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RESEARCH ARTICLE

PATHOGENIC ACTION OF Cx, PG AND PMG ENZYMES OF *Fusaium udum* and *Fusarium oxysporum f.sp.ciceri*

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ABSTRACT

Wilt disease affects the pulse crops at greater extent mainly due to *fusarium* species. *Fusarium* species releases pectolytic and cellulolytic enzymes responsible for disintegration of middle lamella and matrix of cell wall of particular host crop and infect them. Present study mainly use to calculate the role of cellulase(Cx) ,polygalactouronase (PG) and pectin methyl galacturonase(PMG) in *fusarium* for causing wilt disease. Result shows that with increasing incubation time, the activity of all three enzymes have been enhanced. Polygalacturonase shows highest activity i.e. 24.1 in case of *fusarium oxysporum f.sp.ciceri* and 38.7 in case of *fusarium udum* at 90 minutes time interval. While cellulase shows lowest activity 4.8 in case of *fusarium oxysporum f.sp.ciceri* and 7.1 in case of *fusarium udum* at 30 minutes interval. In case of *fusarium oxysporum f.sp.ciceri* r^2 value is 0.985, 0.984, 0.980 for PG, PMG and Cx. While *fusarium udum* shows r^2 value 0.993 for PG, 0.988 for PMG and 0.966 for Cx. All three enzymes activity increases with increasing time of experiment. Activity of three enzymes are higher in *fusarium udum* in comparison to *fusarium oxysporum f.sp.ciceri*. This shows that *fusarium udum* have higher pathogenic effect. .

INTRODUCTION

The Role of cell wall degrading enzymes in pathogenesis is well documented (Albersheim *et al.,* 1969). Pectolytic and cellulolytic are the most important enzymes of the cell wall. The appearance of these enzymes during pathogenesis is considered to be immediately related to the disintegration of structural components. Pectic substances in the middle lamella and polygalacturonate rich compounds in the matrix of cell wall are seen to be degraded. The enzymes involved in pathogenesis are produced by many phytopathogenic fungi. Waggoner and Dimond (1955) reported that pectic enzyme produced in *Fusarium* culture and disease plant but not in healthy plant. Addition of *Fusarium* cultural filterate and commercial pectic enzymes caused wilting in tomato plants (Gothoskar *et al.*, 1955; Scheffer and Walker, 1953) and due to this blocked the xylem vessel in tomato plant (Pierson *et al.*, 1955).

Hussain and Dimond (1960) studied the activity of cellulase on cultural filterate of *Fusarium oxysporum f. sp. lycopersici* on tomato plant. The cellulase produced by *Fusarium* helps in induced wilt disease, provide nutrient supplement for its continued development in the host tissue and also involved in escape of pathogen from vascular tissue at the advanced stage of disease. Sayed *et al.*(1979) assayed *in vivo* and *in vitro* production of pectic enzymes i.e. pectin methyl esterase (PME) and poly methyl galacturonase (PMG) and its role in causing pathogenesis. One pathogenic form of *Fusarium udum* with its two non-pathogenic form of two *fusarium spp*. isolated from pigeon pea plant had been taken. Result showed that both enzymes were present in three species but enzyme activity was much higher in pathogenic form i.e. *Fusarium udum* rather than non-pathogenic *Fusarium species*. The objective of present study is to evaluate the role of cellulase, pectin methylgalacturonase and polygalacturonase enzymes in causing pathogenesis in *fusarium udum* and *fusarium oxysporum* f. sp. *ciceri* responsible for causing wilt disease in pigeonpea and chickpea crop.

MATERIAL AND METHODS

Czapek-dox medium containing following components *viz.* $NaNO_3$ (2gm), K_2HPO_4 (1gm), KCl(0.5gm), $MgSO_4$(0.5gm), Sucrose (15gm) added in one liter distilled water and the broth medium 25 ml. has been taken into 150ml. Erlenmeyer flask and then autoclave at 15lb/m^2 pressure and 121˚C temperature. The flasks are inoculate with 5mm. diameter of 7 days old colony of the pathogen grow on czapek-dox agar medium. The inoculated flasks are incubate at 28±1˚C for 7 days under stationary condition. On 7th day the cultural filtrate are decant and directly centrifuged at 5000 rpm for 20 minutes at 4˚C. The supernatant obtained is used as enzyme preparation. The enzyme was assayed by standard viscometric method (Hancock and Miller, 1963).

Enzyme assay

Ostwald viscometer is fit in a water bath set at 30˚C (optimum temperature for activity of pectic and cellulolytic enzymes).

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i. For polygalacturonase (PG) 1.5 ml enzyme extract with 3.5ml of 1.2% Sodium polypectate as substrate, 1.5 ml distilled water, 1.5 ml sodium acetate buffer (pH 4.5).
ii. For pectin methylgalacturonase (PMG) 1.5 ml enzyme extract with 3.5ml of 1.2% Citrus pectin as substrate, 1.5 ml distilled water, 1.5 ml sodium acetate buffer(pH 4.5).
iii. For cellulase activity(Cx)1.5 ml enzyme extract with 3.5ml of 1.2% Carboxymethyl cellulose as substrate, 1.5 ml distilled water, 1.5 ml sodium acetate buffer(pH 5.5).

The enzyme activity is expressed as percentage reduction in viscosity after definite time interval upto 90 minute. By following formula, calculate the percent loss in viscosity as –

$$N= (ETo – ET_t)/ (ET_o-ET_w) X100$$

Where,

ET_t = Efflux time in second for treatment t
ET_w = Efflux time in second for water

RESULTS AND DISCUSSION

Result from above study indicate that polygalactouronase (PG) activity in terms of percent loss in viscosity is 10.41 at 30 minutes, 22.5 at 60 minutes and 38.7 at 90 minutes and r^2 value of enzyme with increase in time is 0.993 in case of *Fusaium udum* while *Fusaium oxysporum f.sp.ciceri* shows the percent loss in viscosity is 5.6 at 30 minutes , 16.8 at 60 minutes and 24.1 at 90 minutes with r^2 value is found 0.985 (Fig.1). The activity of Pectin methyl galacturonase (PMG) is 8.9 at 30 min , 19.4 at 60 min and 34.7 at 90 min in case of *fusarium udum* and r^2 value of enzyme with increase in time is 0.988 while *fusarium oxysporum f. sp. ciceri* shows the percent loss in viscosity is 5.4 at 30 min , 14.9 at 60 min and 22.6 at 90 min with r^2 value is found 0.984 (Fig.2). The activity of cellulase (Cx) is 7.1, 14.3 and 28.3 respectively at time interval of 30, 60 and 90 min in case of *fusarium udum* and r^2 value of enzyme with increase in time is 0.966 while *fusarium oxysporum f. sp. ciceri* shows the percent loss in viscosity is 4.8, 11.9 and 21.4 respectively at time interval of 30, 60 and 90 min in case of cellulase (Cx) with r^2 value is found 0.980 (Fig.3).

Enzyme production in terms of percent loss in viscosity by *Fusarium udum* and *Fusarium oxysporum f.sp. ciceri* shows that activity of polygalactouronase (PG) is higher followed by pectin methyl galacturonase while cellulase has lowest activity in case of both *Fusarium* species. All the three enzymes level is higher in case of *Fusarium udum* that show that *Fusarium udum* have higher pathogenic effect in comparison to *Fusarium oxysporum f.sp.ciceri*. In case of *Fusarium udum* the loss in viscosity is 38.7 in case of polygalacturonase (PG) followed by PMG (34.7), Cx (28.3) at 90 minutes time interval, while in case of *Fusarium oxysporum f.sp.ciceri* the loss in viscosity is 24.1, 22.6 and 21.4 with PG, PMG and Cx at 90 minutes time interval. Cellulase (Cx) showed minimum loss in viscosity was 7.1 in case of *Fusarium udum* and 4.8 in case of *Fusarium oxysporum f.sp.ciceri* at 30 minutes time interval.Kaiser and sengupta(1970) reported that both pectin methyl esterase(PME)and poly methyl galactouronase (PMG) were found to be present in seedling inoculated with pathogenic formae specials of *fusarium oxysporum f. sp. udum* as well as non-pathogenic formae specials of

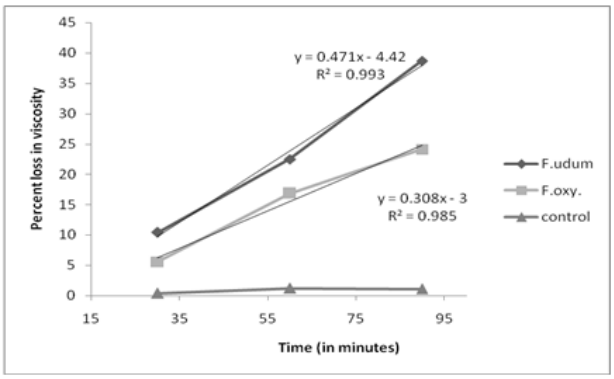

Fig.1: Activity of polygalacturonase(PG) at different time interval in *fusarium udum* and *fusarium oxysporum f.sp.ciceri*

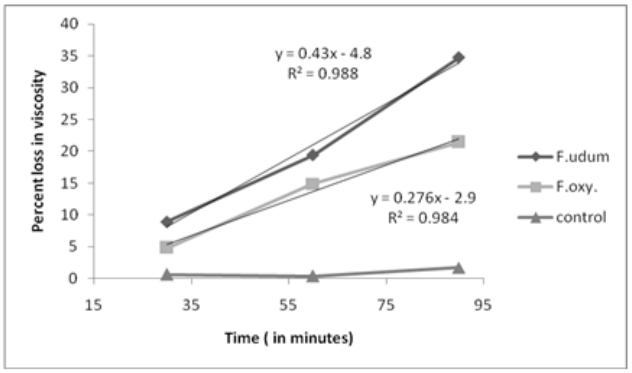

Fig.2 : Activity of polymethylgalacturonase (PMG) at different time interval in *fusarium udum* and *fusarium oxysporum f.sp.ciceri*

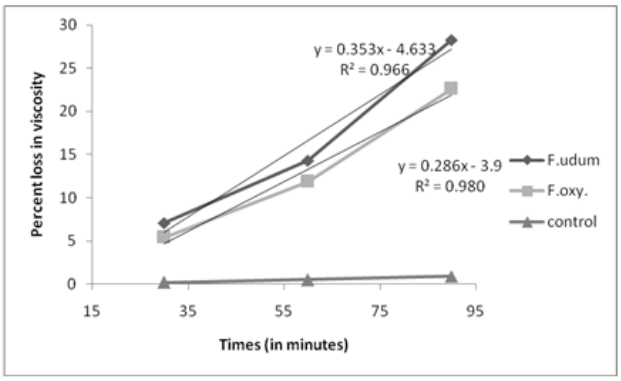

Fig. 3: Activity of cellulase (Cx) at different time interval in *fusarium udum* and *fusarium oxysporum f.sp.ciceri*

Fusarium oxysporum, but the amount of these enzyme was higher in seedling inoculated with *Fusarium oxysporum f. sp. udum* as compared to those inoculated with the non-pathogenic formae speciales. *Fusarim udum* was known to produce pectic enzymes both *in vitro* and *in vivo* condition (Singh and Hussain,1968). Chacko *et al.*(1978) reported that the activity of cellulolytic and pectinolytic enzymes in soyabean isolate of *Colletotrichum dematium f.sp.truncata* and showed that enzymatic activity of both the enzymes was maxium in infected leaf after nine days of inoculation and maximum activity was more after 180 minutes of preparing the reaction mixture.

Present study shows that in both the cases the activity of all three enzymes are increasing with escalating the time interval. Activity of polygalactouronase (PG) is higher followed by pectin methyl galacturonase, while cellulase has lowest activity in case of both *fusarium* species. .On the basis of above study it may be conclude that enzymes play an important role in inducing pathogenesis. *Fusarium udum* contains higher amount of all three enzymes compared to *Fusarium oxysporum f. sp. ciceri* which showed higher pathogenic effect.

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