,Bilaspur	14.84(22.61)	31.70(34.25)	55.60(48.20)	69.57(56.50)
I5	Pomegranate, Solan	11.59(19.84)	24.72(29.79)	57.58(49.34)	61.65(51.72)
I6	Mango,Hamirpur	16.62(24.02)	30.22(33.34)	55.67(48.24)	68.66(55.94)
I7	Mango,Hamirpur	15.66(23.28)	29.78(33.05)	52.73(46.55)	68.07(55.59)
I8	Mango ,Kangra	19.70(26.32)	36.83(37.34)	51.88(46.06)	68.47(55.83)
I9	Mango,Shimla	12.71(20.84)	27.66(31.71)	50.87(45.48)	65.46(53.10)
I10	Mango,Kangra	19.66(26.29)	36.59(37.20)	52.60(46.47)	60.55(51.07)
I11	Mango,Solan	11.69(19.94)	24.74(29.80)	56.68(48.82)	60.53(51.06)
I12	Mango,Sirmour	14.23(22.11)	28.72(32.38)	51.28(45.72)	68.64(55.43)
I13	Mango,Sirmour	10.65(18.98)	23.74(29.13)	55.61(48.21)	60.43(51.00)
I14	Capsicum,Shimla	12.49(20.66)	28.82(32.45)	52.60(46.47)	66.64(54.71)
I15	Mango,Solan	17.57(24.74)	31.599(34.18)	58.89(50.11)	69.39(56.39)
C.D0.05		2.88	2.10	2.03	2.12

Table 2. Pectin methyl esterase activity produced by Colletotrichum isolates.

Isolate no.	Amount of 0.02 N NaOH (ml)
I1	4.4
I2	3.9
I3	4.1
I4	6.1
I5	4.3
I6	5.4
I7	5.7
I8	5.8
I9	4.0
I10	4.2
I11	3.2
I12	5.5
I13	5.6
I14	3.9
I15	4.9

Activity of Pectin Methyl Esterase (PME)

It was apparent (Table 2) that all the fifteen isolates differed in their PME enzyme activity. It has the maximum activity in isolate I4 (6.1 ml) isolated from mango from Bilaspur district followed by that of I8 (5.8 ml) isolated from mango from Kangra district while minimum activity was shown by isolate I13 isolated from mango from Sirmour (5.6 ml).

For successful pathogenesis, the pathogen has to overcome the host barriers like cell wall, pectin layer and protein matrix. The collective action of different cell wall degrading enzymes helps the pathogens for their easy penetration and subsequent colonization in the host tissue. Colletotrichum spp. has been reported to produce different pectinolytic degrading enzymes which play an important role in host-parasite interactions. The PG enzyme hydrolytically cleaves pectin in such a manner that the 1, 4 glycosidic bonds of the chain split and the PME removes the esterified methyl group from the chain hydrolytically (Chandrasekharan et al, 2000). Sahoo *et al* (2010) reported that *C. capsici* on infection in chilies produced pectinolytic enzymes in higher amount which resulted in the dissolution of host cell wall and middle lamella and thus helped in its entry and establishment in the host causing severe disease symptoms.

CONCLUSION

Different isolates of pathogen differed in exhibiting activity of pectin degrading enzymes (PG, PME), which corresponds to the lesion size produced by respective isolates on mango fruit being highest in mango isolate I4. Thus, studies conclude that variations in virulence among isolates of *C. gloeosporioides* causing anthracnose in mango may be related to differences in pectinolytic activity.

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