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CELLWALL MACERATION AND ELECTROLYTE LEAKAGE BY ENDOPOLYGALACTURONASE FROM *ALTERNARIA CEPULAE* CAUSING LEAFBLIGHT DISEASE IN ONION

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ABSTRACT
Pectic enzymes play a significant role in the pathogenesis of many plant diseases. Amidst various pectic enzymes, endopolygalacturonase is an important enzyme during the leaf blight disease of onion caused by *Alternaria cepulae.* The toxic effects of purified Endopolygalacturonase (EPG) on maceration of the intercellular cement of pectic materials in onion cells, carrot, apple cells and electrolyte leakage in onion cells were estimated with a conductivity bridge. The purified enzyme powder takes three hours to 12 hours for maceration. The endo PG preparation macerates more quickly the cell membrane and releases the electrolytes at 1:1 concentration than 1:50 and 1:100 dilutions.

KEYWORDS: *Alternaria cepulae,* endopolygalacturonase (endoPG, EPG), electrolyte leakage, cellwall maceration.

INTRODUCTION

Endopolygalacturonase (Poly α,1,4, galacturonide glycano hydrolase, EC 3.2.1.15) plays a significant role in pathogenesis of many plant diseases (Cooper, 1980; Boothby 1984; Mills 1985). It is found that endopolygalacturonase is one of prime macerating enzyme produced by *Alternaria cepulae* during leaf blight disease of onion (Ponnappa and Anilkumar, 1977; Annadurai *et al*., 1999). The endopolygalacturonase extracted from infected tissues produced the disease symptoms when applied to healthy tissues (Polak, 1985). Pathogenicity is directly related to the amount of endopolygalacturonase produced (Bateman, 1966). Production of enzyme is indicated by measuring the activity of the enzyme since they are directly proportional to each other (Bateman and Bashan, 1976).

Endopolygalacturonase macerates and injures the cell wall resulting in the leakage of electrolytes.This leakage of electrolytes can be studied from infected or enzyme treated tissue in the bathing medium with the help of conductivity bridge (Bashan, 1975; Hall,1973) Leakage of electrolytes indirectly indicates the amount of endopoly galacturonase secreted by organism. Toxicity tests like maceration and electrolytes leakage by endoPG on onion leaves have been carried out.

MATERIALS AND METHODS

Toxic studies of endoPG Maceration Test

Plugs of 1cm diameter were cut from Potato tubers, Apples, the cortex of carrot roots onion leaves. They were immersed in water for 5 minutes. Then the plugs were dried with whatman filter paper. Discs of 0.5 mm thick plugs were cut with the help of hand microtome. Each set containing 3 discs was transferred to 2 ml of test solution in a watch glass. The time taken for the discs to lose coherence with a gentle pull of the needle was noted. The relative activity of different samples is inversely proportional to the time taken for the loss of coherence.

Electrolyte Leakage Experiment

The following method was adopted to measure the electrolyte leakage from onion leaves treated with endoPG.

Procedure

1 gm of fresh onion leaves grown in pots in the laboratory were cut into 2 Cm long pieces. The pieces were immersed in the following solutions.The purified enzyme lyophilised and diluted to 1:1 V/V,1:50 and 1:100 W/V in deionised water was taken 343 in 250 ml Erlenmeyer flasks. The onion leaf pieces were taken out at intervals of one hour and washed five times in deionised water and kept in deionised water for 10 minutes (ambient solution).The onion leaf pieces were taken out from the ambient solution and again kept in their respective endoPG solutions in 250ml flasks on the shaker revolving at 15 rpm.The ions present in the ambient solution were measured in Kohlrausch conductivity bridge (P.I CO model).The same procedure was repeated at the intervals of 1 hour for 12 hours.

The specific conductance was calculated in the following manner. After keeping the conductivity cell in the ambient solution and setting the resistance(R) at 100 ohms, the jockey was moved to different places of the string in the meter scale till a maximum sound is heard from the earphone. The place where the maximum sound heard was taken as l_1 in the meter scale. From that l_2 may be calculated. Then the resistance was set a 1000 ohms, the same procedure repeated and l_1 and l_2 was noted.

The specific conductance was calculated as follows:

$$K = \frac{1}{} \text{ X cell constance}$$

$$R_X$$

Where

K = specific conductance

$$R_X = R \times \frac{l_1}{l_2}$$

cell Constance - 0.9458 (constant)

Where

R = the resistance in ohms

l_1= position of the jockey in the meter scale where the maximum sound was heard

l_2 = the remaining of length in the scale

RESULTS AND DISCUSSION

The effect of endoPG of *A. cepulae* on the maceration of intercellular cement of pectic materials in potato,carrot and apple is given in Table 1. It reveals that purified enzyme powder diluted suitably takes three hours to twelve hours for maceration. The maceration activity is significant (P< 0.001).The results suggest that endoPG is capable of macerating the pectic materials.The results are in agreement with the results of previous attempt (Cervone, 1978).

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TABLE 1. Effect of purified EPG of *A. cepulae* on efflux of electrolytes from onion leaves

			Hours									
Sl.No.	Macerating solution	d.F	1hour	2hours	3hours	4hours	5hours	6hours	7hours	8hours	12hours	significance
1	1:1(w/v)	5	7.10±0.08	7.29±0.14	7.36±0.16	7.44±0.18	7.59±0.12	7.86±0.05	7.92±0.08	8.20±0.12	8.24±0.14	++
2	1:50(w/v)	5	1.47±0.05	1.58±0.15	1.97±0.16	2.39±0.09	3.15±0.12	3.26±0.08	3.67±0.09	4.66±0.14	5.15±0.18	++
3	1:100(w/v)	5	0.99±0.04	1.10±0.06	1.28±0.02	1.68±0.08	2.51±0.12	2.86±0.05	3.16±0.03	4.66±0.15	4.82±0.16	++

Values expressed are the mean value(X) of 6 individual experiments ±SD
d.f=Degrees of freedom=n-1 observation
Significance ++ =P<0.001
The results expressed are the specific conductance in µ mhos/gms of fresh weight of onion tissue

TABLE 2. Macerating effect of endoPG on the intracellular cement of pectic materials in potato, carrot, apple and onion

			Onion		Potato		Carrot		Apple	
Sl.No.	EPG dilution	d.f.	Macerating Time in hr ± SD	Significance	Macerating Time in hr ± SD	Significance	Macerating Time in hr ± SD	Significance	Macerating Time in hr ± SD	Significance
1	EPG PURIFIED 1:1(w/v)	2	5±0.80	-	4±0.8	-	3.45±0.52	-	3.30±0.85	-
2	1:50(w/v)	2	8±0.95	++	7±0.81	++	6.45±1.56	++	6.30±0.92	++
3	1:100(w/v)	2	12±1.35	++	12±1.85	++	11.30±1.36	++	12.10±1.14	++

Values expressed are the mean value of macerating time (in hours) taken for endoPG to macerate the intercellular cement of pectic materials between the cells of potato, Carrot, Apple and Onion ± SD to lose their coherence. df=degrees of freedom = n-1 observations. Significance ++=P>0.001