Induction of early blight resistance in tomato by *Quercus infectoria* gall extract in association with accumulation of phenolics and defense-related enzymes

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Abstract

Treatment of tomato leaves with aqueous extract (0.5%) of the galls of *Quercus infectoria* significantly reduced infection from subsequent inoculation with *Alternaria solani*, the tomato early blight pathogen. When the leaves were challenge-inoculated with *A. solani* 3 d after application of *Q. infectoria* gall extract (QIGE), the percent defoliation decreased from 33.6 to 7.3. Two to three day pre-treatment with QIGE reduced the percent defoliation by 77 percent. The biochemical responses of tomato plants to QIGE were also studied. In tomato plants treated with QIGE, phenolic content increased rapidly, reached the maximum at 2 d after treatment. Phenylalanine ammonia-lyase (PAL) activity increased significantly from 1 d after treatment and the maximum enzyme activity was recorded 2 d after treatment at which period a 3-fold increase in PAL activity was observed when compared to the control. Peroxidase (PO) activity was also significantly increased 1 d after treatment and the maximum activity was reached 2 d after treatment. Peroxidase isozyme analysis indicated that PO-1 was increased dramatically in tomato leaves 1 d after treatment and maintained at the same level throughout the experimental period of 6 d. When tomato leaves were treated with QIGE, a two-fold increase in chitinase and β-1,3-glucanase activities was recorded 2 and 3 d respectively, after treatment. The enhanced activities of defense-related enzymes and elevated levels of phenolics in QIGE-treated tomato plants between 1 and 3 d after treatment suggest that these induced biochemical defenses may be involved in the suppression of early blight by QIGE.

List of abbreviations: GlcNAc- N-acetylglucosamine; QIGE- *Quercus infectoria* gall extract; PAL - phenylalanine ammonia-lyase; PO - peroxidase; PR proteins- pathogenesis-related proteins

Introduction

Plants have evolved a wide array of biochemical defenses to combat the invading pathogens. Some of the biochemical defense mechanisms are constitutive and existing in normally developed healthy plants. These include phenolics, phenolic glycosides, unsaturated lactones, saponins, cyanogenic...
glycosides, glucosinolates, 5-alkylated resorcinols and dienes (Osborn 1996). In addition to the pre-formed biochemical defences, a number of defense responses are induced in plants in response to infection by pathogens. Cell wall can be reinforced by lignification, accumulation of callose and hydroxy-proline-rich glycoproteins (HRGPs) and hypersensitive cell death is triggered to isolate the infected area to help prevent spreading of the pathogen (Mittler and Lam 1995). Several antimicrobial compounds such as phytoalexins and pathogenesis-related proteins are then produced. If these reactions occur in a timely manner, the infection by pathogens will not proceed further. However, if the defense reactions occur too late or are suppressed, the infection process will proceed successfully (Somssich and Hahlbrock 1998). The defense responses in plants can also be induced by certain avirulent pathogens (Manandhar et al. 1998), non-pathogens (Manandhar et al. 1998), root-colonizing rhizobacteria (Van Loon et al. 1998) and chemicals (Oostendorp et al. 2001). Induced resistance in plants can be subdivided into two broad categories. The pathogen-induced resistance has been termed ‘systemic acquired resistance’ (SAR) and the plant growth-promoting rhizobacteria-mediated resistance is known as ‘induced systemic resistance’ (ISR) (Hammerschmidt 1999). SAR is characterized by broad-spectrum disease resistance and is mediated via a salicylic acid dependent process (Mauch-Mani and Metraux 1998), whereas ISR is mediated by a jasmonate/ethylene sensitive pathway (Pieterse and Van Loon 1999). SAR in plants can also be induced by exogenous application of salicylic acid, acetyl salicylic acid, polyacrylic acid, methyl salicylate,jasmonic acid and jasmonic methyl ester, benzo[1,2,3]thiadiazole-7-carbothioic acid-S-methyl ester (BTH), 2,6-dichloroisonicotinic acid (INA), DL-β-amino-n-butyric acid (BABA) (Metraux et al. 2002).

Recently, we purified an antifungal protein from the galls of Quercus infectoria Oliv., belonging to Fagaceae family. The purified protein strongly inhibited the growth of several agronomically important fungal pathogens viz., Rhizoctonia solani, Curvularia lunata, Colletotrichum musae and Alternaria solani (K. Yamunarani, R. Jaganathan, R. Bhaskaran, P. Govindaraju and R. Velazhahan, unpublished data). The present study reports the induction of resistance in tomato against Alternaria solani infection by application of the extract of galls of Q. infectoria (QIGE). The effect of QIGE on accumulation of phenolics, and activities of peroxidase (EC 1.11.1.7), phenylalanine ammonia-lyase (EC 4.3.1.5), chitinase (EC 3.2.1.14) and β-1,3-glucanase (EC 3.2.1.39) in tomato was also investigated.

Materials and Methods

Plant material

The tomato (Lycopersicon esculentum L.) cultivar PKM1, susceptible to early blight was used in all experiments. The seeds were obtained from the Horticultural College and Research Institute, TNAU, Periyakulam, India. The plants were grown in a greenhouse in earthen pots (30 cm in diameter) filled with sterilized soil at 27 - 33 °C with a 14 h photoperiod.

Fungal culture

Alternaria solani (Ellis & Martin) Jones & Grout was isolated from diseased tomato leaves on potato dextrose agar (PDA) medium and incubated at 26 ± 2 °C. Spore suspension of the pathogen was prepared in 10 ml of sterile distilled water by gently rubbing 10-d-old fungal culture on PDA slants with a sterilized glass rod and filtering through two layers of muslin cloth to remove mycellial bits. The spore concentration was adjusted to 5 × 10^4 spores ml^-1 using a haemocytometer.

Preparation of extract from galls of Q. infectoria

Galls of Q. infectoria Oliv. were ground to a fine powder in a coffee grinder and 5 g of powder were mixed with 5 ml of distilled water and allowed to stand overnight with occasional mixing. After incubation the suspension was squeezed through two layers of muslin cloth. From this stock, aqueous extract of 0.5 % was prepared in distilled water and used for spraying.

Induction of resistance to A. solani

To determine the level of induced resistance 25-d-old seedlings of tomato were sprayed with