Effects of *Pseudomonas fluorescens* CHA0 on the Resistance of Wheat Seedling Roots to the Take-all Fungus *Gaeumannomyces graminis* var. *tritici*

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**Abstract**: The aim of this study was to verify that wheat seedlings treated with *Pseudomonas fluorescens* CHA0 (CHA0 hereafter) before inoculation with *Gaeumannomyces graminis* var. *tritici* (Ggt), a pathogen of take-all, acquire induced resistance to Ggt. The soil with wheat seedlings growing on it was drenched with a suspension of CHA0 cells and inoculated with Ggt 24 h later. Then, the seedlings were grown in a glasshouse, and severity of take-all disease, fresh weights of root and shoot and lengths of root and shoot, and also the activities of soluble peroxidase (SPOX), ionically cell-wall-bound peroxidase (CWPOX), β-1,3-glucanase, β-1,4-glucanase and the concentration of total phenolic compounds in the root of the seedlings were examined. The results indicated that the treatment with CHA0 before inoculation with Ggt mitigated the disease severity significantly, and increased the root and shoot lengths and root and shoot fresh weights. The treatment with CHA0 increased the activities of SPOX, CWPOX, β-1,3-glucanase, β-1,4-glucanase and phenolic compounds in the wheat roots and the activities of SPOX and β-1,4-glucanase activities were highest at day 4 and those of CWPOX and β-1,3-glucanase at day 6 after inoculation with Ggt. The concentration of total phenolic compounds was also highest at day 6 after the inoculation with Ggt. The results suggest that the take-all suppressing effect of CHA0 may be related to enhanced defense response of the wheat roots.

**Key words**: Biocontrol mechanisms, Induced resistance, Plant growth-promoting rhizobacteria (PGPR), *Pseudomonas fluorescens* CHA0, Take-all, Wheat.

Take-all is a common crown and root rot disease of wheat. It is caused by the fungal pathogen, *Gaeumannomyces graminis* (Sacc.) Von Arx and Oliver var. *tritici* Walker (Ggt), which is widespread in soil and is closely associated with the roots of native grasses. Because of limited means of control, take-all is considered to be one of the most serious diseases of wheat. Currently, no economically viable control measures are available, and often chemical management is expensive and ineffective. In this context, management with biocontrol agents is increasingly capturing the attention of researchers as an alternative strategy, which is also ecologically sound and environmentally safe.

The root colonizing bacterium, *Pseudomonas fluorescens* strain CHA0 (referred to as CHA0 hereafter), which was isolated from a disease suppressive soil, has been studied in detail as a model strain for the biological control of several plant diseases, such as black root rot of tobacco and take-all of wheat (Voisard et al., 1994; Haas and Keel, 2003). In this strain, as well as in other biocontrol pseudomonads, antifungal secondary metabolites, e.g., 2,4-diacetylphloroglucinol, hydrogen cyanide and pyoluteorin are important for biocontrol activity (Defago et al., 1990).

Researchers over the past two decades have demonstrated that plant possess latent defense mechanism against pathogen, which can be activated by exposure of plants to stress or infection with pathogens. This phenomenon, called “induced resistance”, operates through the activation of defense genes and the accumulation of defense compounds (Van Loon et al., 1998; Buell, 1999). Pretreatment of susceptible plants with plant growth-promoting rhizobacteria (PGPR) can enhance resistance to subsequent attacks, not only at the site of treatment, but also in tissues distant from the initial infection site. Many plant enzymes are involved in defense reactions against plant pathogens. These include oxidase enzymes such as soluble peroxidase (SPOX) and ionically cell-wall-bound peroxidase (CWPOX), which catalyze the formation of lignin and other oxidative phenols that contributed to the formation of defense barriers for reinforcing the cell structure.
Effects of the strain on the resistance against take-all of pathogens, but there have been few studies on the CHA0 on resistance of different plants against various pathogens. The amount of total phenolic compounds in wheat has been correlated with host resistance to a variety of diseases such as take-all (Rengel et al., 1994).

For the induction of resistance, CHA0 appears to be promising and there are a number of reports on the potential of this strain as a biotic inducer of host resistance against fungal, bacterial, viral and nematicidal pathogens (Maurhofer et al., 1994; Hammerschmidt et al., 2001; Iavicoli et al., 2003; Siddiqui and Shaukat, 2003). Previous studies have examined the effects of CHA0 on resistance of different plants against various pathogens, but there have been few studies on the effects of this strain on the resistance against take-all of wheat.

In this study, we examined whether or not the induced resistance mechanism is responsible for the capacity of CHA0 to control take-all disease of wheat by monitoring SPOX, CWPOX, β-1,3-glucanase, β-1,4-glucanase activities and phenolic contents of the roots, which are markers of induced resistance.

Materials and Methods

1. Source of Ggt, CHA0 and growth condition of wheat

*Gaeumannomyces graminis* var. *tritici* (Ggt) isolated from a wheat field in Iran was provided by M. Ghalandar (Agricultural Research Center, Markazi Province, Iran). *Pseudomonas fluorescens* CHA0 obtained from G. Defago, Swiss Federal Institute of Technology, Zurich, Switzerland. In our previous studies, it decreased the severity of take-all in the glasshouse and field-microplot conditions (Sari et al., 2006). The bacterial and fungal isolate was maintained in sterilized distilled water (SDW) and potato dextrose agar (PDA) at 4°C, respectively. The spring wheat (*Triticum aestivum* cv. Shiraz) seeds were surface disinfected in sodium hypochlorite (0.5%) for 4 min and germinated in 9 cm diameter sterile plate containing filter paper, each moistened with 5 mL SDW. After 24 hr, the germinated seeds were sown into individual 10 cm diameter pots containing a sterilized mixture of field soil, leaf compost, sand and organic manure at rate of 1:2:2:2. The seedlings were grown in a glasshouse under natural day light (approximately 12 hr photoperiod) at 20/15°C (day/night) and roots were kept at 25 ±1°C from January to February 2006 in Tehran, Iran (Ryder et al., 1999). To adjust root temperature at 25 ±1°C, we kept the pots in a water bath that was continuously cooled and stirred and had a thermoregulated heating element. Temperature was monitored continuously and maintained within ±1°C. These pots with drainage, each containing 10 seedlings, were placed in the other pots without drainage holes to prevent water uptake from the water bath but to still allow drainage the root media (Borges and Chaney, 1989). Pots were watered as needed.

2. Treatment with CHA0, inoculation with Ggt and disease assessment

CHA0 was cultured in potato dextrose broth on a rotary shaker (150 r min⁻¹) for 48 hr at 25°C. The cells were pelleted by centrifuging at 2,500 g for 10 min, suspended in SDW, repelletized and resuspended in SDW. The population of the bacterial cells was adjusted to 10⁸ colony forming units (CFU) per mL. The soil with wheat seedlings growing on it was drenched with 30 mL of CHA0 cell suspension (Milus and Rotherock, 1997; Radjacommare et al., 2004), which is called CHA0 treatment hereafter. Control plants received SDW instead of the suspension (shown by Co).

Ggt was cultured in 1/5 PDA (diluted-PDA), and the soil with wheat seedlings growing on it was inoculated with ten 5-mm diameter disks of diluted PDA taken from the edge of vigorously growing colonies of Ggt, 24 h after the treatment with CHA0 (CHA0+Ggt treatment) or without pretreatment with CHA0 (Ggt treatment). Before inoculation with Ggt, surface soil of

| Treatments | Disease severity Score | Root fresh weight (g plant⁻¹) | Root length (cm) | Shoot fresh weight (g plant⁻¹) | Shoot length (cm) |
|------------|------------------------|-------------------------------|-----------------|-------------------------------|-----------------|
| Co         | 0                      | 0.7                           | 12.8            | 1.8                           | 19.3            |
| CHA0       | 0                      | 0.9                           | 14.0            | 2.1                           | 21.1            |
| Ggt        | 4.5                    | 0.4                           | 10.5            | 1.2                           | 16.0            |
| CHA0+Ggt   | 2.5                    | 0.6                           | 11.3            | 1.5                           | 17.8            |
| p-value    | 0.01                   | 0.0004                        | 0.0009          | <0.0001                       | 0.0004          |
| LSD (p=0.01) | 0.9                | 0.1                           | 0.1             | 0.2                           | 1.5             |

Values are average of four replicates and each replicate is average of 10 measurements of independent seedlings.
the pot was removed to facilitate Ggt penetration into the seminal roots. The agar disks were covered with the thin layer of sterilized soil (Rengel et al., 1994; Aberra et al., 1998).

The experiment was arranged as a completely randomized design with four treatments (Table 1) and four replicates. Plants were harvested five weeks after the treatment with CHA0, and the severity of disease on roots was assessed using the scale modified from Rothrock (1986) where the score was 0, 1, 2, 3, 4 and 5 when there were no symptoms, lesions on <25% of the root, lesions on 25% to <50% of the root, lesions on 50% to <75% of the root, lesions on 75% to 100% of the root, and lesions on 100% root and on crown, respectively. Root and shoot fresh weights and root and shoot lengths were also determined. The root length of each replicate was taken as the average of 10 measurements of the longest seminal-root of independent seedlings which were grown in the same pot.

3. Enzymatic activities

Activities of soluble peroxidase (SPOX), ionically cell-wall-bound peroxidase (CWPOX), β-1,3-glucanase and β-1,4-glucanase were determined. The root systems of wheat seedlings treated with CHA0-Ggt as described above were used for SPOX and glucanase extraction. Sodium phosphate buffer (0.05 M, pH 6) and sodium acetate buffer (0.05 M, pH 5) were used to extract SPOX and glucanase, respectively. Each treatment consisted of four replicates, and each replicate contained 10 seedlings. The roots of 10 seedlings grown in the same pot were sampled 1, 2, 3, 4, 6, 8 and 11 days after inoculation with Ggt. To isolate SPOX, we ground 0.5 g of the sampled roots at 4ºC with an appropriate amount of the buffer solution and centrifuged the homogenate at 14,000 g for 20 min at 4ºC. The supernatant was transferred to a 1.5 mL vial and stored at –20ºC until subsequent assay (Madhaiyan et al., 2004). To isolate CWPOX, we washed the pellet with distilled water at 4ºC until no SPOX activity was detected in the supernatant, and then washed the pellet with 1.5 mL of 1 M NaCl. At each step of extraction, the homogenate was centrifuged at 14,000 g for 15 min at 4ºC. The supernatants of each fraction were considered to be the ionically cell-wall-bound protein (Reuveni, 1998). The supernatants were transferred to a 1.5 mL vial and stored at –20ºC until subsequent assay.

The specific SPOX and CWPOX activities were measured according to Janda et al. (2003) using guaiacol (Merck, Darmstadt, Germany) as a substrate. Enzyme activities were expressed as changes in absorbance of the reaction mixtures at 470 nm per minute per milligram of total protein.

β-1,3-glucanase activity was determined by the laminarin-dinitrosalicylate method described by Abeles and Forrence (1970) with the following modification. The reaction mixture consisted of 30 μL 4% laminarin (Fluka, Buchs, Switzerland) which was dissolved in sodium acetate buffer (0.05 M, pH 5) and 30 μL plant extract, was incubated at 40ºC. After 30 min, the reaction was stopped by adding 187 μL of dinitrosalicylic acid reagent (DNS, prepared by adding 300 mL of 4.5% NaOH to 880 mL of a solution containing 8.8 g dinitrosalicylic acid (Merck, Darmstadt, Germany) and 22.5 g potassium sodium tartrate) with subsequent heating for 5 min. The reaction mixture was diluted with 2 mL of distilled water and vortexed. The absorbance of resultant colour was recorded at 500 nm. A standard curve of absorbance at 500 nm to glucose concentration was used to calculate the specific enzyme activity, which was expressed as milligram glucose equivalent released per minute per milligram total protein (Anguelova-Merhar et al., 2000).

β-1,4-glucanase activity was assayed using carboxymethylcellulose (CMC) as a substrate. The reaction mixture (200 μL) containing 100 μL crude extract and 100 μL 0.1% CMC stock solution (Sigma, St. Louis, Mo, USA) which was dissolved in sodium acetate buffer (0.05 M, pH 5), was incubated at 50ºC for 30 min. Then, the reaction was stopped by adding 600 μL of DNS reagent with subsequent heating for 5 min. The reaction mixture was diluted with 2 mL of distilled water and vortexed. The absorbance of resultant colour was recorded at 500 nm. A standard curve of absorbance at 500 nm to glucose concentration was used to calculate the specific enzyme activity which was expressed as milligram glucose equivalent released per minute per milligram total protein (Yedidia et al., 2000).

The protein content in crude extract was determined according to Bradford (1976) with bovine serum albumin as a standard.

4. SPOX assay by native-PAGE

SPOX isozymes in the root were assayed in the manner of Seevers et al. (1971) with the following modification. Native-polyacrylamide gel electrophoresis (Native-PAGE) with a 12% running and 6% stacking gel at 4ºC was carried out at 75 V and 100 V in stacking and running gel, respectively. The crude extract of the roots from the above-mentioned treatments which was sampled at 1, 4 and 11 days after inoculation with Ggt, was used for the assay. Equal amount of total protein (20 μg) from each treatment (CHA0, Ggt, CHA0±Ggt and healthy control (Co) was added to each gel lane. After the dye front reached the end of running gel, the gel was treated with 25 mM citrate-phosphate buffer (pH 5.4) containing 5 mM guaiacol for 30 min. Then, 2 mL of 30% H₂O₂ was added dropwise with constant shaking until bands representing SPOX activity appeared (Madhaiyan et
al., 2004; He and Wolyn, 2005). Immediately after resolution of bands, the rate of front (Rf) value of each isozyme was determined and the gels were photographed.

5. Assay of total phenolic compounds
Wheat roots (0.5 g) from each replicate were homogenized in 8 mL of 80% acidified methanol. The homogenate was centrifuged at 4,000 g for 5 min at room temperature and then 1 mL of the methanolic supernatant was added to 5 mL of distilled water and 250 μL of Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), and the solution was incubated at room temperature. After 5 min, 1 mL of a saturated solution of Na₂CO₃ and 1 mL of distilled water were added and the reaction mixture was incubated for 1 hr. The absorbance of the developed blue colour was measured using Royal Milton Company Spectrophotometer (Unterfoehring, Germany) at 725 nm using a blank, water and reagent only. Caffeic acid (Fluka, Buchs, Switzerland) was used as reference phenolic compounds. The total phenolic compounds of samples were expressed as milligram caffeic acid per gram of root fresh weight (Swain and Hillis, 1959; Malick and Singh, 1980).

6. Statistical analysis
ANOVA was conducted using General Linear Model (GLM) procedure of SAS version 9.0 (Statistical Analysis System Institute Inc., Cary, NC, USA). Enzymatic assays on the whole were carried out twice for each sample and the mean of two assays was used for statistical analysis as value of each replicate. In enzymatic and phenolic compound assays, the experiments were arranged as a 4 × 7 factorial with four treatments (CHA0, Ggt, CHA0 + Ggt challenge and Co) and seven sampling times in a completely randomized design with four replicates (Little and Hills, 1978).

Results
1. Biological control of take-all by CHA0 treatment
Table 1 shows the disease severity and growth of wheat seedlings treated with CHA0, Ggt and CHA0 + Ggt, and those in the control (Co), examined five weeks after inoculation with Ggt. The disease severity at 5 weeks after inoculation with Ggt that was 4.5 in Ggt treatment, was greatly reduced to 2.5 in CHA0 + Ggt treatment. The seedlings in CHA0 treatment and Co were healthy (severity = 0). The treatment with CHA0 promoted root and shoot growth compared with the control, though Ggt treatment inhibited the growth (Table 1). CHA0 + Ggt treatment also promoted the root and shoot growth compared with Ggt treatment.

Fig. 1. Time course of change in the enzymatic activities in the wheat roots treated with CHA0, Ggt and CHA0 + Ggt and in the control (Co). Enzyme activities were determined as described in the text. Data are average of four replicates. Error bars indicate ± SE. (a) soluble peroxidase (SPOX). (b) ionically cell-wall-bound peroxidase (CWPOX). (c) β-1,3-glucanase. (d) β-1,4-glucanase.
2. Enzyme activities

(1) Soluble peroxidase (SPOX)

SPOX activity in the roots of wheat seedlings was very low at day 1 after Ggt inoculation in all treatments, but increased day by day reaching the maximum at day 4, followed by gradual decrease thereafter. The activity in CHA0 + Ggt treatment was higher than that in other treatments, and the difference was very large at day 4–11. SPOX activity in CHA0 treatment was slightly lower than that in Ggt treatment during the first four days after Ggt inoculation, but was higher at day 6–11. The activity in the control was always lower than that in other treatments except for at day 1.

(2) Itonically cell-wall-bound peroxidase (CWPOX)

CWPOX activity remained at low level during the first three days after Ggt inoculation in all treatments and control, but it increased thereafter reaching the maximum at day 6 followed by gradual decrease (Fig. 1b). The activity at day 4–11 was higher in CHA0 + Ggt treatment than in other treatments, and the activity at day 6 in CHA0 treatment was greatly higher than in other treatments. The activity in Ggt treatment was higher than that in CHA0 treatment during day 4–11, and that in the control was the lower than in the other treatments during this period except for day 8 (Fig. 1b).

(3) β-1,3-glucanase

The activity of β-1,3-glucanase was low at day 1 after Ggt inoculation in all treatments, and increased gradually thereafter reaching the maximum at day 6 followed by gradual decrease. The activity in CHA0 + Ggt treatment was higher than that in other treatments throughout the experimental period, and the difference between the activity in CHA0 + Ggt and other treatments was very large at day 6–11. The activity in CHA0 treatment was slightly lower than that in Ggt treatment at day 3 and 4, but slightly higher thereafter (Fig. 1c). The activity in the control was lower than in other treatments and was nearly a half of that in CHA0 + Ggt treatment at day 6.

(4) β-1,4-glucanase

The activity of β-1,4-glucanase was low at day 1 in all treatments, and that in CHA0 + Ggt treatment increased gradually at day 2 to 3, and rapidly at day 3 to 4, which declined gradually thereafter. On the contrary, the activity in other treatments increased slightly at day 2–4 and decreased to the initial level at day 8–11. The promoting effect of CHA0 in the presence of Ggt (compare CHA0 + Ggt treatment with Ggt treatment) was prominent (Fig. 1d), but the effect of CHA0 in the absence of Ggt was slight (compare CHA0 treatment with Co).

3. Isozymes of SPOX

Native PAGE indicated that SPOX from wheat roots contained four isozymes, P1, P2, P3 and P4 with Rf 0.04, 0.13, 0.27 and 0.60, respectively, as shown in

![Fig. 2. Native-PAGE assay of soluble peroxidase isozymes (SPOX) at day 1, 4 and 11 after inoculation with Ggt in the wheat roots treated with CHA0, Ggt, CHA0 + Ggt and in the control (Co). Rf, distance of isozyme migration divided by distance of migration of the dye front.](image)

Fig. 2. As a whole, the intensity of each isozyme in each treatment at each day corresponded to those of SPOX shown in Fig. 1a. However, the intensity of P2 was the highest among the isozymes in all treatment, showing the SPOX activity shown in Fig. 1 was mainly depended on the activity of P2. This shows that activity of P2 was greatly increased by the treatment with CHA0 + Ggt at day 4–11, although it was lower than those in Co and Ggt at day 1. Ggt treatment increased the amount of P1, P3 and P4 compared with Co at day 4 after inoculation with Ggt. However, the intensities of these isozymes decreased at day 11. CHA0 treatment increased P1, P3 and P4 levels in either absence or presence of Ggt at day 4–11; compare the intensities of these isozymes in CHA0 treatment with that in Co, and those in CHA0 + Ggt treatment with those in Ggt treatment. The intensity of P2 in Co was very high and was decreased by CHA0 treatment at day 1 and 4, although it was low and increased by CHA0 treatment at day 11.

4. Total phenolic compounds

The content of total phenolic compounds in the root increased from day 2 reaching the maximum at day 6, and gradually decreased thereafter in all treatments. The phenol content of root was greatly higher in CHA0 + Ggt treatment than in other treatments, and was the lowest in Co at day 4–11. At day 11, the content in CHA0 + Ggt treatment was about twofold of that in Co. The phenol content of roots in Ggt treatment was always slightly higher than that in CHA0 treatment.

Discussion

The activity of soil-borne plant pathogen is biocontrolled by multiple mechanisms, such as antibiosis, activities of hydrolytic enzymes and volatile metabolite, and by induced resistance. In this study, we examined the ability of _P. fluorescens_ CHA0 (CHA0) to induce the resistance of wheat against take-all disease in glasshouse. We have previously reported that soil
drench with CHA0 cell suspension significantly decreased take-all severity in the wheat roots in both glasshouse and field-microplot conditions. Keel et al. (1992) also showed that CHA0 reduced the severity of wheat take-all under glasshouse condition. However, CHA0 dramatically suppressed the growth of Ggt in dual culture test (Sari et al., 2006). According to Van Loon et al. (1998) the best evidence for rhizobacterium-mediated induced resistance is obtained when the rhizobacterium has only slight effect on the pathogen in vitro but shows appropriate biocontrol activity in glasshouse and field conditions. Furthermore, our previous study indicated that volatiles from CHA0 could strongly inhibit mycelial growth of Ggt (Sari et al., 2006). Whether this is due to the same volatile that Ryu et al. (2003) showed stimulate plant growth, is not known at present.

CHA0 suppresses a variety of soil-born plant disease such as black root rot of tobacco (Defago et al., 1990) and Rhizoctonia damping-off of tomato (Siddiqui and Shaukat, 2002). The present experiment showed that the root and shoot fresh weights and the root and shoot lengths were increased by CHA0 treatment. According to Glick (1995), plant growth promotion by PGPR includes the bacterial synthesis of plant growth regulators, indole-3-acetic acid (IAA), cytokinin, and gibberellin and the breakdown of plant-produced ethylene by 1-aminoacyclopropane-1-carboxylate deaminase produced by bacteria. These possibilities may be involved in our results.

We found that treatment of the roots with CHA0 activated SPOX activity in the wheat roots. Similarly, peroxidase activity was increased by the treatment with Pseudomonas corrugata in cucumber roots infected with Pythium aphanidermatum (Chen et al., 2000) and by the treatment with Bacillus pumilus 7Km in wheat root inoculated with Ggt (Sari et al., 2007). Although root colonization by Ggt induced SPOX activity, the SPOX activity decreased from day 4 to 10 after inoculation with Ggt. These findings suggest that wheat is infected with Ggt but acquired induced resistance to Ggt itself with time. This is in contrast to the suggestion of previous study that salicylic acid induces the resistance against Ggt in wheat (Seah et al., 1996). The take-all disease severity was decreased by the presence of CHA0 (CHA0 + Ggt treatment), but SPOX activity induced by Ggt treatment was increased by the presence of CHA0. These results imply the induced resistance to Ggt is intensified by the treatment with CHA0.

CWPOX in the seedling roots was also increased by Ggt treatment but more greatly by the presence of CHA0 (CHA0 + Ggt treatment). De Pinto and De Gara (2004) noted that peroxidase accumulation in cell wall could play a critical role in regulating the wall stiffening process during cell differentiation and increase plant resistance to fungal penetration.

The activities of β-1,3-glucanase and β-1,4-glucanase were the highest in CHA0 + Ggt treatment. This agrees with the findings of Maurhofer et al. (1994) who worked with tobacco necrosis virus and CHA0, and Radjacom mare et al. (2004), who worked with ragi blast pathogen and P. fluorescens Pf1. Our results indicated that β-1,3-glucanase and β-1,4-glucanase activities increased gradually in the healthy control plants (Co). The increase of these activities in the uninfected wheat roots may be attributed to the constitutive expression of these enzymes in the roots, which appears to be typical for Shiraz wheat cultivar. These results agree with the finding of Anguelova-Merhar et al. (2001) that β-1,3-glucanase was constitutively expressed in resistance cultivar, whereas in susceptible cultivars substantial β-1,3-glucanase levels were induced strongly by infection. Thus we feel that the constitutive expression of β-1,3-glucanase and β-1,4-glucanase is an important factor contributing to a fast defense response. Pathogenesis-related (PR) proteins such as β-1,3-glucanase and β-1,4-glucanase are host-coded proteins with direct action against the major fungal cell wall compounds, diverse glucans. Also another function of these enzymes might be the release of elicitor-active fragments from the fungal cell wall to intensify plant defense reactions. The present study also showed that β-1,3-glucanase and β-1,4-glucanase may be involved in the enhanced resistance against take-all in the wheat roots treated with CHA0.

The isozyme patterns of soluble peroxidase in the wheat roots confirmed the results of colorometric determination of the enzyme activity. All SPOX isozymes (P1–P4) were greatly increased by CHA0 + Ggt treatment. Also the intensities of P1, P3 and P4 isozymes were high in the Ggt treatment compared with healthy control roots (Co) at day 4 after inoculation with Ggt. However, the intensities of

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**Fig. 3.** Time course of change in the amount of phenolic compounds in the wheat roots treated with CHA0, Ggt and CHA0 + Ggt and in the control (Co). Phenol contents of the roots were expressed as milligram caffeic acid per gram root fresh weight (RFW). Data are average of four replicates. Error bars indicate ± SE.

- O sterile water (control).
- □ CHA0.
- ■ Ggt.
- ■ CHA0 + Ggt.
these isozymes were low in the extract from the roots in Ggt treatment at day 11. These results imply time difference in the elevation of SPOX activity and the onset of resistance mechanisms between the wheat roots treated and not treated with CHA0, and confirm the colorometric results. Gogoi et al. (2001) showed that peroxidase isozyme patterns were different between the wheat cultivars showing compatible and incompatible interaction with karnal bunt pathogen. Our results provide another example in cereals correlating peroxidase isozymes with resistance to Ggt.

In our study, phenolic compound accumulation in wheat root was greater in CHA0+Ggt treatment than in the Ggt treatment. Phenolic compounds are naturally antifungal compounds and their accumulation in the plants reduces pathogen attacks (Mpiga et al., 1997). In addition to direct effects of phenolic compounds on fungal pathogens, they are oxidized to form more toxic, quinones, by peroxidases (Gogoi et al., 2001).

Plants inoculated with the pathogen (Ggt), in the presence of the antagonist, CHA0, showed significantly higher levels of SPOX, CWPOX, β-1,3-glucanase, β-1,4-glucanase activities and phenolic compounds in their roots than those from the other treatments. This finding meets the information already available (Sticher et al., 1997), which indicates that tomato plants exposed to the antagonist are in the induced state. The results also showed that in CHA0, treatment, especially from 6 to 11 days after inoculation with Ggt, the roots accumulated a smaller amount of enzymes than in the CHA0+ Ggt. According to Van Loon et al., (1998) resistance is commonly induced only when plants are inoculated with a dosage of bacteria that exceeds a threshold population size. On the other hand, the previous works indicated that one possible explanation for the noted stimulation of certain bacterial species by some fungi may be that those bacteria are activated by species-specific fungal exudates (Andrade et al., 1997). According to these assertions, it is assumed that the presence of the active fungal pathogen is necessary to preserve the bacterial population size at high levels and therefore to preserve the induced state of plant resistance for a long period.

The induced resistance obtained can be local and not necessarily systemic. One way of constructing an experiment to draw that the induced resistance is systemic is that of a split root experiment. If the roots of all experimental plants are split and an antagonist was applied to one split and a plant pathogen to the other, direct interaction between the antagonist and pathogen is physically impossible. If then the antagonist + pathogen treated plants have less disease symptoms than the pathogen-treated plants, this conclusion can be drawn (Attitalla et al., 2001). The results of our split root study showed that when CHA0 was applied to one split and the pathogen to the other, take-all severity decreased approximately 25% (20% lower than when Ggt and CHA0 were applied to the same pot) compared with that treated with the pathogen alone (data not show). Perhaps in addition to direct effects of CHA0 on wheat resistance mechanisms, interaction of the Ggt with the CHA0 outside the plant resulted in a reduction in the rate of entry of Ggt cells into the plant. This reduction is enough for the plants to have time to develop a sufficient physiological response to early contact with the pathogen at the root surface. Due to the lower rate of pathogen-cell entry, there was enough time for the plant to develop resistance mechanisms to tackle the pathogen (Attitalla, 2004). This possibility was also supported by the results of enzymatic activities where CHA0 treatment alone was insufficient to induce the enzymatic activities at significantly higher levels than in the healthy control.

This study added to the attractiveness of using P. fluorescens CHA0 as a biological control agent for wheat take-all. The induced resistance may be established in addition to the competition for space and nutrients, antibiosis and plant growth promotion. This strategy in controlling take-all disease may be more attractive in an integrated approach to the control of take-all. In the future, further fundamental and multi-component researches on the interaction among P. fluorescens CHA0, the pathogen and the host are required.

Acknowledgements

We are grateful to Prof. Dr. G. Defago for the provision of Pseudomonas fluorescens CHA0. We also thank M. Ghalandar for providing Gaeumannomyces graminis var. tritici. Special thanks are due to Mrs. S. Hill for her assistance in the correction of this manuscript. This work was financed from the Department of Plant Protection, Abourayhan Campus, University of Tehran.

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