African Journal of Agricultural Research Vol. 7(43), pp. 5849-5857,13 November, 2012
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR12.322
ISSN 1991-637X ©2012 Academic Journals

Full Length Research Paper

Biochemical basis of defense response in plant against *Fusarium* wilt through bio-agents as an inducers

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Accepted 8 October, 2012

Plant resists pathogen infection through physical and chemical defenses that may be application of some biotics and abiotics inducers. It has been found that pre treatment with bioagents *Trichoderma harzianum,* (Kan.), *T. harzianum* (Del.), *T. harzianum* (Pant), *Trichoderma viride* (Kan.), *T. viride* (Del.), *T. viride* (Pant), *Aspergillus niger* AN-27 (Kan.) *Chaetosphaeridium globosum* (Del.) and *Pseudomonas fluorescens* (Del.) provided induced resistance in plant against *F. o.* f.sp. *lycopersici* resulting declined disease incidence from 100 to 7.69%. The maximum inhibition was noted by *T. harzianum* (Kan.) isolates. The induction of resistance was associated with certain biochemical changes in tomato leaves. Treatment with bio-agents as inducers prior to challenge inoculation sensitized the seedling to produce increased level of soluble proteins. The maximum increase in soluble protein content was found in (T_1) *T. harzianum* (Kan.) treated plant showing 35.04 mg/g of fresh leaves against 20.49 mg/g of fresh leaves in case of control-II. A high content of phenols which are the indication of first stage of defense mechanism was also recorded in treated plant with maximum in *T. harzianum* (Kan.) treatment representing 2.62 g/mg of fresh leaves against 1.38 in control-I at 20 days of inoculation. The disease severity showed negative correlation with soluble protein (r=-0.6364) and total phenol (r=-0.7653). Protein profiling by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) revealed that the soil treated with *T. harzianum* (Kan.) to synthesize some new protein representing maximum number of 22 bands. The presence or absence of the bands in protein profiling might be responsible for resistance response against *Fusarium oxysporum* f.sp. *lycopersici* in tomato.

Key words: Biochemical change, biotic inducers, disease incidence, *Fusarium* wilt, protein profiling.

INTRODUCTION

Induced resistance as a technique of phyto-immunity has received great attention for management of plant diseases. Various types of biological agents, virulent or avirulent strains of pathogens, plant extracts, crude extracts and chemicals which are not considered as fungicides are used for induction of resistance in various crops (Metraux et al., 1991; Cohen, 1994; Van Loon et al., 1998; Attitalla et al., 1998; De Cal and Melgarejo, 2001). The pre-application of some biotic and abiotic inducers also provided induced resistance in plants against many pathogens (Kuc, 1995; Biswas et al., 2003; Van Loon, 1983; Kessman et al., 1990).

Doubrava et al. (1988) found that treatment of first leaves with spinach extracts provide induced resistance against *Colletotrichum orbiculare* in cucumber and resistance mechanism was confirmed due to presence of oxalic acid in spinach extract. Aqueous extracts of barley leaves induced oversized papillae formation in barley which in turn produces resistance against powdery mildew (Yokoyama et al., 1991).

Recently, bio-agents are using as an inducers in induced resistance for management of several diseases.

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Kuc (1995) reported that repeated treatment of winter wheat with *Bacillus subtilis* was highly effective against powdery mildew. Similarly, effect of *B. subtilis* was also noticed against powdery mildew of winter and summer barley. Application of various fungal fluids provides induced resistance in rice plants against *Drechslera oryzae* (Trivedi and Sinha, 1976).

Metraux et al. (1991) reported induction in systemic resistance by *Pseudomonas syringae* pv. s*yringae in* rice against *Pyricularia oryzae*. Pre inoculation spray with avirulent or virulent pathogens induces local resistance against powdery mildew in barley and wheat (Ouchi et al., 1974). Resistance against blue mold of tobacco can be achieved by stem infection with live sporangiophores of the causal organism of blue mold, *Peronospora tabacina* (Tuzun et al., 1989). Biochemical changes associated with induced resistance in crop plant against pathogens by biotics and abiotics agents has been reported by several workers (Kuc, 1995; Choudhary et al., 2010; Olga and OZerestksorskay, 1995, Arzoo et al., 2012, Mandal and Sinha, 1991).

Biswas et al. (2003) found that pre inoculation sprays of crude extract sensitized seedlings to produce elevated levels of protein, soluble protein and phenol contents. New proteins of different molecular weight (MW) that is, 110, 105, 38 and 32 KDa were measured by SDS-PAGE analysis. These observations imply that certain biochemical alteration in the host might be associated with defense mechanism due to the effect of crude extract. Therefore, the present investigation was undertaken on this aspect.

MATERIALS AND METHODS

Collection of diseased plant sample

The present investigation was undertaken during 2007 to 2010 at Department of Plant Pathology, CSA University of Agriculture and Technology, Kanpur. The pathogen *Fusarium oxysporum* f. sp. *lycopersici* was isolated from diseased plant showing typical wilt symptoms collected from Vegetable Research Farm, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India.

Isolation and purification of the pathogen

The diseased plant's stem showing typical wilt symptom was washed thoroughly with distilled water and then cut out into small pieces by a sterilized knife and each piece is having small bits of diseased and healthy tissues. These pieces were dipped in 0.1% mercuric chloride ($HgCl_2$) solution for 30 s and then thoroughly washed thrice in distilled water to remove the traces amount of $Hgcl_2$ solution. Excess moisture was removed by putting these pieces between two folds of sterilized blotting paper under aseptic conditions. The pieces then transferred to sterilized Petri plates containing 2% potato dextrose agar (PDA) medium in inoculation chamber with the help of sterilized forceps. Two pieces were placed aseptically in each Petri plates and incubated at room temperature (20±1°C). On appearance of the colony around the bits, the pathogen was purified by the transfer of hyphal tip in Petri plates which was previously poured with sterilized PDA in aseptic condition. The purified culture was then maintained at 25º±1ºC in refrigerator. On appearance of the colony in Petri plate, the pathogen was examined under compound microscope and identified on the bases of its morphological and cultural characteristics.

Identification of the pathogen

The fungus was observed under a compound microscope and identity of the pathogen was established on the basis of morphological and cultural characteristics described by Snyder and Hansen (1940). The culture of the pathogen was maintained on PDA at 27 ± 1°C for further investigation.

Preparation of pathogen inoculums

The Petri plate containing 14 days old culture of the *F.o.* f.sp. *lycopersici* was taken and flooded with sterile water. The mycelia along with spores were scrapped off with the help of sterile forceps and collected in a beaker. The suspension was then sieved with the help of a strainer to remove PDA clods. The collected spore suspension was diluted with distilled water and required concentration of spore suspension was measured with the help of a haemocytometer. About 250 µl spore suspension was pipette into the counting chamber. The counting chamber of the haemocytometer was covered with a cover slip. The haemocytometer was further mounted over a compound microscope. Average number of spores per square was counted and the spore suspension was adjusted to 10, 00, 000 conidia ml^{-1}.

Preparation of spore suspension of biotic inducers

The experiment was conducted by using nine isolates of bioagents that is, three isolates each of *Trichoderma harzianum* and *Trichoderma viride* and one each isolate of *Aspergillus niger*, *Chaetosphaeridium globosum* and *Pseudomonas flurescens* were collected from three different geographical areas of the country namely sub-himalayan region (Pant Nagar), Northern Alluvial zone (Delhi) and Central plain zone (Kanpur). Each isolate; *T. harzianum* and *T. viride* were collected from Kanpur, IARI New Delhi, and Pant Nagar, *A. niger* was obtained from CSAUA&T Kanpur, *C. globosum* and *P. flouresence* were obtained from IARI, New Delhi. All the cultures of bioagents were maintained on PDA through sub culturing from time to time and stored at 4ºC in a refrigerator.

Measurement of disease incidence

The experiment was conducted in the glasshouse complex, Department of Plant Pathology, C.S.A. University of Agriculture and Technology, Kanpur. The seeds of tomato variety 'Azad-T-6' were sown in 30 cm earthen pots, which was previously filled with a mixture of sandy loam and farm yard manure in the ratio of 2:1. In each pot, 10 properly spaced seeds were sown and watered regularly. After one month, plants were sprayed with different plant extract solutions (1:5 w/v) separately. Two controls were kept, in one case, plants were sprayed with distilled water only served as control-I and in another case, and plants were inoculated with conidial suspension of fungus served as control-II. Three replications were kept for each treatment. After two days of spraying, all the treated plants except control I were inoculated with spore suspension of the pathogen. Then all the plants were kept on glass house bench at 25 ± 1°C. The concentration of conidia was maintained at 10, 00, 000 conidia/ ml.

The measurement of disease incidence was taken after 10, 15 and 20 days of pathogen inoculation. The disease incidence was recorded by using 0 to 4 scale as described by Weitang et al. (2004) where zero representing no infection and four denoting plants completely infected. The 0 to 4 scale of the disease incidence was classified as follows:

0: No infection.
1: Slight infection, where 25% leave become wilted and one or two leaves became yellow.
2: Moderate infection, two or three leaves became yellow, 50% of leaves became wilted.
3: Extensive infection, the all plant leaves became yellow, 75% of leaves become wilted, and growth is inhibited.
4: Complete infections, the whole plant leaves become yellow, 100% of leaves become wilted, and the plants die.

The percentage of disease incidence was determined using the formulas as given by Weitang et al. (2004):

$$\text{disease incidence } (\%) = \left[\frac{(\sum \text{scale} \times \text{number of plants infected})}{(\text{highest scale} \times \text{total number of plants})} \right] \times 100.$$

Biochemical changes due to induced resistance

Tomato leaves were collected from different treatments and the changes in the content of soluble protein and phenol in leaves were estimated at 5, 10, and 15 days after pathogen inoculation profiling of soluble protein was also done by sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to find out whether some new protein was associated with resistance or not.

Estimation of total phenol

The accumulation of phenols in tomato plants after treatment with different inducers followed by inoculation of pathogen was estimated following procedure developed by Bray and Thorpe (1954) with slight modification. In this method, the total phenol estimation was carried out with Folin-Ciocalteu reagent (FCR), which was measured at 650 nm.

Exactly, 1.0 g of leaf sample of tomato was ground in a pestle and mortar in 10 times volume of 80% ethanol. It was then centrifuged to homogenate the suspension at 10,000 rpm for 30 min at room temperature. Supernatant was separated and re-extracted for 5 times with required volume of 80% ethanol, centrifuged and the supernatant was pooled. It was then evaporated near to dryness and residues were dissolved in 5 ml of distilled water. Different aliquots (0.2, 0.6 and 1.0 ml) were pipette out into test tubes and the volume in each tube was made to 3 ml with distilled water.

Subsequently, 0.5 ml of FCR was added and after three minutes, 2 ml of 20% Na_2CO_3 solution in each tube was thoroughly mixed. Then tube were placed in boiling water for 1 min and then cooled at room temperature. Then absorbance at 650 nm against blank was measured using ultra violet visible (UV-VIS) spectrophotometer and the standard curve using different concentration of catechol was prepared. From the standard curve the concentration of phenols in the test sample was determined and expressed as Milligrams phenols per gram of sample materials.

Estimation of total soluble protein

Protein extraction

Tomato leaves from different treatments were harvested, washed with distilled water several times and blotter dried before protein extraction. A quantity of 1.0 g of each sample was cut into small pieces and grinded in pestle and mortar using 1:5 (leaves:extraction buffer). The suspension was centrifuged at 10,000 rpm for 30 min at 4°C. The supernatant was collected and used for quantification and profiling of protein.

Quantification of protein

The method developed by Lowary et al. (1951) was used with slight modification for quantification of the total soluble protein content. The working standard solution was pipette out 0.2, 0.6, and 1.0 ml and put into series of test tubes. A quantity of 0.2, 0.6 and 1.0 ml of the sample extract was also pipette out and kept into other test tubes separately. Then volumes in all the tubes were made up to 1 ml with distilled water. A tube with 1 ml of distilled water served as a blank. Later on, 5 ml of solution C was added in each test tube and incubated at room temperature for 10 min. Thereafter, 0.5 ml of FCR was mixed well immediately and incubated at room temperature for 30 min in dark place. The absorbance at 660 nm against the blank was read and standard graph was drawn to calculate the amount of soluble protein in sample and represented as mg/g of fresh leaf sample.

Protein profiling

Profiling of soluble proteins was also done in various treatments. Analysis of total soluble proteins through sodium dodecyl sulphate polyacrylamide gel electrophoresis was carried out for the study of variable response of resistance to *F. oxysporum* f.sp. *lycopersici*. SDS PAGE was done to get banding pattern of soluble protein pattern. Soluble protein was electrophoresed by 12% SDS polyacrylamide gel, based on the method of Laemmli (1970).

Gel preparation

In order to prepare stacking and resolving gel, quantities of different chemicals used are shown in Table 1. All the chemicals were mixed well and poured into vertical cassette leaving behind 3 to 4 cm from upper side. Subsequently, stacking gel solution was poured over the resolving gel. A comb was inserted into the gel mould to create wells for sample loading.

Sample loading

Take 75 ml of extracted soluble protein in an eppendoff and mixed with 25 µl of sample buffer and 5 µl of tracking dye (Bromophenol blue). Before loading the sample, it was boiled for 1 min at 100°C to activate the protein molecules. Exactly 20 µl of sample was poured in each well. Then electrophoresis was carried out in Tris-glycine buffer at 30 mA current in stacking gel and 40 mA in separating gel. The electrophoresis was stopped after the tracking dye reached the bottom of the gel. The gel was then separated gently from the electrophoresis unit and placed in staining solution. After destaining, gel was illuminated with diffused fluorescent light and photographed.

Statistical analysis

All the experiments were conducted in triplicates along with equal number of appropriate controls. The data obtained was subjected to analysis of variance technique using completely randomized design (CRD) following Gomez and Gomez (1976).

Table 1. Preparation of stacking and resolving gel using different chemicals.

Chemical	Quantity	
	Stacking gel	Resolving gel
Acrylamide-bisacrylamide 30%	2.6 ml	20.0 ml
Tris-Hcl	5.0 ml (0.5 M, pH 6.8)	12.5 ml (1.5 M, pH 8.8)
10% SDS	0.20 ml	0.50 ml
TEMED	10.0 µl	25 µl
10% APS	100.00 µl	250 µl
Distilled water	12.1 ml	16.7 ml
Total	20.0 ml	50.0 ml

Table 2. Effect of biotic inducers on disease incidence of fusarium wilt.

Treatment	Average infected plants per plot	Disease incidence
T. harzianum (Kan.)	2	7.69
T. harzianum (Del.)	3	11.53
T. harzianum (Pant.)	4	11.11
T. viride (Kan.)	3	14.2 8
T. viride (Del.)	4	14.81
T. viride (Pant)	4	13.33
A. niger (Kan.)	3	10.71
C. globosum (Del.)	5	16.66
P. flouroscence (Del.)	4	13.79
Check-I	25	100.00
Check-II	0	00.00
Standard error (S.E) (diff.)	0.443	1.651
Critical difference (CD)	0.920	3.425

RESULTS AND DISCUSSION

Effect of biotic inducers on development of disease

Pre application of biotic inducers revealed that the drastic decline in wilt formation from 7.69 to 100% in tomato plant under glasshouse condition (Table 2). The susceptible variety Azad T-6 developed on an average 100% wilt incidence in case of *F. o.* f.sp. *lycopersici* treated plant where as, *T. harzianum* (Kan.) treated plant showed only 7.69%. On the other hand, *T. harzianum* (Pant.) treated plant was showing 11.11% wilt incidence which is also statistically at par with *T. harzianum* (Pant.). Thus, the protection provided by biotic inducers indicated the resistance was expressed by decrease in the disease incidence.

Several workers have also been reported that application of biotics and abiotics inducers reduces disease incidence in tomato against *Fusarium* wilt (Arzoo et al., 2012; Kumar and Biswas, 2010). He et al. (2002) found that reduced disease severity of *F. oxysporum* f.sp. *asparagi* in *Asparagus officinalis* inoculated with non pathogenic strains of *F. oxysporum.* The efficacy of different bio-agents against Fusarium *wilt* pathogen in tomato has also been reported by several workers (Sallam, 2011; Zaker and Mosallanejad, 2010; Latha et al., 2009; Seleim et al., 2011).

Biochemical changes associated with induction of resistance by bio-agents as inducers

Total soluble protein

The soluble protein contents of treated tomato leaves with inducers were studied after 10, 15 and 20 days of application. Biochemical analysis of treated leaves revealed that spore suspension of inducers significantly increased the amount of soluble protein in tomato seedlings (Table 3). The contents of soluble protein in *F. o.* f.sp. *lycopersici* treated plant (control-I) and healthy plant (control-II) were 20.49 and 23.74 mg/g of leaf at 10 days and 21.34 and 24.45 mg/g of fresh leaf at 15 days and 20.05 and 22.78 mg/g fresh of leaf at 20 days of treatment representing minimum amount of soluble protein among all the treatments. The maximum soluble protein content was found in the *T. harzianum* (Kan.) treated leaves which were 35.04, 34.34 and 33.54 mg/g of fresh leaf at 10, 15, and 20 days of sowing, respectively.

Table 3. Effect of seed treatment with biotic inducers on total soluble protein content of tomato leaves after 10, 15 and 20 days of application.

Treatment	Total soluble protein content (mg/g of fresh leaves)			Percent increase in protein contents	
	10	15	20	Over diseased plants (Check-I)	Over healthy plants (Check-II)
T. harzianum (Kan.)	35.04	34.34	33.54	67.28	47.23
T. harzianum (Del.)	28.05	29.10	27.90	39.15	22.47
T. harzianum (Pant)	33.58	33.90	32.10	60.09	40.91
T. viride (Kan.)	28.89	30.11	27.90	44.13	26.86
T. viride (Del.)	30.19	31.20	29.70	48.12	30.37
T. viride (Pant)	32.90	33.94	30.85	53.86	35.42
A. niger (Kan.)	31.55	32.72	31.20	59.10	36.96
C. globosum (Del.)	30.48	31.69	29.82	48.72	30.90
P. flouroscence (Del.)	32.74	33.30	30.15	50.37	32.35
Check-I	20.49	21.34	20.05	-	-11.98
Check-II	23.74	24.45	22.78	13.61	-
S.E (Diff.)	0.213	0.244	0.278		
C.D.	0.443	0.507	0.577		

The percent increase in protein content due to the effect of *T. harzianum* (Kan.) was 67.28 and 47.28% over Check-I and Check-II, respectively at 20 days of application. The plant treated with *T. harzianum* (Pant.) isolate registered 33.58, 33.90 and 32.10 mg/g of fresh leaves at 10, 15 and 20 days of application respectively, showing second highest among the treatments. The rest of the treatment were also found increase amount of soluble protein content over diseased (control-I) and healthy (control-II) plants. The healthy plant also show content increase amount of soluble protein over diseased plant but their effects were not as superior as to inducers treated plant.

From the Table 3, it was cleared that increasing trend of soluble protein was found from 10 to 15 days of application but it was again decrese from 15 to 20 days of application, representing the highest soluble protein content was found at 15 days of application. The increase amount of soluble protein decreases the incidence of disease which might be assumed that some proteins are associated with induction of resistance against the pathogen. Antoniew et al. (1980) considered that pathogen related proteins (PR protein) are involved in plant defense response to pathogens. Boller (1985) was also opined that proteins are associated with defense in plants against fungi and bacteria. Metraux et al. (1988) and Tuzun et al. (1989) also reported that proteins forms of chitinases and β -1, 3 glueanase may be involved in the defense of plants against fungi and bacteria by their action on the cell walls of invading pathogen.

The productions of chitinase and β-1, 3 glucanase, which are pathogenesis related proteins (PR-proteins) have been studied most extensively. Biochemical change associated with induced resistance in crop plant against pathogens by non conventional of chemical against have been reported by several workers (Adesh, 2008; Steiner and Schonbeack, 1995; Sindhan and Prashar, 1996).

Total phenol content

The total phenol contents of tomato leaves were studied after 10, 15 and 20 days of application of inducers. The results presented in the Table 4 indicated that the total phenol content was maximum in the leaves treated with *T. harzianum* (Kan.) as an inducer with the value of 2.62 mg/g of fresh leaf at 15 days of application. From the table, it is cleared that the concentration of total phenol content was increased from 2.40 to 2.62 mg/g in *T. harzianum* (Kan.) treated leaves from 10 to 15 days of treatment but it was again decrease to 2.52 mg/g of fresh leave at 20 days. The percent increased in phenolic compounds in respect to *T. harzianum* (Kan.) treated leaves over diseased plants and healthy plants were 82.60 and 46.51% at 20 days of application, respectively.

Phenols are well known antifungal, antibacterial and antiviral compounds. The phytoalexins involved in disease resistance are phenolics in chemical constitution. Phenols are involved in disease resistance in many ways like hypersensitive cell death or lignifications of cell walls or increased content of phenol itself toxic also to pathogen (Nicholson and Hammerschimdt 1992). Arzoo et al. (2012) reported that biochemical evidence of defence response in tomato against *Fusarium* wilt induced by plant extract. Girdhari et al. (2008) also reported that increased total phenol content was found in rice leaves after treatment with biotic inducers. Kumar and Biswas (2010) reported that increased total phenol was found in tomato leaves after treatment with inorganic chemicals. Mishra et al. (2011) reported that increased

Table 4. Effect of seed treatment with different isolates of bio-agents on total phenol content of tomato leaves after 10, 15 and 20 days of application.

Treatment	Total phenol content (mg/g of fresh leaves)			Percent increase in phenol contents	
	10	15	20	Over diseased plants (Check-I)	Over healthy plants (Check-II)
T. harzianum (Kan.)	2.40	2.62	2.52	82.60	46.51
T. harzianum (Del.)	2.10	2.34	2.32	68.11	34.88
T. harzianum (Pant)	2.28	2.56	2.30	66.66	33.72
T. viride (Kan.)	2.15	2.27	2.17	57.24	26.16
T. viride (Del.)	2.06	2.24	2.29	65.94	33.13
T. viride (Pant)	2.21	2.40	2.35	68.11	36.62
A. niger (Kan.)	2.27	2.35	2.21	60.14	28.42
C. globosum (Del.)	2.08	2.27	2.06	49.27	19.76
P. flouroscence (Del.)	2.19	2.31	2.25	63.04	30.81
Check-I	1.42	1.51	1.38	-	-19.76
Check-II	1.89	1.92	1.72	24.63	-
S.E. (diff.)	0.098	0.133	0.156		
C.D. ()	0.203	0.275	0.324		

Table 5. Protein profiling by SDS-PAGE as an effect of biotic inducers.

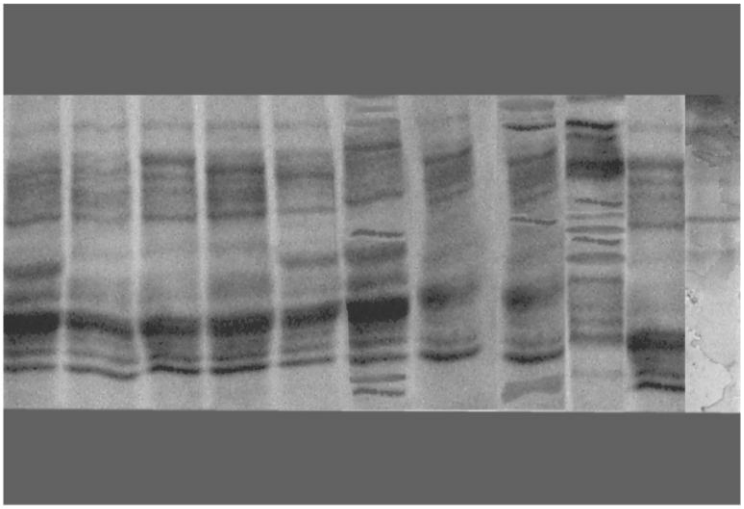
S/N	Bio-agents	Number of bands
1	*T. harzianum* (Kan.)	22
2	*T. harzianum* (Del.)	18
3	*T. harzianum* (Pant.)	16
4	*T. viride* (Kan.)	19
5	*T. viride* (Del.)	20
6	*T. viride* (Pant)	21
7	*A. niger* (Kan.)	18
8	*C. globosum* (Del.)	14
9	*P. flouroscence* (Del.)	15
10	Check-I	04
11	Check-II	09
S.E. (diff.)		0.921
C.D		1.910

total phenol was found in varieties of wheat showing resistance spot of blotch. Therefore, increased content of phenol might be involved in the expression of disease resistance in plant.

Protein profiling

Protein profiling was done to determine whether some new protein was associated with resistance to *F. o.* f.sp. *lycopersici* in tomato variety Azad T-6 or not. SDS-PAGE is used for finding the banding pattern of proteins. It has been found that the banding patterns of protein of different treatments are variable (Table 5 and Figure 1). The number of protein bands presents in each treatments range from 04 to 22. The highest number of bands was found in *T. harzianum* (Kan.) treated plant and minimum number of bands are in *F. o.* f.sp. *lycopersici* treated plant. The banding pattern of proteins from figure represented that some new proteins of higher molecular weight was found in *T. harzianum* (Kan.) treated plant which was not found in rest of any other varieties. Similarly, some new bands were also found in *T. harzianum* (Del.) (18) bands, *T. harzianum* (Pant.) (21bands), *T. viride* (Kan.) (19 bands), *T. viride* (Del.) (16 bands), *T. viride* (Pant) (20 bands), *A. niger* (Kan.), (18 bands), *Chaetomium globosum* (Del.) (14 bands) and *P. flouroscence* (Del.) (15 bands) treated plant.

The presence or absence of protein bands might be the activities of inducers in plant which may also be key responsible factors for defense mechanism of tomato



L to R- . T. harzianum (Kan.), T. harzianum (Del.), *T. harzianum* (Pant.), *T. viride* (Kan.), *T. viride* (Del.), *T. viride* (Pant.), *A. niger* (Kan.), *C. globosum* (Del.), *P. fluoresens* (Kan.), Check-II and Check-I.

Figure 1. Banding patterns of soluble protein by SDS-Page.

Table 6. Correlation of disease incidence with soluble protein content and total phenol content.

Variable	Days	Correlation coefficient (r) with disease incidence	Regression equation
Soluble protein	10	-0.6375	Y= 125.84-3.34x
	15	-0.6059	Y= 115.92-2.92x
	20	-0.6364	Y= 119.09-3.20x
Total phenol	10	-0.7969	Y= 150.87-56.96x
	15	-0.7649	Y= 141.75-49.71x
	20	-0.7653	Y= 140.96-51.02x

against *F. o.* f.sp. *lycopersici*. Biswas et al. (2003) also reported that some new proteins were associated with resistance to *Bipolaris sorokiniana* induced by crude extracts of *Chaetomium globosum*. They also found that some new proteins of different molecular weight that is, 110, 105, 38, 35 and 32 kDa were resolved by SDS PAGE analysis which was missing in unchallenged healthy and diseased seedlings and in some other treatments. The possible role of the new proteins for induction of resistance was speculated. Antoniew et al. (1980) considered that PR-proteins are involved with defense in plants to pathogens.

Induction of systemic resistance in tobacco after inoculation with *Pseudomonas tabaci* was followed by an increase in concentration of PR- proteins was reported by Tuzun et al. (1989). A 23 kDa protein was detected in leaves of tobacco which was previously immunized with TMV (Spiegel et al. 1989). Mishra et al. (2011) also found that the different types of banding pattern in wheat varieties represent variable types of resistance response to spot blotch. Arzoo et al. (2012) reported that increase in number of protein bands in various treatments compared to control-I and Control-II and also reported the increased number of bands indicates that some new types of proteins are synthesized at the time of induction resistance.

Correlation coefficient of disease incidence with soluble protein and total phenol

The correlation regression equation revealed that negative correlation (r), -0.6375 (10 days), -0.6059 (15 days) and -0.6364 (20 days) between disease incidence and soluble protein content. Similarly, disease incidence was decreased with increased level of total phenol. There was also negative correlation showing (r), -0.7969 (10 days), -0.7649 (15 days) and -0.7653 (20 days) between disease severity and total phenol content at different days of treatment. The corresponding simple regression equations showed that the increase level of soluble protein and total phenol content has negative role in disease development (Table 6). Similar observation were also found in rice against brown leaf spot (Kumawat et

al., 2010) in tomato against *Fusarium* wilt (Kumar and Biswas, 2010; Arzoo et al., 2012) in wheat against spot blotch (Mishra et al., 2011).

Conclusion

Pre treatment with bioagents as inducers provided induced resistance in plant against *F. o.* f.sp. *lycopersici* resulting declined disease incidence. Prior application of inducers to challenge inoculation sensitized the seedling to produce increased level of soluble proteins and total phenol content. Both factors (Phenol and protein) also showed negative co-relation with disease incidence. Protein profiling by SDS-PAGE revealed that some new protein are synthesized due to application of inducers.
The presence or absence of the bands in protein profiling might be responsible for resistance response against *F. oxysporum* f.sp. *lycopersici* in tomato.

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