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Induced systemic resistance: an innovative control method to manage branched broomrape (*Orobanche ramosa* L.) in tomato

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Abstract

The biochemical responses of tomato (*Lycopersicon esculentum* Mill.) roots to different concentrations of the hormonal elicitors, salicylic acid (SA) and indole acetic acid (IAA), as presoaking treatments, on the healthy and *Orobanche ramosa*-infected plants were studied in pot experiment under control conditions. Application of SA and IAA activate the production of proline and soluble proteins in tomato roots, where their levels were more pronounced in the infected than the healthy plants. Moreover, both elicitors increased the levels of phenolics and flavonoids accompanied by stimulations in the activities of phenylalanine ammonia lyase (PAL), peroxidase (POX) and polyphenol oxidase (PPO) in tomato roots, where the magnitude of induction were more obvious in infected plants than healthy one. Also, microscopic examination revealed the anatomical differences observed in both untreated healthy and infected plants as well as in plants treated with SA and IAA. The present results indicate the effectiveness of SA and IAA in enhancing resistance of tomato to retard the incidence of infestation by *Orobanche.* These biochemical and histological changes involved in plant defense mechanisms may be one of the basic ways participate in the action of SA and IAA for increasing resistance in tomato against *Orobanche ramosa*.

Keywords: *Orobanche ramosa*; *tomato, Induced resistance, Phenolics, PR-proteins*.
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Introduction

Branched broomrapes (*Orobanche* spp.) are root parasitic plants that depend entirely on their host for water and nutrient requirements and cause severe yield losses on important crops including tomato (Cagan and Toth 2003). *Orobanche* parasite affects primary metabolism, particularly carbohydrates and nitrogen metabolism by repression of photosynthetic process and induction of various pathogenesis-related (PR) proteins (Van Loon 1997; Castillejo et al. 2004; Mauromicale et al. 2008). The rapid accumulation of PR-proteins is a complex defense mechanism of plants in response to pathogenic infection (Jung et al. 1993). These PR-proteins defined as proteins encoded by the host plant genome and expressed both locally and systemically, where their expression is correlated with the development of SAR against further infections by pathogens (Van Loon et al. 2006).

Several studies revealed that parasite attachment to the host root promote the plant defense responses which are similarly observed in plant-pathogen interaction (Sauerborn et al. 2002; Perez-de-Luque et al. 2004). The natural resistance of plants to pathogens and parasitic weeds is based on the combined effects induced mechanisms among which the systemic acquired resistance (SAR) that controlled by signaling pathway and depends on endogenous accumulation of salicylic acid

(Durrant and Dong 2004; Yang et al. 2010). Phenolic compounds including flavonoids are structurally diverse group of plant secondary products that can play a variety of roles in plant defense against pathogens, such as phytoanticipins, phytoalexins, structural barriers and activators for plant defense genes (Junqueria et al. 2004; Treutter 2006).

Induction of enzymes, that activate the phenylpropanoid pathway, have been reported to be among plant defense mechanisms by conferring mechanical and/or chemical barriers in host tissues against *Orobanche* (Goldwasser et al. 1999; Castillejo et al. 2004). The level of phenol metabolizing enzymes, phenylalanine ammonia lyase (PAL), peroxidase (POX), and polyphenol oxidase (PPO) are induced upon treatment with chemical and/or biological elicitors that influences the levels of phenolic compounds in the metabolic pool (Mandal and Mitra 2007; Nafie and Mazen 2008). A variety of plant defense-related processes have been implicated by POX activity including: hypersensitive response, cross-linking of phenolics with glycoproteins and cell wall lignification (Castillejo et al. 2004; Perez-de-Luque et al. 2006b). Also, enhancement of peroxidase activity has been reported in many plants exposed to external biotic and abiotic stresses (Passardi et al. 2004; Kosesakal and Unal 2009).

Indole acetic acid (IAA) and salicylic acid (SA) are endogenous plant growth regulators and play an important role in many physiological processes under different biotic and abiotic stresses (El-Tayeb et al. 2006; Gravel et al. 2007; Mandal et al. 2009). Salicylic acid (SA) is considered to be a plant signal molecule and involved in induction of SAR, which activates many defense compounds including phenolic acids, coumarins, flavonoids and lignin (Serghini et al. 2001; Katoch et al. 2005). Beckman (2000) added IAA to the list of host factors in the time space model of host parasite interactions in an infected vascular element and the surrounding parenchyma cells, since a reasonable role in defense process has been established by this hormone. Exogenous application of SA and IAA have been demonstrated to enhance plant resistance against pathogens by acting as potent inducer of systemic resistance (Katoch et al. 2005; Ragab et al. 2009; Ueno et al. 2011). Limited research has been directed to investigate the effect of SA and IAA as hormonal elicitors of plant resistance to parasitic weeds. Therefore, the purpose of this study was to elucidate the potential of SA and IAA as external hormonal inducers on the biochemical and histological changes involved in the resistance of tomato (*Lycopersicum esculentum* Mill.) to *Orobanche ramosa*, under controlled conditions.

Materials and methods

Plant materials

Tomato (*Lycopersicon esculentum* Mill.) seeds were kindly obtained from the Department of Vegetables, Agriculture Research Center. *Orobanche ramosa* L. seeds were collected from an infested tomato field in Faculty of Agriculture Experimental Station, Giza, Egypt.

Surface sterilized tomato seeds were soaked for 6 hrs either in the different concentrations of salicylic acid (SA), indole acetic acid (IAA) or in water to provide as a control. SA and IAA were obtained from Sigma Chemical Co. (St. Louis, USA) and used at three different concentrations: 0.05, 0.5, 1.0 mM for SA and 0.03, 0.06, 0.09 mM for IAA. The presoaked seeds were air dried before sowing.

Pot experiments

Presoaked tomato seeds were grown in plastic pots (8 cm diameter x 10 cm depth). Each pot contained 500 g of a mixture of sand and clay (3:1, w/w) soil. The pots were divided into two sets, one set used for healthy plants and the second set for the infected plants. Each set was subdivided into seven groups, one group

serve as control and the other six groups used for the presoaked seed treated with the three different concentrations of either SA or IAA. In the pots of infected set, the upper soil layer was mixed homogenously with *O. ramosa* seeds (0.05 g seeds/pot). This experiment was carried out under controlled conditions with a day/night temperature of 25±2 ^{0}C, with supplemental light provided for 14 hrs in a growth chamber. All pots were arranged in a randomized complete block design with five replicates per treatment. Plants were watered when necessary during the course of the experiment.

After sixty days from sowing, the healthy and infected plants were harvested and their roots separated and washed thoroughly with water, then the *Orobanche* tubercles cutted out. The fresh root samples were ground to fine powder in liquid nitrogen and stored at -70 ^{0}C for enzyme assay. Other root samples were oven dried at 70 ^{0}C for constant weight, then ground to fine powder and kept for biochemical analyses.

Extraction and determination of phenolics

Phenolic aglycones were extracted and determined following the protocol illustrated by Sauvesty et al. (1992) using 80% ethanol. One ml of each pure extract was mixed with 1 ml of the 10% Folin-Ciocalteau phenol reagent and 1 ml of 20% anhydrous sodium carbonate, then completed up to known volume with distilled water. The absorbance of the blue colour was measured after 30 min at 750 nm against water-reagent blank. Phenolic aglycone content of each sample was calculated from the protocatechuic acid standard curve, then expressed as mg g^{-1} dry weight.

Extraction and determination of flavonoids

Total flavonoid content was measured using aluminum chloride colorimetric assay (Sakanaka et al. 2005). Dry root samples were extracted with methanol, then 0.25 ml of the clear extract was mixed with 1.25 ml of distilled water in a test tube, followed by addition of 75 µl of 5% (w/v) sodium nitrite solution. After 6 min of incubation, 150 µl of 10% (w/v) aluminum chloride solution was added and the mixture allowed standing for a further 5 min before addition of 0.5 ml of 1 M sodium hydroxide. The mixture was completed up to 2.5 ml with distilled water. The absorbance was measured immediately at 510 nm and the concentration of total flavonoids was calculated using standard curve of myricetin, then expressed as mg g^{-1} dry weight.

Extraction and determination of proline

Free proline content was extracted and determined according to the method proposed by Bates et al. (1973). A known dry powdered weight of root tissue was homogenized in 10 ml of 3% aqueous sulfosalicylic acid for 10 min followed by centrifugation. Two ml of the clear supernatant was mixed with 2 ml of freshly prepared acid ninhydrin reagent and 2 ml glacial acetic acid, then boiled on water-bath for 1 hr. After cooling in ice-bath, the mixture was mixed with 4 ml toluene and vigorously stirred for 20 seconds. Thereafter, the chromophore-containing toluene was aspirated from the aqueous phase, and its absorbance measured at 520 nm against toluene as blank. The proline content was determined from a standard curve of proline and calculated as mg g^{-1} dry weight.

Extraction and determination of soluble proteins

Extraction of water-soluble proteins was carried out according to the method described by El-Tayeb et al. (2006). A known dry weight of root sample was boiled in 1 ml distilled water for two hrs, then centrifuged at 6000 *g*. Determination of soluble proteins is carried out using the modified Folin-Lowry method adopted by Hartree (1972). One ml of the clear protein extract was mixed with 0.9 ml of alkaline sodium

carbonate solution, and heated in a water-bath at 50°C for 10 min. After cooling, 0.1 ml copper sulphate-potassium sodium tartrate solution was added to the mixture and allowed to stand for 10 min at room temperature, followed by addition of 3 ml of 10% Folin-phenol reagent with immediate mixing. After 30 min, the absorbance of the blue colour was recorded at 750 nm against water-reagent blank. The soluble protein content was determined using standard curve of bovine serum albumin, then expressed as mg g^{-1} dry weight.

Extraction and determination of phenylalanine ammonia-lyase (PAL)

Extraction and assay of PAL (EC 4.3.1.5) were carried out as described by Solecka and Kacperska (2003). A known weight of liquid-nitrogen powdered root tissues was homogenized with mortar and pestle in the extraction buffer (50 mM Tris–HCl buffer, pH 8.9, 5 mM EDTA, 5 mM ascorbic acid). The homogenate was filtrated and centrifuged at 12,000 *g* for 20 min at 4 °C and the supernatant was used as a source of crude enzyme for assaying PAL activity. The reaction mixture contained 100 µl crude enzyme extract and 0.9 ml substrate solution (16 mM L-phenylalanine, 50 mM Tris-HCl buffer, pH 8.9, 3.6 mM NaCl). Incubation was performed at 37 °C for 1 hr and the reaction was stopped by the addition of 500 µl of 6 N HCl. The absorbance was measured at 290 nm. One unit of enzyme activity equals the amount of PAL that produced 1 µM of *trans*-cinnamic acid in 1 hr, and is expressed as µM *t*-cinnamic acid $h^{-1}mg^{-1}$ fresh weight.

Extraction and determination of peroxidase (POX)

Extraction of POX (EC 1.11.1.7) was carried out according to the method outlined by Kar and Mishra (1976). One gram of the liquid nitrogen frozen root tissues was ground at 4 °C with 1 ml of 0.1 M sodium phosphate buffer, pH 7.0, using a mortar and pestle. The homogenate was centrifuged at 10,000 *g* at 4 °C for 15 min and the clear supernatant used for measuring POX activity. The colorimetric assay described by Wakamatsu and Takahama (1993) was used for determination of POX activity. The reaction mixture contained 50 µl of the crude enzyme extract and 10 ml of assay mixture (40 mM potassium phosphate, pH 7.2; 0.1 mM EDTA; 5 mM guaiacol; 0.3 mM hydrogen peroxide). Peroxidase activity was determined by measuring the absorbance at 470 nm, then expressed as ABS unit min^{-1} g^{-1} fresh weight.

Extraction and determination of polyphenol oxidase (PPO)

Polyphenol oxidase (EC 1.14.18.1) was extracted as described by Kar and Mishra (1976) with slight modification. A known weight of frozen root tissues was homogenized in ice-cold 0.1 M sodium phosphate buffer, pH 7.0, at 4 °C. After centrifugation at 10,000 *g* for 10 min at 4 °C, the clear supernatant used for PPO assay. Enzymatic activity was assayed using the method proposed by Nguyen et al. (2003). The assay mixture contained 0.5 ml of the crude enzyme extract and 2.5 ml of substrate solution (0.05 M phosphate buffer, pH 6.0, containing 0.05 M catechol). The mixture was incubated at 30 °C for 30 min, then the absorbance measured at 420 nm and expressed as ABS unit h^{-1} g^{-1} fresh weight.

Fixation and staining of microtome sections

Tomato root segments with attached *Orobanche* tubercles were fixed in FAA (50% ethanol: 5% formaldehyde: 10% glacial acetic in water) for 48 hrs (Perez-de-Luque et al. 2006a). The fixed segments were dehydrated in ethanol series (50, 80, 95, 100, 100% for 12 hr for each), then transferred to an embedding solvent through xylene-ethanol series (30, 50, 80, 100, 100% for 12 hr for each) and finally saturated with paraffin. Root specimens were cut into 5 µm-thick sections with a rotary microtome and attached to adhesive-

treated microscope slides. Staining of sections was carried out according to the method outlined by Goldwasser et al. (2000). The sections were placed on a drop of water on glass slides and dried on a 45^{0}C hot plate. Each slide was stained with 1% toluidine blue O (TBO) in phosphate buffer (pH 5.5) for 5 min, washed with water, then overlaid with a covering glass and dried on hot plate. Slides were examined under light microscope (Leica DM-LB, magnification X100 to X1000).

Statistical analysis

The computer program SPSS (version 18) was used for statistical analyses of studied parameters. All the data were subjected to analysis of variance (ANOVA). The treatment means were compared by Duncan's multiple range test at P = 0.05. When needed, data were transformed by log (x+1) before statistical analysis. The values expressed as means of 5 replicates ± standard error.

Results

Changes in the levels of proline and soluble proteins in response to SA and IAA treatments in the roots of 60-day-old healthy and infected tomato plants are illustrated in Fig.1. Infected plants showed a pronounced increase in in the levels of proline and soluble proteins as compared with the non-infected control plant. The production of both compounds was more obvious by SA and IAA treatments, where the accumulation was greater in the infected plants than healthy ones. Increasing the concentration of SA and IAA significantly increased the proline and soluble protein contents reaching their maximum values at the higher concentrations, where at 1.0 mM SA, the level increases to about 92 and 60%, respectively, while the 0.09 mM IAA treatment raised their levels to 2-fold for proline and 74% for protein, over their respective untreated-infected plants.

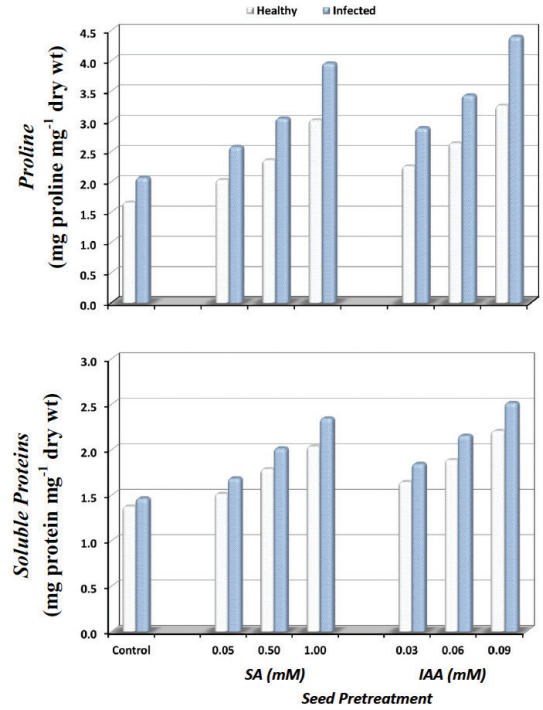

Figure 1. Effect of SA and IAA seed treatments on the ***proline*** and ***soluble protein contents*** in the ***roots*** of 60-day-old healthy and *Orobanche*-infected tomato.

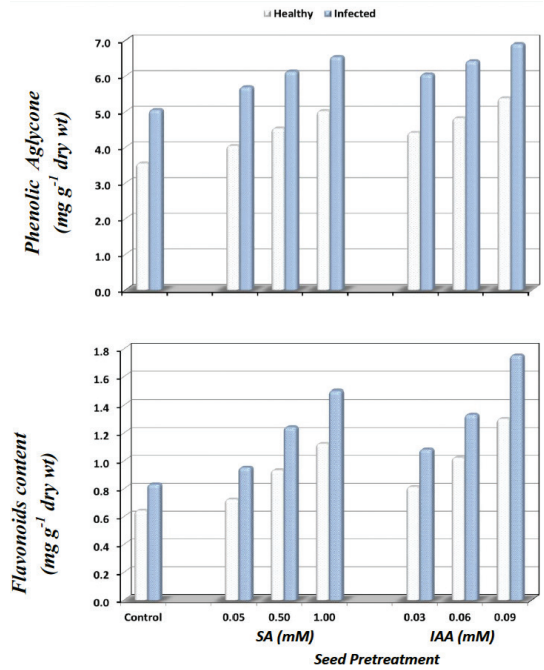

Figure 2. Effect of SA and IAA seed treatments on ***the phenolic*** and ***flavonoid*** contents in the ***roots*** of 60-day-old healthy and *Orobanche*-infected tomato.

The data also showed noticeable increase in the production of both phenolics aglycones and flavonoids upon infection of tomato plants with *Orobanche ramosa*, where the magnitude of increase was 42 and 30 %, respectively as compared with healthy plants Fig.2. Presoaking of tomato seeds with different concentrations of SA or IAA significantly raised the levels of both compounds. This enhancement was more pronounced in the infected than in the healthy tomato plants, where the maximum rate of accumulation of both phenolics and flavonoids were 42 and 75%, respectively in plants treated with 1.0 mM SA and were 52 and 2-fold, respectively with 0.09 mM IAA treatment compared with untreated-infected plants.

The changes in the activities of phenylalanine ammonia lyase (PAL), peroxidase (POX) and polyphenol oxidase (PPO) in both healthy and *O. ramosa*-infected tomato plants treated with SA and IAA in are presented in Fig.3. Presoaking of tomato seeds in the different concentrations of either SA or IAA significantly enhanced the PAL activity, where the maximum stimulation was more pronounced under the highest concentrations of SA and IAA treatments, relative to untreated healthy plant. Similar trends of stimulation in the activities of POX and PPO observed in the healthy plants treated with SA and IAA, as compared with the untreated control one.

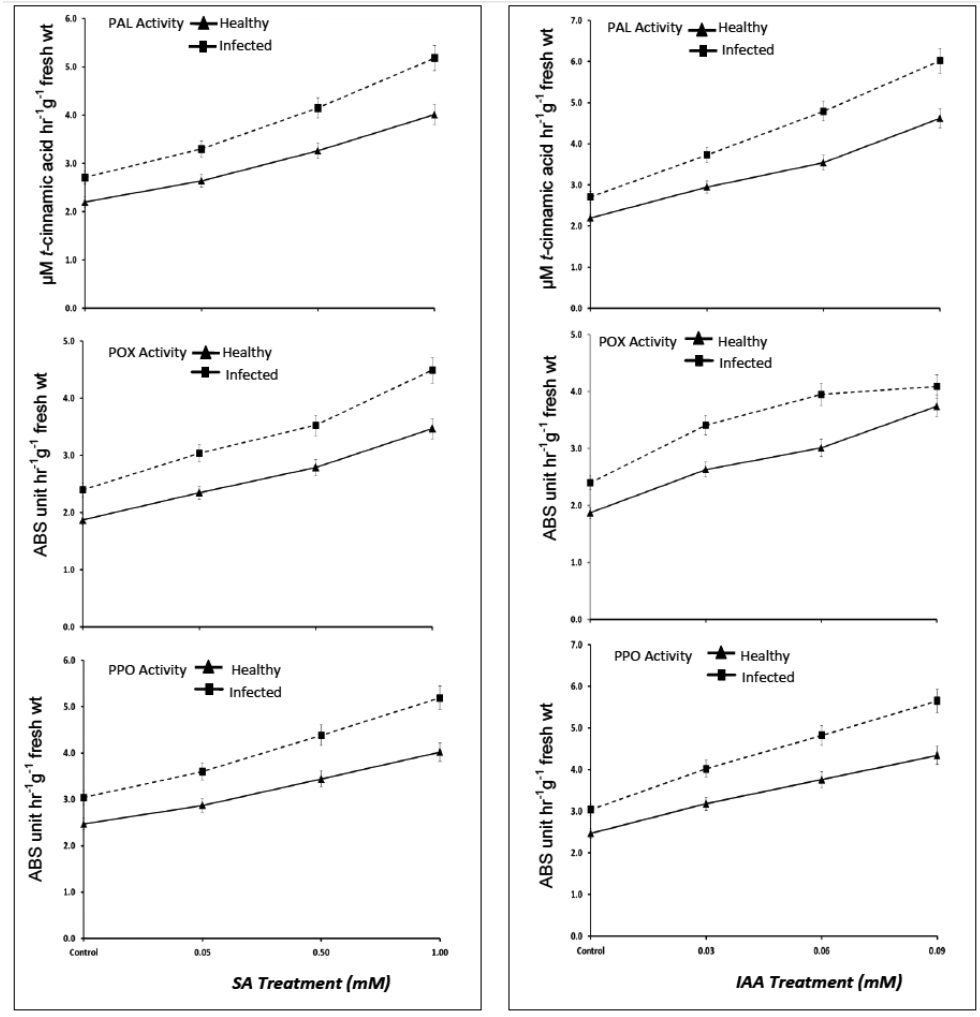

Figure 3. Effect of SA and IAA seed pretreatment on the ***PAL, POX*** and ***PPO*** activities of both healthy and *Orobanche*-infected tomato roots.

In addition, infection of tomato plants with *O. ramosa* stimulated the activities of PAL, POX and PPO by 23, 28 and 23%, respectively, as compared with their respective levels in the healthy plants. SA and IAA treatments significantly enhanced the activities of these enzymes in the host roots, where the maximum improvement induced by the 0.09 mM IAA, followed by the 1.0 mM SA treatment, relative to those in the untreated-infected plants.

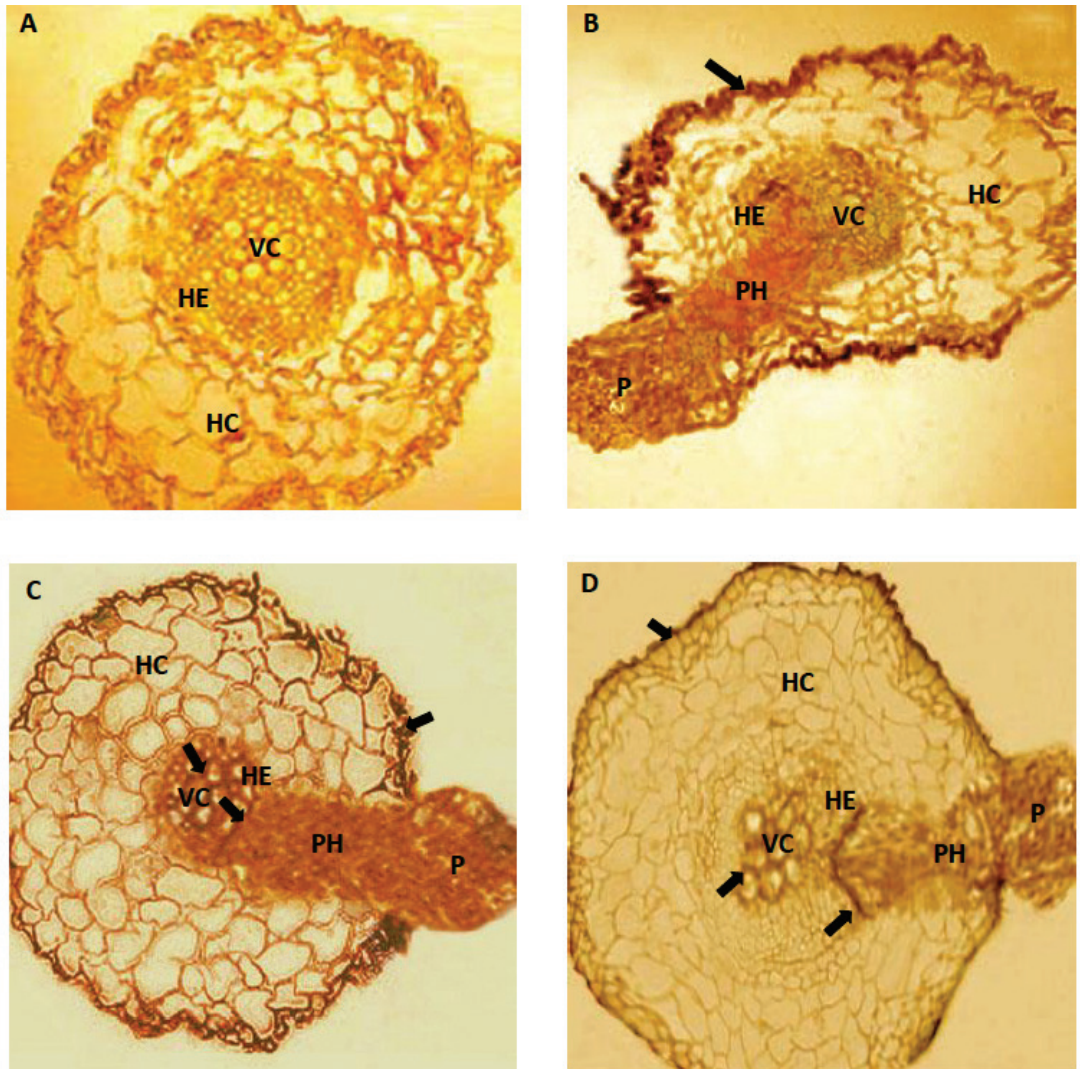

Figure 4. Transverse sections of tomato roots infected with *Orobanche ramosa*: **(A)** healthy tomato roots, **(B)** untreated-infected roots **(C)** infected roots treated with 1.0 mM SA, **(D)** infected roots treated with 0.09 mM IAA. Black arrows indicate the dark secretions observed in the root apoplast. **VC**, Vascular Cylinder; **HE**, Host Endodermis; **HC**, Host Cortex; **P**, Parasite; **PH**, Parasite Haustorium.

The interaction between *Orobanche ramosa* and tomato roots and those treated with 1.0 mM SA and 0.09 mM IAA is shown in Fig.4. Light microscope sections clarified that *O. ramosa* haustorium enters tomato root epidermis and grows perpendicularly through the cortex and endodermis then directly into the vascular cylinder (Fig. 4B). On the other hand, in sections of tomato roots treated with 1.0 mM SA and 0.09 mM IAA, the *O. ramosa* haustorium enters the root epidermis and cortex but fails to penetrate the endodermis layer

(Fig. 4C and 4D). Staining with TBO showed a reddish brown coloration in the apoplast interface between parasite haustorium and host tissues.

Discussion

It is well documented that branched broomrape (*Orobanche ramosa)* causes a drastic failure in tomato crop (Cagan and Toth 2003; Mauromicale et al. 2008). Induced resistance is a phenomenon in which the natural defense mechanisms of plants are activated by biotic or abiotic inducers. The chemical inducers including Benzothiadiazole (BTH) and acibenzolar-*S*-methyl (ASM) were reported to activate the systemic acquired resistance (SAR) in plants by increasing the induction of pathogenesis related (PR) proteins, therefore diminish the damage caused by *Orobanche* spp. (Sauerborn et al. 2002; Perez-de-Luque et al. 2004; Kusumoto et al. 2007). In the present study, salicylic acid (SA) and indole acetic acid (IAA) were used as seed pretreatments to evaluate their ability to induce plant resistance against *O. ramosa*.

In previous study, we showed that priming of tomato seeds with SA and IAA considerably inhibited the number and growth of *O. ramosa* tubercles accompanied with improvement of tomato root growth due to the increase in the host root lignification and some PR-proteins including chitinase and β-1,3- glucanase (Al-Wakeel et al. 2012). The disturbances in the host physiology due to *Orobanche* infection are related to alteration in protein metabolism, where the increase in soluble proteins level in the infected tomato roots may be attributed to the induction of many PR-proteins. Moreover, treatments with SA and IAA significantly increased the soluble protein level in the healthy and *Orobanche*-infected plants, relative to their respective untreated plants, indicating the ability of these treatments to increase the resistance of tomato plant. It has been reported that plants develop a complex variety of events involving synthesis and accumulation of new soluble proteins that have a direct or indirect role in inducing plant resistance (Van Loon and Van Strien 1999). Proteomic study on pea response to *O. crenata* revealed the up-regulation of many PR-proteins (Castillejo et al. 2004; Perez-de-luque et al. 2006b). Expression of defense genes in host plants that increase the plant resistance to parasitic weeds have been recognized by Griffitts et al. (2004), Lejeune et al. (2006) and De-Zelicourt et al. (2007). In addition, Radwan et al. (2010) demonstrated a considerable increase in soluble protein levels, under SA treatment, in faba bean plant infected with virus.

Proline is one of the metabolic products that frequently accumulate in response to variety of environmental stresses. The accumulation of proline in the roots of the infected tomato plants treated with SA and IAA, as compared to untreated host, could be contributed to decreasing the osmotic potential of host root to restrict the movement of water to *Orobanche ramosa*. In this respect, proline can be considered one of important factors involved in plant defense mechanisms by formation of rigid cross-links between cellulose of secondary cell wall and hydroxyproline-rich glycoproteins (Bowels 1990). Moreover, Sharma et al. (1998) revealed that the accumulation of proline permits osmotic adjustment and provides protection for some enzymes by formation of metal-proline complex.

Increased the levels of phenolics and flavonoids in the infected tomato root, relative to the healthy one, supports the significant role of these compounds in increasing the resistance of tomato to broomrapes. The obvious increase in the total phenolics and flavonoids levels in the infected than healthy tomato roots that treated with SA and IAA, indicating their effectiveness in inducing resistance. In this regards, soluble and cell wall-bound phenolics

have been shown to accumulate in plant roots that play important role in the parasitic infection process and host resistance (Sahm et al. 1995; Rispail et al. 2007). Similarly, the effects of SA and IAA on the accumulation of soluble phenolic level have been reported on onion plant after foliar application of both elicitors (Amin et al. 2007).

Furthermore, Skadhauge et al. (1997) ascribed the role of flavonoids in barley as defense compounds against *Fusarium* spp. to the formation of a hard and crystalline structure that act as barrier against pathogen attachment. Also, using chitosan treatment as elicitor activated defense response in *Oryza sativa* leaves due to increase the production of flavonoids beside other phytoalexins (Agrawal et al. 2002). Recently, Radwan et al. (2010) recorded accumulation of flavonoids level in faba bean in response to virus infection and SA treatment.

Phenylalanine ammonia-lyase (PAL) is the first enzyme of phenylpropanoid pathway and plays a significant role in regulating the phenolics biosynthesis in plants. Induction of PAL is correlated with increased resistance to pathogenic infection (Yamunarani et al. 2004; Wang and Zhang 2010). Both SA and IAA treatments caused obvious stimulation in the PAL activity in tomato roots, where the induction of PAL was more pronounced in the infected-treated plants than the uninfected-treated ones. Activation and expression of *PAL* mRNA in grape berry treated with SA, improved the development of acquired resistance (Wen et al. 2005). Similar increase of PAL activity was demonstrated in response to application of several chemical inducers such as SA, BTH and chitosan that improved the resistance of several plant species against pathogens (Mandal and Mitra 2007; Nafie and Mazen 2008; Mandal et al. 2009).

Oxidative-reduction enzymes play an important role in induced resistance involved in plant's defense reactions against pathogens. Peroxidases are found in all terrestrial plants and have many diverse functions, including H_2O_2 detoxification and formation of ROS that cause an oxidative burst in response to pathogens (Bolwell et al. 1995). Moreover, peroxidases act as bifunctional enzymes that can oxidize various substrates and produce ROS (Passardi et al. 2004; Kosesakal and Unal 2009). Polyphenol oxidase (PPO) is a wide spread enzyme found in plant cells and enhances the oxidation of the *O*-diphenol compounds into more toxic forms, *O*-quinons (Chranowski et al. 2003). The present study revealed a significant stimulation in POX and PPO activities in the infected tomato roots relative to healthy one, indicating their importance in the plant defense machinery. In addition, treatments of tomato seeds with all SA and IAA significantly stimulated POX and PPO activities, where the magnitude of increases was more obvious in the infected plants than the uninfected ones. These stimulatory effects confirm the effective role of SA and IAA treatments in improvement plant resistance against *Orobanche* infection.

In this respect, the present results are in agreement with the previous studies of Goldwasser et al. (1999), Castillejo et al. (2004) and Perez-de-luque et al. (2006b). They demonstrated that the resistant cultivar of vetch (*Vicia* spp), pea (*Pisum sativum*) had higher levels of defense proteins, including peroxidases which increase their susceptibility to *Orobanche* infection. Similarly, considerable increases in the activity of POX and PPO were recorded in basil plants whose seeds have been treated with SA (8 mM) and IAA (400 ppm) against root rot diseases (Ragab et al. 2009). Moreover, exogenous application of chemical and biological elicitors was greatly stimulated the activity of POX and PPO in host plants that reduced root rot disease (Mandal and Mitra 2007; Mandal et al. 2009; Ragab et al. 2009).

Reinforcement of cell wall of tomato roots through induction of phenolic compounds and lignin by biological and chemical elicitors had been demonstrated by Mandal and Mitra (2007) that increase the plant resistance responses. Anatomical investigations by Goldwasser et al. (2000) ascribed that, in the susceptible genotype, the parasite haustorium penetrated through the endodermis into the host vascular cylinder, while the haustorium in the resistant genotype was blocked at the root endodermis layer due to the secretion of chemical material that preventing the parasite from establishing. The present study showed the ability of *Orobanche* haustorium to penetrate the endodermis of tomato roots to reach its vascular system, an essential mechanism for functional parasitism by this obligator parasite. Obstruction of the penetrating haustorium into the vascular bundle in the roots treated with SA and IAA indicates the degeneration or death of the parasite. Our previous study showed a noticeable increase in lignin level in the infected tomato roots by SA and IAA treatments that consider as a mechanical barrier restricting haustorial penetration through host root tissues (Al-Wakeel et al. 2012).

In conclusion, this study reveals that presoaking of tomato seeds with the hormonal inducers: SA and IAA have the potential to induce systemic resistance in tomato against *O. ramosa.* This response could contribute to be a useful way for improving the plant growth and yield in the *Orobanche* infected areas.

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