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PEROXIDASE, POLYPHENOL OXIDASE ACTIVITY, PROTEIN PROFILE AND PHENOLIC CONTENT IN TOMATO CULTIVARS TOLERANT AND SUSCEPTIBLE TO *FUSARIUM OXSYPORUM* F.SP.*LYCOPERSICI*

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Abstract

The total phenol content, peroxidase and polyphenol oxidase enzyme activities and the total protein profile in tomato cultivars (*Lycopersicon esculantum* Mill.), tolerant and susceptible to *Fusarium* wilt disease was studied. The tolerant cultivars of tomato viz., FEB-2, FEB-4, FloraDade and NF-31 had significantly higher phenol content as well as peroxidase and polyphenol oxidase activities than the susceptible ones (Sel-7, Sel-18 and Punjab Chhuhara). The maximum peroxidase activity was recorded in the resistant cultivar, Flora Dade (02.073unit/ml) and minimum in the susceptible cultivar,Sel-18 (0.241unit/ml). Major differences in soluble protein banding pattern were observed in the susceptible and resistant cultivars. The hierarchical cluster analysis was performed using NTSYS-pc(V.1.8) software. The dendrogram using the average linkage between the groups, showed proximity of resistant cultivars viz., FEB-4,FEB-2, Flora Dade and NF-31 to the wild species with respect to similarity of banding patterns. The three susceptible cultivars viz., Sel-7, Punjab Chhuhara and Sel-18 were grouped separately.

Introduction

Fusarium oxyporum f.sp.*lycopersici* (Sacc.) W.C. Synder and H.N. Hans, a soil borne plant pathogen in the class Hyphomycetes, causes *Fusarium* wilt especially in tomato. It is a devastating disease causing considerable economic losses ranging from 10-80% yield loss in many tomatoes producing area of the country (Keshwan & Chaudhary, 1977). It is of worldwide importance, where at least 32 countries had reported the disease, which is particularly severe in countries having warm climate. At one time, the disease nearly destroyed tomato production in part of Florida and the southeastern states of United States. Most forms specials of *Fusarium oxyporum* colonize the vascular system of their host, thereby causing wilting of the plant. Tomato (*Lycopersicon esculentum* Mill.) is only host, for this *Fusarium oxyporum* f. sp. *lycopersici*. Of this *Fusarium oxysporum* f.species. three races are known, notably race *I*-1, *I*-2 and *I*-3 (Labate *et al*., 2007). The objective of this research was to study the biochemical basis of *Fusarium* wilt resistance in tomato in search for a biochemical marker. Such study will result in a better understanding of the mechanism of the defense-response in tomato. Knowledge of this interaction will be helpful in the development of the new concepts for disease resistance in crop protection and may also contribute to a better understanding of factors determining the expression of resistant genes. Morphological traits have commonly been used for cultivar identification, the majority of which are controlled by several genes (Tsaftaris, 1987) and most are influenced in varying degrees by environmental conditions, however the biochemical markers, have no such disadvantage.

Materials and Methods

Plant material: Tomato accessions consisting of cultivated varieties viz., FEB-2, FEB-4, Flora Dade, NF-31, Sel-7, Sel-18 and Punjab Chhuhara (with varying disease scores), screened against *Fusarium oxyporum* f.sp. *lycopersici* race 1, under artificial disease stress conditions were selected from a replicated field trial of vegetable improvement section of Indian Institute of vegetable Research, Varanasi, UP, India. The resistance/susceptibility was evaluated (Benerjee *et al*., 1990) based on percent plants infected (PPI), percent disease intensity (PDI) and coefficient of disease index (CODEX) values. The fresh leaf samples from disease free plants were harvested from the field trial and transferred to the laboratory on ice for further analyses.

Extraction and estimation of phenolics: The total phenolic content was analyzed spectrophotometrically using the Folin-Ciocalteu colorimetric method (Singleton, 1999) with some modifications. 0.5 g sample was grinded in 10 time volume of 80% ethanol. The homogenate was centrifuged at 10000 rpm for 20 minutes and the supernatant was saved. The residue was re-extracted in 80% ethanol, centrifuged and the supernatant was pooled. Evaporated the supernatant to dryness, dissolved the residue in water. To 3.0 ml extract 0.5ml Folin-Ciocalteu's reagent, was added and after 3 minutes added 20% Na_2CO_3 solution. Mixed thoroughly, placed the tubes in a boiling water both for 1 minutes, cooled and measured the absorbance at 650 nm. All values were expressed as means (µg catechol/g tissue).

Peroxidase assay (EC1.11.1.7): Peroxidase (EC1.11.1.7; donor: hydrogen-peroxidase oxido-reductase) enzyme was assayed as per the procedure described by Putter (1974), with slight modifications. The reaction mixture consisted of 2.5ml of a mixture containing 20mM Guaiacol in 0.01M Sodium phosphate buffer, pH 6.0 and 0.1M Hydrogen Peroxide. Enzyme extract (0.1 ml) was added to initiate the reaction. The absorbance change was recorded at 436 nm.

Crude enzyme extract was diluted to adjust the change in absorbance at 436 nm in the range of 0.1 to 0.2

absorbance units per minute. The boiled enzyme extract served as blank. Peroxidase enzyme activity was expressed as change in absorbance at 436 nm per minute/g fresh tissue.

Polyphenoloxidase assay (EC1.10.3.2 or EC1.14.18.1**):** The Polyphenoloxidase (PPO) (EC1.10.3.2 or EC1.14.18.1) activity was assayed as per the procedure of Haplin & Lee (1987). The reaction mixture consisted 1.5 ml of 0.1M Sodium phosphate buffer (pH 6.5) and 200µl of the enzyme extract. 0.01ml Catechol was added to the reaction mixture to start the reaction. PPO activity was expressed as change in absorbance at 412 nm per minute/g fresh weight of tissue.

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE): Fresh leaf sample 100mg was extracted in 0.1M Sodium phosphate buffer (pH 7.0) at 0-4^{o}C. The homogenate was centrifuged for 20 minutes at 12000rpm. The supernatant was decanted and used for SDS-PAGE (Laemmli, 1970). The protein content of the sample was determined by the method of Lowry *et al*., (1951). Protein (100µg) from different tubes was taken and mixed with 10µl of sample buffer in eppendorf tube, boiled for 3-4 minutes and incubated at 4^{o}C for 30 minutes. The samples containing equal amount of protein were loaded into wells of 12% polyacrylamide gel. Electrophoresis was carried out at constant voltage of 75 volts for two hours. The gels were stained with 0.2% coomassie brilliant blue (R-250) solution and distained with acetic acid/ water. The hierarchical cluster analysis was performed on gel documentation system using NTSYS-pc software (v. 1.8) and the dendrogram was prepared using average linkage between groups based on presence/absence of protein bands in different lanes of the gel.

Results and Discussion

The total phenolics content in 7 diverse tomato cultivars are shown in Fig. 1. Results showed that the resistant cultivars had higher phenolic content as compared to the susceptible cultivars. Variety FEB-2 had maximum phenolic content (1591.00µg catechol/g tissue) followed by Flora Dade (1482.23µg catechol/g tissue) and NF-31(1456.70 µg catechol/g tissue). The minimum amount of phenolics was recorded in the Sel-7 (1111.86µg catechol/g tissue) and Punjab Chhuhara (1111.90µg catechol/g tissue), respectively. Both cultivars are statistically at par. The differences in total phenolics content between resistant and susceptible cultivars statistically significant. Our results are in close agreement to earlier studies by a number of workers, where it has been shown that a resistant variety had a higher level of phenolics than the susceptible one (Banerjee & Kalloo, 1989; Singh *et al*., 2002). The exact mode of action of phenolics compounds in reducing the incidence of *Fusarium* wilt is not yet very well known. However, phenols in quinone forms and in oxidized state are effective in checking the pathogen, including the inactivation of enzymes produced by it (Sequeria, 1983).

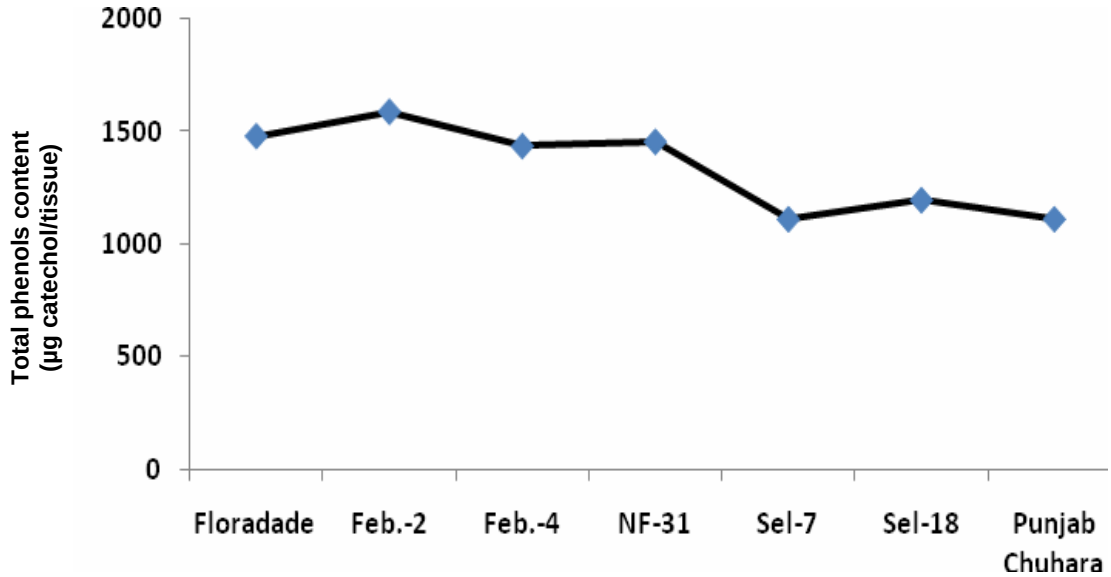

Fig. 1. Total Phenols content in tomato cultivars.

The peroxidase (PO) (EC1.11.1.7; donor: hydrogen-peroxidase oxido-reductase) activity was assayed in the leaf samples of all the seven cultivars and it was noticed that the PO activity was significantly higher in the resistant cultivars as compared to the susceptible ones (Fig. 2). The maximum peroxidase (PO) activity was recorded in the resistant cultivar Flora Dade (2.073 units/g) and minimum in the susceptible cultivar Sel-18(0.241 units/g). Peroxidase plays an integral part in the biosynthesis of plant cell wall components viz., lignin, suberin and cross-linked extension (Lamport *et al*., 1986). The lignifications and wall thickening are part of defense response to pathogens particularly fungi (Gasper *et al*., 1982). Besides these, peroxidases also play an important role in one of the earliest observable event of plant's defense response i.e., oxidative burst (Wojtaszek, 1997). Earlier Bose & Rajan (2000) indicated the possibility of using peroxidase isozyme as markers for resistant and moderately resistant varieties of tomato.

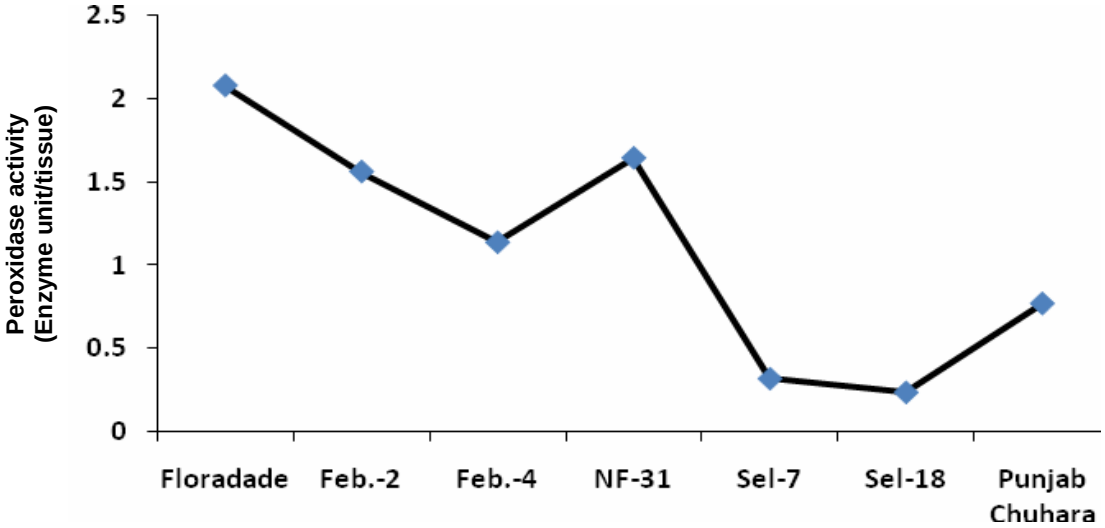

Fig. 1. Peroxidase activity in tomato cultivars.

Polyphenol oxidase (PPOs) (EC1.10.3.2 or EC1.14.18.1) catalyzing the oxygen dependent oxidation of phenols to quinines are ubiquitous among angiosperms and assumed to be involved in plant defense against pests and pathogens (Yedidia, et al., 2003). In order to investigate the role of PPO in plant disease resistance, the polyphenol oxidase activity was also assayed in the leaf samples of the resistant as well as susceptible cultivars. Higher PPO activity was recorded in the resistant cultivars viz., FEB-2, FEB-4, Flora Dade and NF-31 as compared to the susceptible (Sel-7, Sel-18 and Punjab Chhuhara) (Fig. 3). Ramamoorthy *et al*., (2002) studied the induction of defense proteins and chemicals by *P. fluorescens* isolate Pf1 against challenge inoculation with *Fusarium oxyporum* f.sp.*lycopersici* in tomato. *Pseudomonas fluorescens* isolate Pf1 was found to protect tomato plants from wilt disease caused by *Fusarium oxyporum* f.sp. *lycopersici.* Phenolics were found to accumulate in bacterized tomato root tissues challenged with *Fusarium oxyporum* f.sp. *lycopersici* at one day after pathogen challenge. The accumulation of phenolics reached maximum at the 5th day after pathogen challenge. In pathogen inoculated plants, the accumulation started at the second day and drastically decreased 4 days after the pathogen inoculation. Activities of Phenylalanine Ammonia-lyase (PAL), Peroxidase (PO) and Poly phenoloxidase (PPO) increased in bacterized tomato root tissues challenged with the pathogen at one day after pathogen challenge and activities of PAL and PO reached maximum at the 4th day while activity of PPO reached maximum at the 5th day after challenge. Their results suggest that induction of defense enzymes involved in phenylpropanoid pathway accumulation of phenolics and RR-Proteins might have contributed to restriction of invasion of *Fusarium oxyporum* f.sp. *lycopersici* tomato roots.

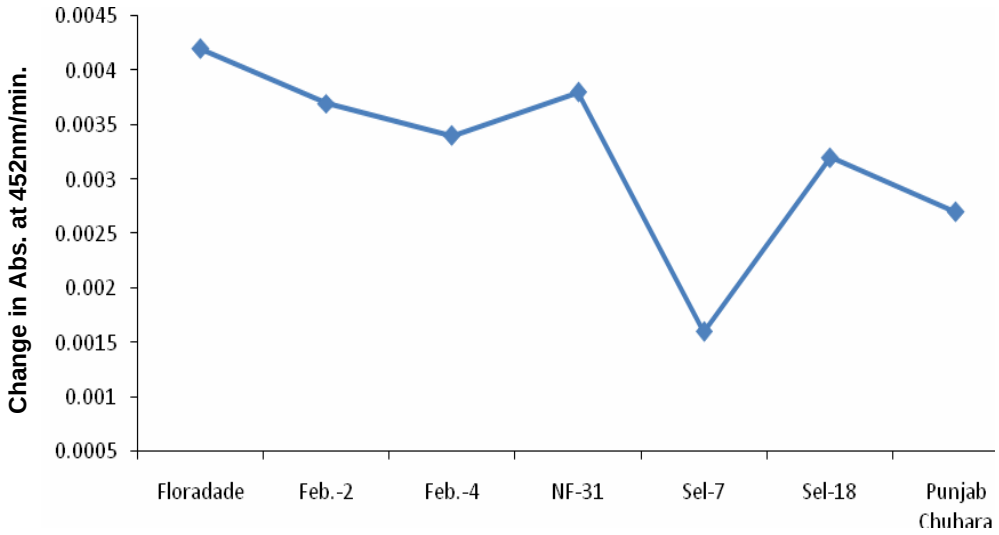

Fig. 2. Polyphenol oxidase (PPO) activity (Change in Abs. at 452nm/min.) in tomato cultivars.

Protein may be associated with the *Fusarium* wilt resistance in tomato were analyzed in three susceptible and four resistant cultivars along with ten wild accessions of tomato utilizing Sodium Dodecyle Sulfate Polyacrylamide gel electrophoresis. Major differences in soluble protein banding pattern were observed in the susceptible and resistant cultivars. The hierarchical cluster analysis was performed using NTSYS-pc (v. 1.8) software. The dendrogram (Fig. 4) using the average linkage between the groups, showed proximity of resistant cultivars viz., FEB-2, FEB-4, Floradede and NF-31to the wild species with respect to the similarity of banding patterns. The three susceptible cultivars viz., Sel-7, Punjab Chhuhara and Sel-18 were grouped together. The proximity matrix was calculated in terms of Jaccard coefficient.

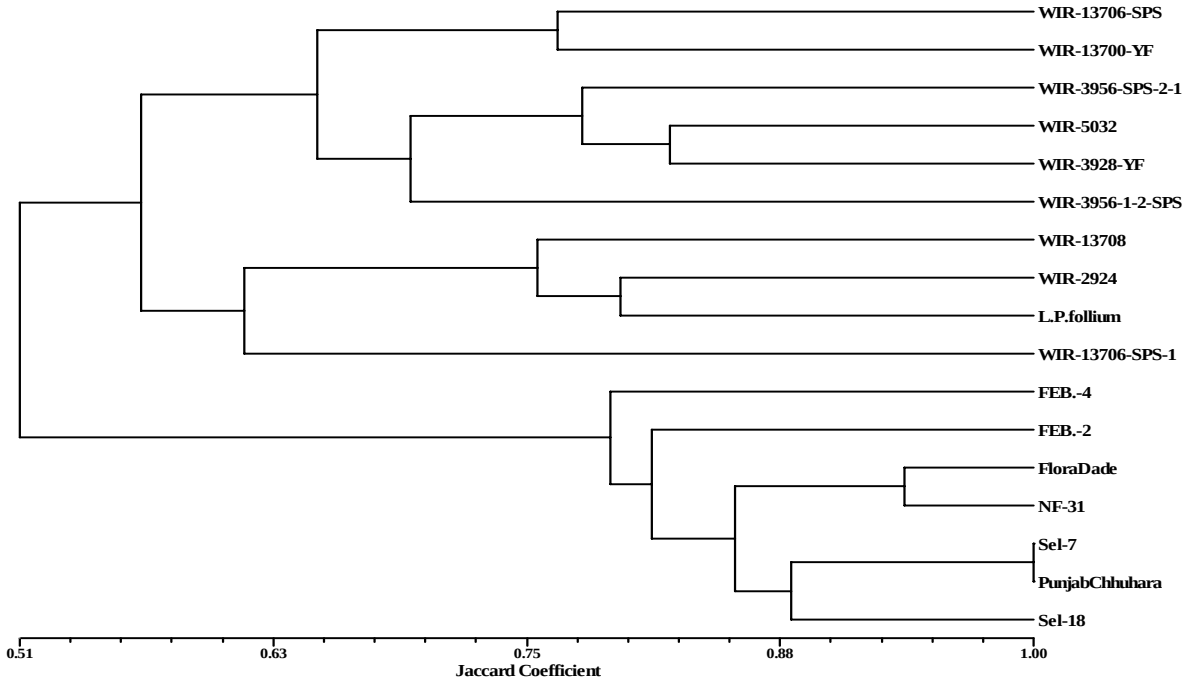

Fig. 4. Hierarchical cluster analysis of Tomato.

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