CHANGES IN EXPRESSION OF PATHOGENESIS-RELATED GENE 1, PATHOGENESIS-RELATED GENE 2, PHENYLALANINE AMMONIA-LYASE AND CATALASE IN TOMATO IN RESPONSE TO PECTOBACTERIUM CAROTOVORUM SUBSP. CAROTOVORUM

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SUMMARY

Pectobacterium carotovorum subsp. carotovorum (Pcc) is one of the most destructive pathogens causing stem rot disease on tomato. In this study, expression profiling of pathogenesis-related gene 1 (PR-1), pathogenesis-related gene 2 (PR-2), phenylalanine ammonia-lyase (PAL) and catalase (CAT) in a partially resistant (Sun) and a susceptible (Early orbano) tomato cultivar in response to Pcc were compared. Our results showed more expression of PR-2, PAL and CAT in the partially resistant cultivar than the susceptible cultivar. On the other hand, no significant difference was found in expression of PR-1 between the both cultivars. These findings suggest the involvement of PR-2, PAL and CAT in defense responses of tomato against Pcc that might be considered in plant breeding programs.

Keywords: catalase, pathogenesis-related gene 1, pathogenesis-related gene 2, Pectobacterium carotovorum subsp. carotovorum, Phenylalanine ammonia-lyase.

INTRODUCTION

During pathogen infection of plants, expression of some defense genes is changed to overcome pathogen attack. The role of pathogenesis-related (PR) proteins in plant defense mechanism is demonstrated (Ebrahim et al., 2011). Most PR proteins are acid-soluble, low molecular weight and protease-resistant. PR proteins have been divided into 17 families so far, based on their sequences and functions (Ebrahim et al., 2011). PR-1 protein class was identified in the early 1980s (Antoniw et al., 1980). It has been found in some plant species such as barley, wheat, maize, tobacco, rice, pepper and tomato (Liu and Xue, 2006). Although PR-1 is expressed abundantly upon pathogen attack (Mitsuhara et al., 2008; Asai et al., 2014), the exact mode of action of this protein is yet to be understood. PR-2 as a β-1,3-glucanase enzyme was diagnosed firstly in tomato (Antoniw et al., 1980) and afterward discovered in a variety of plants like peanut, chickpea and common bean. Induction of PR-2 in response to various pathogens is proved (Elvira et al., 2008). Phenylalanine ammonia-lyase (PAL) is an important enzyme in the regulation point between primary and secondary metabolism that catalyses the non-oxidative deamination of phenylalanine to trans-cinnamate (Dixon and Paiva, 1995; Huang et al., 2010). PAL gene expression responds to pathogen invasion and numerous abiotic stresses (Dixon and Paiva, 1995; MacDonald and D’Cunha, 2007). Furthermore, rapid and strong accumulation of reactive oxygen species (ROS) is a crucial plant defense mechanism against pathogen invasion (Bolwell et al., 2002). Although early research about ROS focused on its direct toxicity, recent studies confirmed the role of ROS as a signaling molecule. ROS involves in hypersensitive response (HR) (Lamb and Dixon, 1997), systemic acquired resistance (SAR) (Alvarez et al., 1998), phytoalexin production (Daudi et al., 2012) and callose deposition (O’Brien et al., 2012). Scavenging enzymes like catalase (CAT) play a key role in balancing of ROS to employ it as a signaling molecule (Mittler et al., 2004). Expression of CAT in plants upon pathogen attack is shown (Kwak et al., 2009; Cheng et al., 2012).

Tomato is a major vegetable crop in the world that suffers from numerous pathogens. Stem rot caused by Pectobacterium carotovorum subsp. carotovorum (Pcc) is one of the most important diseases of tomato worldwide (Pérombelon and Salmond, 1995). In recent years, the promoting concern about harmful environmentally effects of pesticides and also appearance of pesticides-resistant pathogen strains has led to search for new more effective and safer techniques. Use of resistant cultivars is one of the popular methods to reach this goal. Additionally, identification of the genes that play role in resistance is a key approach to might be considered in plant breeding programs. There are no reports about the effect of Pcc on expression of PR-1, PR-2, PAL and CAT in tomato. Hence, the aim of this study was to investigate expression of PR-1, PR-2, PAL and CAT in tomato in response to Pcc. Moreover, Expression of
these genes in cultivars Sun (as a partially resistant cultivar) and Early orbano (as a susceptible cultivar) were compared.

MATERIALS AND METHODS

Plant materials and pathogen inoculation. The tomato (Lycopersicon esculentum) cultivars Early orbano and Sun were used in this study. Tomato seeds were surface sterilized in 1.0% sodium hypochlorite for 10 min and rinsed four times in sterile distilled water. The seeds were sown in quartz sand in 15-cm plastic pots. The inoculum of Pcc 1675 (purchased from Persian Type Culture Collection) was provided in sterile distilled water at a concentration of about 10^8 CFU/ml and each stem of six-week-old plants was infiltrated with 50 µl of the bacterial suspension. Sterile distilled water was employed as a negative control. The plants were incubated in a growth chamber at 28°C with a 16 h photoperiod at 70% relative humidity. The length of about 10^8 CFU/ml and each stem of six-week-old plants was provided in sterile distilled water at a concentration of about 10^8 CFU/ml and each stem of six-week-old plants was infiltrated with 50 µl of the bacterial suspension. Sterile distilled water was employed as a negative control. The plants were incubated in a growth chamber at 28°C with a 16 h photoperiod at 70% relative humidity. The length of rotten lesions (cm) on stems was recorded at 5 and 10 days post pathogen inoculation (dpi).

RNA extraction and cDNA synthesis. Stems were harvested at 24, 48, 72 and 96 h post pathogen inoculation (hpi) with three repetitions. Total RNA was isolated by a RNA extraction kit (DENAziest, Iran) according to the manufacturer’s protocol. After treatment of RNA with DNase I (Fermentas, Lithuania) to eliminate genomic DNA contamination, its quality and quantity was evaluated by ethidium bromide staining of agarose gel and spectrophotometrically. First-strand cDNA synthesis was performed using a cDNA synthesis kit (Fermentas, Lithuania), according to manufacturer’s guidelines.

Real-time RT-PCR analysis. Real-time RT-PCR reactions were performed with 30 ng of cDNA, 600 nM of each primers, 10 µl (2x) of the SYBR green Master Mix (Ampliqon, Denmark) and RNase free water in a final volume of 20 µl. Primers used in this study had the following sequences: 5′GGATCGGACACGTCCTTAC and 5′GCAACATCAAAAAAGGAAATAAT (Molinari et al., 2014) for PR-1, 5′AAGTATATAGCTGTTGGTAATGAA and 5′ATTCTCATCAAAACATGGCGA (Molinari et al., 2014) for PR-2, 5′ACGGTGTGCCATCTAATCTG and 5′AGCTCCTTTTCTGGCTGAAA (Aimé et al., 2013) for PAL, 5′TGGAAGCCAACCTGTGTTG and 5′ACTGGGATCAACGGCAAGAG (Zhang et al., 2014) for β-tubulin. Real-time RT-PCR experiments were carried out in a Bioneer (South Korea) with a program consist of initial denaturation for 5 min at 94°C, followed by 40 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 58°C and extension for 1 min at 72°C; with final extension for 5 min at 72°C. Each Real-time RT-PCR was repeated three times and the results were averaged.

RESULTS

Disease evaluation. The length of rotten lesions on stems of tomato plants cultivar Early orbano inoculated with Pcc was significantly higher than cultivar Sun at 5 and 10 dpi (Fig. 1).

Expression of PR-1. PR-1 expression in cultivar Sun inoculated with Pcc increased 3.6-, 3.2-, 2.5- and 2.2-fold at 24, 48, 72 and 96 hpi, respectively compared to controls. Moreover, PR-1 expression in cultivar Early orbano inoculated with Pcc enhanced 3.5-, 3.0-, 2.6- and 2.2-fold at 24, 48, 72 and 96 hpi, respectively compared to controls. No significant difference was found in expression of PR-1 between cultivars Sun and Early orbano at the all time intervals (Fig. 2).

Expression of PR-2. Expression of PR-2 in cultivar Sun inoculated with Pcc was significantly higher than cultivar Early orbano at the all time points. PR-2 expression in cultivar Sun inoculated with Pcc enhanced 4.1-, 3.3-, 2.8- and 2.1-fold at 24, 48, 72 and 96 hpi, respectively compared to controls. On the other hand, PR-2 expression in cultivar Early orbano inoculated with Pcc increased 2.6-, 2.2-, 1.9- and 1.5-fold at 24, 48, 72 and 96 hpi, respectively compared to controls (Fig. 3).

Expression of PAL. Expression of PAL in cultivar Sun inoculated with Pcc was significantly higher than cultivar
Early orbano at 24 and 48 hpi. PAL expression in cultivar Sun inoculated with Pcc increased 3.4- and 2.8- fold at 24 and 48 hpi, respectively compared to controls. Additionally, PAL expression in cultivar Early orbano inoculated with Pcc enhanced 2.3- and 1.6- fold at 24 and 48 hpi, respectively compared to control. On the other hand, no significant difference was observed in PAL expression between plants inoculated with Pcc and controls at 72 and 96 hpi in the both cultivars (Fig. 4).

Expression of CAT. Expression of CAT in cultivar Sun inoculated with Pcc increased 7.4-, 4.2-, 3.3- and 2.6- fold at 24, 48, 72 and 96 hpi, respectively compared to controls. In addition, CAT expression in cultivar Early orbano inoculated with Pcc increased 4.6-, 2.8-, 2.3- and 1.7- fold at 24, 48, 72 and 96 hpi, respectively compared to controls. Significant difference was found in expression of CAT between cultivars Sun and Early orbano at the all time points (Fig. 5).

DISCUSSION

Plants exhibit numerous responses against pathogen attack, many of which involve the expression of defense genes. Activation of these genes leads to physical and physiological changes in plant which are not favorable for pathogen. The involvement of PR-1, PR-2, PAL and CAT genes in basal resistance of tomato against Pcc was investigated in this study. Moreover, in order to better understand about resistance mechanisms to Pcc, expression profiling of the genes in partially resistant and susceptible cultivars were compared in time course experiments. Our results indicated expression of PR-1 and PR-2 in tomato in response to Pcc. Mitsuhara et al. (2008) displayed up-regulation of PR-1 in rice upon Magnaporthe grisea infection. Overexpression of WRKY48 (as a negative regulator of PR-1) in Arabidopsis leads to enhancing susceptibility against Pseudomonas syringae that is associated with reduced expression of PR-1 (Xing et al., 2008). Elvira et al.
activity in the resistant banana cultivar inoculated with *Mycosphaerella fijiensis* is higher compared to the susceptible cultivar (Torres et al., 2012). In our study, expression of *PAL* occurred only at 24 and 48 hpi that suggests *PAL* expression at early stages of infection may be enough to motivate synthesis of secondary metabolites to prevent pathogen invasion.

ROS production is one of the earliest responses of plants against pathogens (Bolwell et al., 2002). On the other hand, to prevent the harmful effects of ROS and to maintain the equilibrium of its levels, plants produce some enzymes to scavenge the ROS proficiently (Mittler et al., 2004). CAT expression in tomato upon *Pcc* infection is displayed in this study. Induction of scavenging enzymes including CAT in pepper in response to *Xanthomonas campestris* pv. *vesicatoria* is shown (Kwak et al., 2009). CAT is expressed in broad bean upon *Puccinia striiformis* attack (Cheng et al., 2012). In addition, our results showed an increment in expression of CAT in the partially resistant cultivar to *Pcc* when compared to the susceptible cultivar. In sugarcane, CAT activity in resistant cultivar to *Sporisorium scitamineum* is higher than the susceptible cultivar, suggesting that catalase activity may play an important role in resistance to the pathogen (Su et al., 2014). Expression of CAT is detected in both susceptible and resistant cultivars of cabbage to *Xanthomonas campestris* pv. *campestris*, while the expression in the resistant cultivar is relatively higher than the susceptible cultivar (Kaunain Roohie and Umesha, 2015). In summary, expression of *PR-1*, *PR-2*, *PAL* and CAT in tomato in response to *Pcc* was shown in this study. Moreover, we demonstrated more expression of *PR-2*, *PAL* and CAT in a partially resistant cultivar than a susceptible cultivar after inoculation with *Pcc*. These findings suggest the partially resistance to *Pcc* in cultivar Sun may is correlated with higher expression of some defense genes such as *PR-2*, *PAL* and CAT. Identification of resistant tomato germplasm and the mechanisms involved in resistance to *Pcc* would aid in plant breeding programs.

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