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Full Length Research Paper

Interaction of polygalacturonase enzymes from *Fusarium oxysporum with* tomato polygalacturonase inhibiting protein

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Polygalacturonases (PGs) are important pectolytic enzymes produced by phytopathogenic fungi during the process of infection and colonisation of the host plants. In this work to study the inhibiting effect of polygalacturonase inhibiting protein (PGIP) on polygalacturonase (PG) enzyme from *Fusarium oxysporum f.sp lycopersici*, (highly virulent isolates), protein extraction was carried out from various tissues (stem, leaf and root) of 40, 60 and 80 days old tomato plants (*Lycopersicon esculentum* cv. FDT 202). Study of PGIP- PG interaction showed correlation between the plant age and increasing effect of inhibition activity of PGIP, with stem PGIP having the most effect. There were some differences in protein patterns of induced and non-induced tomato plants by *F. oxysporum*. Tomato PGIP showed different inhibitory activity on PG extracted from different phytopathogenic isolates of *F. oxysporum*.

Key words: Polygalacturonase inhibiting protein (PGIP), *Fusarium oxysporum f.sp lycopersici*, polygalacturonase (PG).

INTRODUCTION

Polygalacturonase-inhibiting proteins (PGIPs), present in the cell wall of many dicotyledonous plants (Desiderio et al., 1997), inhibit the activity of fungal endopolygalacturonases and *in vitro* favor the accumulation of elicitor-active oligogalacturonides (Desiderio et al., 1997). PGIPs are proteins structurally related to several resistance gene products recently cloned in plants (Bent, 1996), and belong to a superfamily of leucine-rich repeat (LRR) proteins specialized for recognition of non-self molecules and rejection of pathogens. It has been proposed that PGIPs and resistance gene products may function as integrated components of a cell surface apparatus, part of the plant "immune system," in which the role of each component is defined by both its structure and regulation (Desiderio et al., 1997). PGIPs with distinct regulation and distinct specificity, that is, ability to interact with and inhibit PGs from different fungal sources.

Since PGIPs are present in uninfected plant tissue, it is possible that PGIPs are part of a pre-existing defense mechanism (Stotz et al., 1994).

Here we have presented the inhibiting activity of both PGIP-1 and PGIP-2 of tomato PGIP on PG enzyme of *F. oxysporum* fungal pathogen.

MATERIALS AND METHODS

F. oxysporum was from PTCC (Persian Type Culture Collection). Protein molecular weight marker was from SIGMA. All other chemicals and media components were of analytical grade and obtained from MERCK.

Plant culture

Tomato seeds were grown in greenhouse at 28°C and 14:10 h light and dark.

Growth of fungi for preparation of PG

Highly virulent isolates of *F. oxysporum*, which includes F23, F47, F18 and F15 (Zamani et al., 2001) were grown in PZ medium containing pectin (10 g), $[(NH4)_2SO4]$(2.64 g), (K_2HPO_4)(0.34 g) ,

Figure 1. Tomato shoots infected with *Fusarium oxysporum* (F15) (left) and uninfected (right).

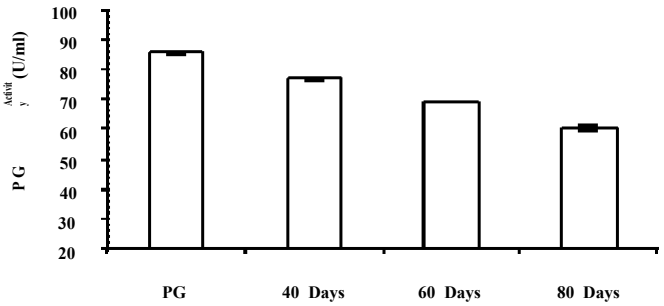

Figure 2. Effect of PGIP extracted from stem on PG activity of *F. oxysporum* (F15). The picture shows PG activity of *F. oxysporum* decreasing with ageing.

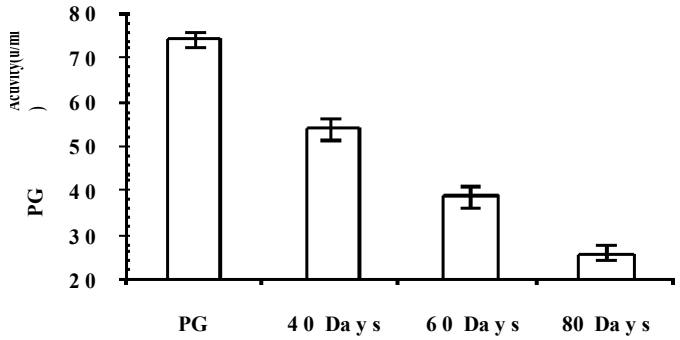

Figure 3. Effect of PGIP extracted from tomato leaf on PG activity of *F. oxysporum* (F15) isolate. The picture shows PG activity of *F. oxysporum* decreasing with ageing.

($MgSO_4$ $7H_2O$) (0.14 g), final volume with dH_20 (1 L) and pH adjusted to 4.5. The culture was grown at 26°C with shaking at 125 rpm for 10 days. Flask was harvested by suction filtration through a Wathman N.113 disc. The filtrate, which contained the extracellular PG enzymes stored at 4°C before PG activity assay.

Inducing the tomato shoots

20 days after planting tomato seeds, the plants were transferred to a soil which was contaminated with isolate F15 spores *F. oxysporum*.

PGIP extraction from tomato

Cell wall proteins including PGIP was isolated from tomato root, stem and leaf according to Abu-Goukh method (Abu-Goukh et al., 1983) and dialyzed against 20 mM Na acetate pH 5. The dialyzed proteins were mixed with a suspension of diethylaminoethyl (DEAE) cellulose pre-equilibrated with 20 mM Na acetate pH 5. The nonabsorbed proteins were precipitated with 70% (NH4)2SO4, resuspended in a small volume of water, dialyzed against PBS buffer.

PG and PGIP interaction

PGIP activity was determined by assaying the PG –catalyzed release of reducing sugars from sodium polypectate in the presence or absence of PGIP. PG a PGIP were mixed and incubated at room temperature for 5 min prior to the addition of substrate. The reaction was stopped after different time intervals and initial reaction rates were determined. The standard assay contained 0.05% (W/V) of sodium polypectate and sufficient PG to produce 80 nmol of galacturonic acid reducing equivalents in 20 min at 30°C. PGIP concentrations were determined by quantitative amino acid analysis.

Protein gel electrophoresis

The extracted tomato PGIP was analyzed by SDS-PAGE (15%) according to Laemmli method (Laemmli, 1970). 5 µl of extracted tomato PGIP with 5 µl 2X SDS-PAGE gel loading buffer was mixed and boiled for 10 min and loaded per well. The bands were visualized by staining with Coomassie Brilliant Blue R250.

Data analysis

The result of the interaction assay between PG and PGIP, with three replicates was analyzed using MSTAT statistical.

RESULTS

For study of pathogenicity of *F. oxysporum* (F15) on tomato plants. The 40, 60 and 80 days old plant infected with fungal isolate showed the reduced growth and root rot in compared with uninfected plants as control (Figure 1). The inhibitory activity of PGIP from different tissues of tomato (stem, leaf and root) on PG enzyme extracted from *F. oxysporum* (F15) was studied.

The PGIP extracted from stems of 40, 60 and 80 days old plants showed that the expression of PGIP protein increases with the age (Figure 2). One-way ANOVA analysis and LSD test proved the significance of the age difference at 95% level.

Similar results were observed using PGIPs extracted from tomato leaf (Figure 3) and root (Figure 4).

The inhibiting activity of PGIP of 80 days old plants for

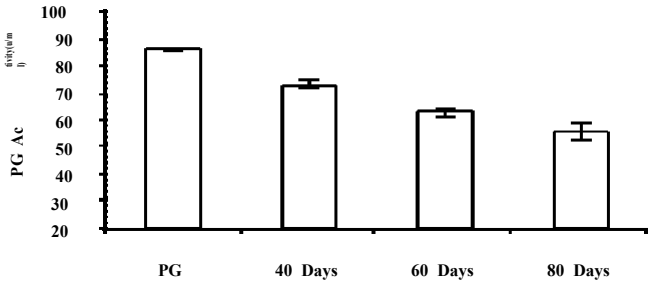

Figure 4. Effect of PGIP extracted from tomato root on PG activity of *F. oxysporum* (F15) isolate. The picture shows PG activity of *F. oxysporum* decreasing with ageing.

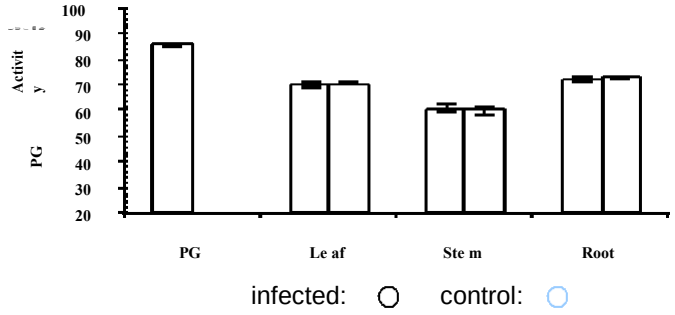

Figure 5. Comparison of PG inhibition by PGIP from infected plants with *F.* oxysporum (F15) and un infected plants. The picture shows no difference between infected and uninfected plants in leaf, stem and root.

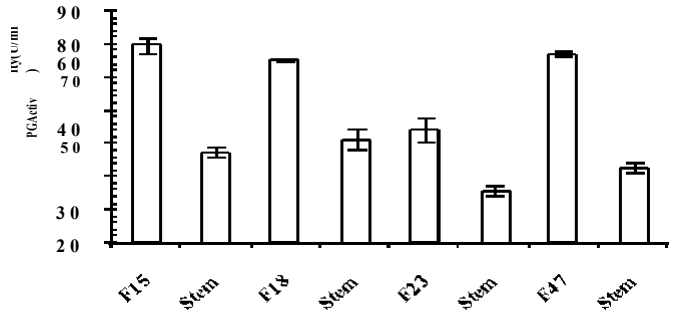

Figure 6. Effect of tomato stem PGIP on PG activity of different *F. oxysporum* isolates. The results show there is most inhibitory effect on F23 isolates of *F. oxysporum*.

infected with *F. oxysporum* (F15) isolate and uninfected was compared. It was shown there is no significant difference between the effect of these two PGIPs in terms of reducing the activity of PG from isolate F15 of *F. oxysporum* (Figure 5). Induction by fungus did not lead to PGIP increased production.

The interaction between PG of highly virulent isolates of *F. oxysporum* and PGIP from stem of 80 days old plants was studied and it was shown that the stem PGIP

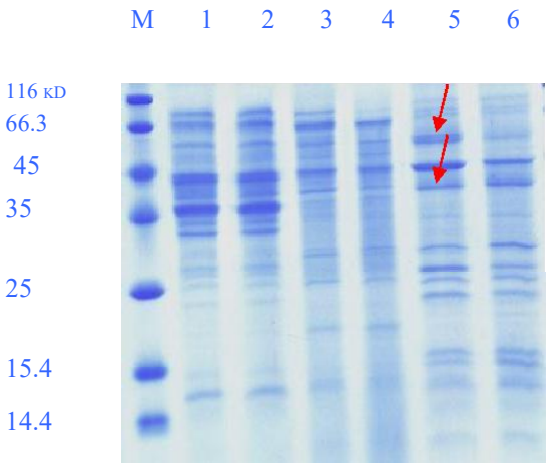

Figure 7. Protein pattern of infected and control plant (arrow show difference) M: marker. Lane 1 is proteins extracted from induced root. Lane 2 is proteins extracted from non- induced root. Lane 3 is proteins extracted from induced stem. Lane 4 is proteins extracted from non-induced stem. Lane 5 is proteins extracted from induced leaf. Lane 6 is proteins extracted from non-induced leaf. The differences only are in induced leaf at two positions between 45 and 66 KDa. Tomato PGIP places between these regions.

reduces 40.5% of PG activity of isolate F23, 31.2% of isolate F18, 34.3% of isolate F15 and 43.8% of isolate F47 (Figure 6).

The result of SDS-PAGE analysis of the root, stem and leaf protein pattern of 80 days old plant infected with isolate F15 of *F. oxysporum* and uninfected plants clarified the difference in the intensities of the bands in the range of 45 to 116 kDa between infected and uninfected plants (Figure 7).

DISCUSSION

The occurrence of PGIPs has been reported in a variety of dicotyledonous plants and in the pectin-rich monocotyledonous plants (onion and leek) (De Lorenzo et al., 2001).

PGIP from tomato plant can protect tomato cell walls from degradation by enzymes produced by *F. oxysporum* (De Lorenzo et al., 2001). The result of this research is consistent with this finding. In the present study it becomes clear that the amount of tomato PGIP increases with age before flowering. PGIP expression are induced by wounding and pathogen infection in soybean (Favaron et al., 1994) and apple fruits (Yao et al., 1999), but not in pear and tomato fruits (Daniels, 1992).

PGIP is a glycoprotein with varying size. The smallest PGIP reported was from peach, with molecular mass 15 kDa, and the largest was isolated from pear with a molecular mass of 91 kDa. However, most of the identified PGIPs fall in the range of 40 to 90 kDa. Tomato

PGIP is in the range of 40 to 90 kDa (Powell et al., 2000). SDS-PAGE pattern showed some difference in two bands between proteins extracted from leaf of control and induced plant in this range. For confirmation, isoelectric focusing is needed to show these bands are PGIP or not.

Control of plant disease is vital in plant agriculture. Commercially produced, registered products, such as fungicides, are frequently recommended for plant disease management. These chemicals have an established history of controlling many economically important diseases, despite the development of tolerance or resistance in pest organisms. Intensified use of fungicides has resulted in the accumulation of toxic compounds potentially hazardous to humans and environments.

Further characterization of tomato PGIP is useful in plant biotechnology field. Literature shows tomato *pgip* gene in not interrupted by intron (Liang et al., 2005; Stotz et al., 1994). According to inhibiting activity of tomato PGIP which was showed in this research, this gene can act as a candidate for decreasing pathogens damages to agriculture products.

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