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# *IN VIVO* PRODUCTION OF PECTOLYTIC ENZYMES IN CRUCIFER SEEDLINGS SHOWING SUSCEPT AND RESISTANT RESPONSES TOWARDS *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* (PAMMEL) DOWSON.

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Extent of *in vivo* secretion of pectolytic enzymes (PMG, PG & PGTE) was assayed in eight different crucifer cvs. having varied host responses after 15--, 17 - and 19 - days of inoculation of *Xanthomonas campestris* pv. *campestris*. It was found that for sustenance of susceptibility, the bacterium evoked moderate to good-production of these enzymes. In contrast, the secretion was meagre-to very low-in tissues of resistant cultivars. The duration of host-pathogen interaction also influenced the secretion of enzymes.

**Key Words :** *Xanthomonas campestris* pv. *campestris*, susceptibility, resistance, crucifers.

Pectolytic enzymes are involved in the break-down of host cell wall during pathogenesis. *In vitro* and *in vivo* secretion of pectolytic enzymes by many phytopathogenic bacteria have been reported (Husain and Kelman, 1958; Abo-El-Dahab, 1964; Reddy *et al.*, 1969; Neduramen and Vidhyasekaran, 1981; Pandey and Prasad, 1983 and Lal and Prasad, 1983). It is proposed to investigate the extent of *in vivo* sec-retion of Pectin-methyl-galacturonase (PMG), Polygalacturonase (PG) and Polygalacturonate trans-eliminase (PGTE) enzymes in eight different crucifer cvs. of varied host responses after inoculation with the bacterium *X. campestris* pv. *campestris*.

## MATERIALS AND METHODS

Surface sterilized and previously pricked seeds of cauliflower cvs. `Dania', `Pusi', `Pusa Deepali' and `Snowball', kohlrabi cvs. `Early Vienna' and `Earliest White' and mustard cvs. `Baruna' and Yellow' were inoculated separately with YDCA-grown (Dye, 1968) 48-h old culture of *Xanthomonas campestris* pv. *campestris*. The seeds were kept for 8-10h in the bacterial suspension for successful inoculation. The cauliflower cv. `Snowball', kohlrabi cv. `Earliest White' and mustard cv. `Yellow' were found to be resistant to the black rot pathogen. The rest of the cvs. were susceptible.

Inoculated and non-inoculated seeds of the three crucifers were sown separately, and after 15-, 17- and 19 days of inoculation, the leaf tissue extract was analysed for PMG, PG and PGTE activities. Extracts of contemporary healthy leaf tissues were simultaneously analysed which served as control. Procedure for extraction and dialysis of leaf extract for obtaining crude enzyme was the same as reported by Srivastava and Prasad (1989).

Analyses for PMG and PG production were done by Hoppler's Viscosimeter type $BH_2$ (GDR make), using pectin (1.2%) and NaPP (1.2%) as substrates respectively. The percentage loss in viscosity of the substrate enzyme complex indicated the extent of enzymatic activity in each case. The procedure of the viscosimetric analysis adopted was the same as reported by Pandey and Prasad (1983) and Lal and Prasad (1983).

PGTE activities were determined by reacting the leaf tissue extract enzyme-substrate (NaPP) complex with thiobarbituric acid (TBA), following the procedure adopted by Sherwood (1967). The reading was taken in a colorimeter (Systronics, Indian make) at different wave-lengths (440 to 600 mm).

## RESULTS AND DISCUSSION

As is evident from table 1, the secretion of PMG and PG was high in all the susceptible crucifer cvs. whereas it was meagre in resistant ones. The production of these enzymes was also found dependent upon the duration of host-pathogen interaction. In most of the cases of suscept cvs. the maximum PMG and PG production was discernible in the leaf-tissue extract of 17- day infected seedlings, where as in suscept kohlrabi variety `Early Vienna', such a case was noticed in 19- day infected seedlings. However, in the

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Table 1: *In vivo* PMG-, PG-& PGTE-activation in leaf tissues of different crucifers after inoculation of *Xanthomonas campestris* pv. *c ampestris.*

| Varieties of hosts | Days after inoculation | % loss in viscosity of enzyme-substrate complex | | PGTE Expressed in units (1 unit = change in absorbance of 0.01) |
|---|---|---|---|---|
| | | PMG | PG | |
| (A) Cauliflower | | | | |
| (i) `Dania' | 15 | 32.23 | 25.77 | 10 |
| | 17 | 43.00 | 33.89 | 17 |
| | 19 | 41.16 | 23.31 | 20 |
| (ii) `Pusi' | 15 | 31.00 | 36.12 | 14 |
| | 17 | 35.28 | 35.24 | 16 |
| | 19 | 19.00 | 35.00 | 21 |
| (iii) `Pusa Deepali' | 15 | 30.00 | 23.03 | 10 |
| | 17 | 43.39 | 33.12 | 18 |
| | 19 | 25.13 | 29.30 | 15 |
| (iv) `Snowball' | 15 | 11.35 | 13.24 | 1.5 |
| | 17 | 9.52 | 17.65 | 1.0 |
| | 19 | 1.30 | 19.82 | 0.5 |
| (B) Kohlrabi | | | | |
| (v) `Early Vienna' | 15 | 32.00 | 23.27 | 7 |
| | 17 | 25.23 | 26.21 | 10 |
| | 19 | 34.12 | 31.12 | 12 |
| (vi) `Earliest While' | 15 | 18.85 | 12.24 | 0.5 |
| | 17 | 20.12 | 16.13 | 0.5 |
| | 19 | 17.43 | 11.55 | 0.5 |
| (C) Mustard | | | | |
| (vii) `Baruna' | 15 | 28.00 | 25.24 | 20 |
| | 17 | 32.03 | 31.01 | 22 |
| | 19 | 14.79 | 28.27 | 16 |
| (viii) `Yellow' | 15 | 17.84 | 8.47 | 1 |
| | 17 | 14.00 | 10.59 | 3 |
| | 19 | 11.15 | 13.72 | 1.5 |

suscept cauliflower variety `Pusy' during the entire period of host-pathogen interplay almost the same level of enurement of PG was noted. Among the resistant cvs. the meagre to low production of PMG declined with increase in the days of host-pathogen interaction. On the contrary, in the similar tissues of the resistant cvs. the low PG expletion increased slightly with the prolongation of the host-pathogen interplay.

In consonance to our observations Srivastava (1981) asserviated concordance between pathogenicity of suscept been *(Lablab niger)* cvs. `7023', `RD' and `7010' towards X. *campestris* pv. *phaseoli* with the degree of pathogen-induced sensitization of PMG in the leaf tissues of the host.

Neduramen and Vidhyasekaran (1981) also found moderate to-fair endo-PG production in tomato fruits after 3-, 5- and 7-days of infection of *Corynebacterium michiganense.* However, Reddy *et al* (1969) failed to deduce an irrefragable interrelationship between pectolytic enzyme production and pathogenicity of *Xanthomonas alfalfae* in tissues of alfalfa plants. However, Husain and Kelman (1957) detected very high level PG action in tomato fruits infected with *Pseudomonas solanacearum.*

As would be evident from the table 1, in suscept varieties of different crucifers equally prominent PGTE production was induced in the leaf tissue of the infected seedlings, the highest liberation being in suscept mustard variety `Baruna'. Contradistinct to this insignificant to meagre - *in vivo* PGTE expletion was noted in the resistant varieties of the crucifers. Whereas in certain varieties of suscepts there was progressive increase in trans-eliminase liberation with increase in period of host-pathogen interaction, in others it diminished. It could, however, be safely inferred that pathogenicity of the bacterium and progress of disease development was to some degree dependent upon PGTE liberation activised by the pathogen inside the host tissue.

Involvement of PGTE secreted by *Erwinia chrysanthemi* in tissue maceration during soft rot development and permeability changes causing cell death has been reported by Mount *et al.* (1970), Starr and Chatterjee (1972) and Hall and Wood (1973). *In vivo* production of PGTE in tomato fruits and seeds infected with *Corynebacterium michiganense* has been recorded by Neduramen and Vidhyasekaran (1981). Srivastava (1980) had found high post-inoculative PGTE activity in stalk tissues of suscept corn cvs. `Rajendra Makka' and `GS-2' after continued infection of different isolates of *Erwinia chrysanthemi* pv. *zeae.* In stalk tissues of resistant cultivar `Pant' little or negligible amount of PGTE was found. These results conform to our findings here and suggest that transeliminases along with PMG and PG are actively involved in disease development in suscept cvs. of cauliflower, kohlrabi and mustard seedlings.

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