Abstract

Early blight is one of the major diseases of tomato that reduce the quality and quantity of the product. Regarding the molecular mechanisms involved in the host defense to the pathogen, little information is available to realize the mechanisms of defense response of tomato to pathogenic fungus *Alternaria solani*. In this study, the reaction of different tomato cultivars to early blight was first evaluated. To better understand the resistance mechanisms of tomato plants against *A. solani*, resistant and susceptible cultivars were selected for biochemical and molecular tests. Susceptible and resistant tomato cultivars were inoculated with pathogenic fungus in the greenhouse. The leaves of plants were sampled at five interval (0, 24, 48, 72 and 96 hours) after inoculation. Then several biochemical changes of plants such as total protein, total phenol, Guaiacol peroxidase enzyme, superoxide dismutase, catalase, Phenylalanine Ammonia-Lyase, and hydrogen peroxide accumulation, as well as the pattern of expression of *PR1b1* and *WRKY33* genes by qRT-PCR during interaction with *A. solani* was examined. Also, the protein profile (proteome) of tomato leaves using two-dimensional electrophoresis were investigated in resistant and susceptible cultivar to the fungus *A. solani*. Biochemical results shown the hydrogen peroxide accumulation, activity of defense enzymes such as Guaiacol peroxidase, Catalase and Superoxide dismutase enzymes, Phenylalanine Ammonia-Lyase, total phenol and the amount of transcripts of genes was found to be increased in challenge with pathogen. Activity of these enzymes and genes were higher in resistant H2O2 accumulated rapidly in the resistant leaf tissues and peaked during the early stages of infection, whereas accumulation was stronger and more intense in the susceptible tissues in later stages. A total of 610 detection protein showed 29 different expression proteins in resistant and susceptible cultivars. These proteins mainly involved in stress and defense, energy and metabolism, photosynthesis, protein biosynthesis, and signal transduction. Proteins involved in stress and defense such as pathogencity related proteins (PR) and biosynthesis of phenolic metabolite were the most frequent. The most important defense mechanisms of the plant against *A. solani* were strengthening of the cell wall and antimicrobial phenolic compounds. These results indicated that the induction of oxidant/antioxidant responses and the activity of genes and proteins in this study are part of the tomato defense mechanism against the necrotrophic fungus *A.solani.* The results of this study can be used to production of resistant varieties of tomato against *A.solani.*

Keywords*:* Early blight, Resistance, Expression gene, Biochemical, Two-dimensional electrophoresis

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Investigation of biochemical, proteomics defense aspects and assessment of expression of some genes involving in tomato defense to the pathogenic fungus *Alternaria solani* using Real Time PCR

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