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# Pathophysiology of *Fusarium oxysporum* f. Sp. *Dianthi* in carnation (*Dianthus caryophyllus* L.)

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ABSTRACT

The bio-chemical changes occurred in the carnation after inoculation with *F. oxysporum* f.sp. *dianthi* was studied under *in vitro*. Carnation plants inoculated with fourteen isolates of *F.oxysporum* f.sp.*dianthi* and monitored for their ability to production of fungal pectin-degrading enzymes *viz.,* Pectin Methyl Esterase (PME), Polygalacturonase (PG) and Pectin Trans Eliminase (PTE) involved in development of disease symptoms. Production of pectinolytic enzymes in carnation plants were assessed from 2 days up to 8 days after inoculation at 48h intervals. The accumulation of these enzymes increased in two days after inoculation and attained a peak at six days after inoculation and slowly declined thereafter in all the inoculated plants. Among the fourteen isolates, YRPFOD2 had maximum ability to increase the activity of pectinolytic enzymes *viz.*, Pectin Methyl Esterase (0.49 µ mole hydrogen ion / min / ml), Polygalacturonase (16.11% reduction in viscosity) and Pectin Trans Eliminase (57.59 % reduction in viscosity) after six days of inoculation in infected plants.

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## INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) is one among the most popular commercial cut flowers of the world, ranking second in commercial importance next only to rose. Carnation is preferred by several exporting countries, on account of its excellent keeping quality, wide range of forms and colours and ability to withstand long distance transportation. Cut carnations, roses and chrysanthemums contribute close to 50 per cent of the world cut flower trade (Jawaharlal *et al.,* 2009). Fusarium wilt is an important soil borne disease occurring prevalently in carnation fields (Kyounge *et al.,* 2001). The wilt pathogen in the host is characterized by severe degradation of the vascular tissues, eventually resulting in hollowing-out of stems and wilting of leaves (Baayen and Elgersma, 1985 and Baayen *et al.,* 1988). Degradation of host tissues generally begins with production of polygalacturonase (PG) enzymes that hydrolyze pectin, a constituent of the middle lamella and primary cell walls (Cervone *et al.,* 1986 and Cooper and Wood, 1975). The action of pectic enzymes is often

already sufficient to cause host tissue maceration and cell death (Brett and Waldron, 1990 and Collmer and Keen, 1986). Polygalacturonase production *in vitro* has been reported for several formae speciales of *F. oxysporum* (Cooper and Wood, 1975; P´erez Art´es and Tena, 1990) including f.sp. *dianthi* (Scala *et al.*, 1981). The marked degradation of xylem in carnations infected with race 2 of *F. oxysporum* f.sp. *dianthi* (Baayen and Elgersma, 1985 and Baayen *et al.*, 1988) prompted us to investigate the involvement of pectin degrading enzymes such as pectin methyl esterase (PME), Polygalacturonase (PG) and Pectin Trans Eliminase (PTE) in this process and to evaluate their prospects involved in susceptibility of carnation to fusarium wilt.

## MATERIAL AND METHODS

**Collection of *F. oxysporum* f.sp. *dianthi* isolates:**

Carnation plants showing the typical symptoms of fuarium wilt were collected from the different carnation growing areas of Tamil Nadu. The diseased specimens collected from different areas were used for the isolation of the pathogen (Table A).

**Table A : Survey on the incidence of carnation wilt disease in Tamil Nadu**

| Sr. No. | Places | Taluk | Isolates |
|---|---|---|---|
| 1. | Senbaganur | Kodaikanal | KOSFOD1 |
| 2. | Vilpatti | | KOVFOD2 |
| 3. | Kavunchi | | KOKFOD3 |
| 4. | Thandikudi | Thandikudi | THTFOD1 |
| 5. | Kamanoor | | THKFOD2 |
| 6. | Kunoor | Kunnoor | KUKFOD1 |
| 7. | Benthimai | | KUBFOD2 |
| 8. | Cherrycross | Ooty | OOCFOD1 |
| 9. | ElKhills | | OOEFOD2 |
| 10. | Kombai | | OOKFOD3 |
| 11. | Kannerimukku | Kothagiri | KOKFOD1 |
| 12. | Arakambai | | KOAFOD2 |
| 13. | Vellakadai | Yercaud | YRVFOD1 |
| 14. | Piliyur | | YRPFOD2 |

**Isolation of the pathogen:**

The wilt pathogen *F. oxysporum* f.sp. *dianthi* was isolated from the diseased plants collected during the survey. The infected plant tissues was surface sterilized with 80 per cent ethanol and transferred on to the potato dextrose agar medium (Peeled potato -250 g, Dextrose -20g, Distilled water-1000ml and pH -7.0) in Petri plate. The Petri plates were incubated at lab temperature (28 ± $2^0$C) and observed periodically for the growth of pure colonies. The pure colonies which developed from the bits were transferred to PDA slants for the maintenance of the culture. The *Fusarium* culture isolated from carnation was identified based on the morphological and cultural characters by Booth (1971).

**Assay of pectin degrading enzymes:**

The bio-chemical changes occurred in the carnation plants after inoculation with fourteen isolates of *F. oxysporum* f. sp. *dianthi* were studied. Carnation plants were cut into small pieces (8 mm). Ten pieces were placed in a sterile Petri dish and 10 ml of culture filtrate was added and macerate the solution which was used as enzyme source for the following assay. Pieces without inoculation were kept as control (Mahadevan, 1965).

**Assay of Pectin Methyl Esterase (PME) activity:**

Twenty ml of pectin solution was pipetted in a 50 ml beaker and pH was adjusted to 7.0. Ten ml of the enzyme solution was then added and the pH was adjusted immediately to 7.0 by adding 1N NaOH. At every 15 min the pH was checked and alkali was added from the burette, when the pH falls below the reference point, while stirring. To adjust the pH, 0.02 N NaOH was used and the volume of alkali consumed was noted, which was equal to the enzyme activity. The enzyme activity was expressed as m mole of hydrogen ion per min per ml of the enzyme preparation (Mahadevan and Sridar, 1982).

**Assay of Polygalacturonase (PG) activity:**

The activity of PG was assayed as per the method described by Mahadevan and Sridar (1982). One g of bulb tissue was transferred to a wearing blender and five ml of 0.1M chilled phosphate buffer (pH 6.6) was added. The material was blended for five min, filtered through two layers of cheese cloth and centrifuged at 2000 rpm for 30 min at $4^oC$. The supernatant was decanted and the clear extract was taken as enzyme source. Four ml of the substrate, one ml of acetate buffer (pH 5.2) and two ml of enzyme source were taken in a viscometer and the contents were mixed gently by drawing air rapidly through the large arm of the viscometer by suction. The efflux time of the mixture

was determined by suction through small arm (zero time). The efflux time of the mixture after 30 min was measured. From this, the enzyme activity was calculated as per cent reduction in viscosity of the substrate from the following formula.

$$V = \frac{T_0 - T}{T_0 - T_{H_2O}} \times 100$$

where,

$T_o$ - Flow time in seconds at zero time,

T - Flow time of reaction mixture at time,

$T_{H_2O}$ - Flow time of distilled water.

**Assay of pectin trans eliminase (PTE) activity:**

PTE activity was estimated by the viscometric method (Mahadevan and Sridar, 1982). One ml of the enzyme solution and four ml of the substrate were pipetted into the viscometer. The loss in viscosity of the pectin solution was determined by using of Vinsell Viscometer of size 300. The activity was expressed as per cent reduction in viscosity.

$$V = \frac{T_0 - T}{T_0 - T_{H_2O}} \times 100$$

where,

V - Per cent loss in viscosity,

$T_o$ - Flow time in seconds at zero time,

T - Flow time of reaction mixture at time,

$T_{H_2O}$ - Flow time of distilled water

## RESULTS AND DISCUSSION

Pectinases catalyses the degradation of pectic polysaccharides, the main component of middle lamella, that is, the intercellular cement that holds in place the cells of the plant tissues (Roumbouts and Pilnik, 1980). Pectic enzymes consist primarily of pectin methyl esterase, polygalacturanase and pectin trans eliminase (Verlent *et al*., 2004). The present investigation revealed that the production of polygalacturanase, pectin methyl esterase and pectin trans-eliminase were increased in the carnation plants inoculated with *F .oxysporum* f. sp. *dianthi*.

The isolates of *F. oxysporum* f. sp. *dianthi* were assessed for their ability to produce PME in carnation plants. The accumulation of PME was increased in two days after inoculation and attained a peak at six days after inoculation and slowly declined thereafter in all the inoculated plants. The PME activity at six days after inoculation ranged from 0.15 to 0.49 µ mole hydrogen ion per min per ml in the isolates. Enhanced activity of PME was recorded in the plants inoculated with YRPFOD2 isolate (0.49 µ mole hydrogen ion / min / ml) after six days of inoculation followed by YRVFOD1(0.46 µ mole hydrogen ion / min / ml). The isolate THKFOD2 produced least amount of Pectin Methyl Esterase (0.15 µ mole hydrogen ion / min / ml) (Table 1). Similar observations were made in carrot roots inoculated with *F. solani* f. sp. *radicicola* by Abraham (1999). Saravanan *et al.* (2004) indicated pectin methyl esterase activity of *F. oxysporum* f. sp. *cubense* in banana plants.

**Table 1 : Production of Pectin Methyl Esterase (PME) by different isolates of *F. oxysporum* f. sp. *dianthi* in carnation plants**

| Isolates | PME (µ mole hydrogen ion $min^{-1}$ $ml^{-1}$) Days after inoculation* | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 8 |
| KOSFOD1 | 0.15 | 0.26 | 0.42 | 0.40 |
| KOVFOD2 | 0.15 | 0.26 | 0.43 | 0.41 |
| KOKFOD3 | 0.12 | 0.19 | 0.28 | 0.24 |
| THTFOD1 | 0.07 | 0.21 | 0.33 | 0.29 |
| THKFOD2 | 0.08 | 0.12 | 0.15 | 0.13 |
| KUKFOD1 | 0.14 | 0.23 | 0.37 | 0.32 |
| KUBFOD2 | 0.10 | 0.19 | 0.23 | 0.20 |
| OOCFOD1 | 0.16 | 0.26 | 0.43 | 0.41 |
| OOEFOD2 | 0.07 | 0.12 | 0.19 | 0.13 |
| OOKFOD3 | 0.14 | 0.23 | 0.39 | 0.36 |
| KOKFOD1 | 0.10 | 0.16 | 0.21 | 0.20 |
| KOAFOD2 | 0.12 | 0.21 | 0.32 | 0.29 |
| YRVFOD1 | 0.18 | 0.28 | 0.46 | 0.43 |
| YRPFOD2 | 0.19 | 0.28 | 0.49 | 0.45 |
| Control | 0..02 | 0.03 | 0.03 | 0.01 |

* Mean of three replications

| C.D. (P=0.05) | |
|---|---|
| Isolates | 0.004 |
| Days | 0.002 |
| Isolates × Days | 0.008 |

The Polygalacturonase activity on inoculated plants was significantly increased from two days to six days after inoculation and slowly declined after eight days in all the fourteen isolates when compared to control plants. Increased polygalacturanase activity was observed (16.11% reduction in viscosity) in plants inoculated with most virulent isolate YRPFOD2 than other isolates of *F. oxysporum* f. sp. *dianthi* tested (Table 2). According to Baayan *et al.* (1997) the development of wilt symptoms in inoculated carnation plants was accompanied by quadratic increased in PG activity. Though the fungal, pectin degrading enzymes degrade the xylem of infected

**Table 2 : Production of Poly Galacturonase (PG) by different isolates of *F. oxysporum* f. sp. *dianthi* in carnation plants**

| Isolates | Polygalacturonase (% reduction in viscosity) | | | |
|---|---|---|---|---|
| | Days after inoculation* | | | |
| | 2 | 4 | 6 | 8 |
| KOSFOD1 | 9.08 | 10.51 | 15.28 | 14.45 |
| KOVFOD2 | 9.12 | 10.78 | 15.41 | 14.72 |
| KOKFOD3 | 8.28 | 9.74 | 14.10 | 13.36 |
| THTFOD1 | 8.75 | 9.92 | 14.66 | 13.79 |
| THKFOD2 | 7.79 | 9.08 | 11.18 | 10.25 |
| KUKFOD1 | 8.87 | 10.21 | 14.81 | 14.12 |
| KUBFOD2 | 8.19 | 9.36 | 13.88 | 12.46 |
| OOCFOD1 | 9.25 | 10.89 | 15.76 | 13.17 |
| OOEFOD2 | 8.01 | 9.32 | 11.45 | 11.01 |
| OOKFOD3 | 8.92 | 10.49 | 15.01 | 14.23 |
| KOKFOD1 | 8.10 | 9.65 | 12.76 | 11.58 |
| KOAFOD2 | 8.53 | 9.87 | 14.45 | 13.67 |
| YRVFOD1 | 9.46 | 11.56 | 15.87 | 15.21 |
| YRPFOD2 | 9.54 | 12.62 | 16.11 | 15.52 |
| Control | 0.30 | 0.36 | 0.34 | 0.30 |

* Mean of three replications
C.D. (P=0.05)
Isolates 0.18
Days 0.09
Isolates × Days 0.37

**Table 3 : Production of Pectin Trans Eliminase (PTE) by different isolates of *F. oxysporum* f. sp. *dianthi* in carnation plants**

| Isolates | Pectin trans - eliminase (% reduction in viscosity) | | | |
|---|---|---|---|---|
| | Days after inoculation* | | | |
| | 2 | 4 | 6 | 8 |
| KOSFOD1 | 38.71 | 50.66 | 53.02 | 49.66 |
| KOVFOD2 | 40.68 | 51.28 | 53.27 | 50.12 |
| KOKFOD3 | 31.88 | 42.91 | 46.41 | 42.69 |
| THTFOD1 | 35.02 | 47.28 | 50.16 | 47.52 |
| THKFOD2 | 25.87 | 30.56 | 38.16 | 34.59 |
| KUKFOD1 | 35.45 | 49.02 | 51.22 | 46.01 |
| KUBFOD2 | 31.05 | 42.17 | 46.01 | 42.15 |
| OOCFOD1 | 40.91 | 51.79 | 56.45 | 50.20 |
| OOEFOD2 | 28.34 | 34.23 | 40.26 | 37.23 |
| OOKFOD3 | 37.69 | 50.12 | 52.34 | 48.45 |
| KOKFOD1 | 30.25 | 38.52 | 45.10 | 41.29 |
| KOAFOD2 | 34.11 | 44.23 | 48.53 | 45.10 |
| YRVFOD1 | 41.59 | 52.01 | 56.12 | 53.17 |
| YRPFOD2 | 44.28 | 52.11 | 57.59 | 56.23 |
| Control | 11.89 | 13.09 | 13.59 | 13.41 |

* Mean of three replications
C.D. (P=0.05)
Isolates 0.70
Days 0.36
Isolates × Days 1.40

plants their role in pathogenesis, provided reliable and rapid biochemical factor for monitoring fungal growth and quantifying the partial resistance of carnation cultivars against *Fusarium* wilt. Mariotti *et al.* (2009) reported that the endo PG of *F. phyllophilum* strain FC-10, previously classified as *F. verticillioides*, is the best characterized *Fusarium* cell wall degrading enzyme. A consistent increase in the activity of the PTE was observed in all the fourteen isolates of *F. oxysporum* f. sp. *dianthi*. The maximum enzyme production (57.59% reduction in viscosity) was recorded in the case of YRPFOD2 isolate on six days after inoculation which corroborated the observation (Table 3). Malathi (2010) also observed the same trend of increase in the activity of pectin trans eliminase in the onion bulbs inoculated with *F. oxysporum* f. sp. *cepae.*

The findings of the present study suggested that, the pectin degrading enzymes in infected carnation plants is likely to be related to the amount of actively growing fungal mycelium present in the stem. Although these enzymes clearly contribute to degradation of xylem of infected plants, the enzyme activity in itself need not be causal, however, to the development of disease symptoms. Regardless of their role in pathogenesis, fungal Pectinolytic enzymes anyhow provide a reliable and rapid biochemical parameter for monitoring fungal growth and quantifying resistance of carnation to *Fusarium* wilt.

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