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ScienceDirect
Plant Science 174 (2008) 456–466
PLANT SCIENCE
www.elsevier.com/locate/plantsci

Overexpression of tomato polyphenol oxidase increases resistance to common cutworm

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Received 23 May 2007; received in revised form 7 January 2008; accepted 11 January 2008
Available online 21 January 2008

Abstract

Polyphenol oxidases (PPOs), which catalyze the oxidation of phenolics to quinones, have been reported to confer resistance to *Pseudomonas syringae* and are thought to be involved in insect resistance. To assess the impact of PPO expression on resistance to the common cutworm (*Spodoptera litura* (F.)) (Lepidoptera: Noctuidae), we used transgenic tomato (*Lycopersicon esculentum* Mill.) plants constitutively expressing sense- and antisense-oriented potato PPO genes. Transgenic plants expressing a sense PPO construct (overexpressing PPO [OP] plants) exhibited 2.0–5.7-fold higher PPO activity levels, whereas antisense PPO transgenic plants (suppressed PPO [SP] plants) exhibited 1.5–7.3-fold lower PPO activity levels than nontransformed controls. The PPO-overexpressing transgenic plants clearly showed an increase in resistance; simple growth rates of common cutworms on OP plants were up to 2.5 and 3.3 times lower than on controls and SP leaves, respectively, and larvae consumed less foliage. In addition, increased PPO activity led to higher larval mortality. The efficiency of conversion of ingested food and efficiency of conversion of digested food of third instars were found to be significantly different among tomato genotypes with differing PPO activity levels, suggesting that PPO activity rendered foliage less nutritious. Moreover, when leaflets at nodes 4 and 8 of SP, NT and OP plants were detached and fed to common cutworm larvae, their PPO activity levels were induced 1.6–2.2-fold. These results suggest a critical role for PPO-mediated phenolic oxidation in resistance to this insect. Manipulation of PPO activity could provide resistance simultaneously to both disease and insect pests, and therefore might be used as a component of effective integrated pest management.

Keywords: Induction; Insect resistance; *Lycopersicon esculentum* Mill.; PPO; *Spodoptera litura*; Transgenic tomato

1. Introduction

Tomato is one of the most widely grown vegetables worldwide. Tomato production in the Asian tropics faces a number of serious and destructive pests such as cotton bollworm (*Helicoverpa armigera* (Hübner)), beet armyworm (*Spodoptera exigua* (Hübner)), common cutworm (*Spodoptera litura* (F.)) and tobacco whitefly (*Bemisia tabaci* (Gennadius)). *S. litura* (Noctuidae, Amphipyrinae, synonym: *Prodenia litura*) known as tobacco cutworm, common leafworm, corn earworm, cotton leafworm and Asiatischer Baumwollwurm, is distributed throughout tropical and temperate Asia, Australia, Africa, the Middle East, Southern Europe and the Pacific Islands [1]. It is polyphagous and has been reported on more than 130 host species from 40 families, including cotton, flax, groundnut, maize, rice, soybean, tea, tomato, ornamentals and weeds [2]. The duration of its life cycle is ca. 5 weeks, with ca. eight generations per year depending on temperature and humidity. Females lay 1000–2000 eggs in masses of 100–300 eggs. Eggs eclose within 4 days in summer and 11–12 days in winter. Larvae pass through five instars in 15–23 days at 25–26 °C. Insects pupate in the soil and the pupal stage last 4–10 days. Population size depends critically on host plant, temperature and humidity [3–6].

In Thailand, defoliation by *S. litura* causes yield losses of up to 50% [7] in tomato. Chemical controls for this pest are inadequate, environmentally damaging, and economically infeasible. Therefore, resistant tomato cultivars are potentially

Abbreviations: AD, approximate digestibility; ECD, efficiency of conversion of digested food; ECI, efficiency of conversion of ingested food; NT, nontransformed; OP, overexpressing PPO; PPO, polyphenol oxidase; RCR, relative consumption rate; RGR, relative growth rate; ROS, reactive oxygen species; SP, suppressed PPO.

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0168-9452/$ – see front matter
doi:10.1016/j.plantsci.2008.01.006

an important component of a cost-effective and environmentally sustainable management program for *S. litura*.

Plant polyphenol oxidases [PPOs (EC 1.14.18.1, EC 1.10.3.2)], which catalyze the O_2-dependent oxidation of phenolics to quinones, have been proposed as a component of elaborate plant defense mechanisms. In tomato, PPOs serve a critical role in disease resistance; antisense suppression of PPO increases susceptibility, and PPO overexpression increases resistance, to *Pseudomonas syringae* pv. *tomato* [8,9]. In addition to bacterial disease, PPO is also inducible upon *Alternaria solani* infection [10], and its activity has been correlated with resistance to diverse pathogens including *Alternaria macrospora*, *Alternaria triticina*, *Ascochyta rabiei*, *Colletotrichum lindemuthianum* and *Sclerospora graminicola* [11–15]. PPO activity has also been associated with resistance against phloem-feeding and leaf-chewing insects in some plants, including tomato [16–20]. In addition, PPOs, like other defense genes, are frequently found to be differentially induced in response to signaling molecules and injuries inflicted by wounding, pathogens or insect pests in various plant species [10,19,21–24]. Recently, Wang and Constabel [25] reported enhanced resistance to forest tent caterpillar (*Malacosoma disstria*) in transgenic *Populus* overexpressing PPO when larvae from older egg masses were used, substantiating the possible role of PPO in plant defense against some insects.

Here, we used transgenic tomato plants constitutively expressing antisense- and sense-oriented PPO genes to evaluate the defensive role of PPO against common cutworm. The local induction of PPO activity by insect feeding was also examined.

2. Materials and methods

2.1. Plant materials and growth conditions

Transgenic tomato (*Lycopersicon esculentum* Mill. cv Money Maker) plants with suppressed PPO (SP plants) and transgenic plants overexpressing PPO (OP plants) were generated as described in [8,9]. Briefly, to generate sense and antisense transgenic plants, a potato PPO cDNA [26] was cloned in the sense and antisense orientation relative to the cauliflower mosaic virus (CaMV) 35S promoter of pART7 and pBI121 vectors, respectively, and introduced into *Agrobacterium tumefaciens* strain LBA 4404 [27] by electroporation. *Agrobacterium* was then used to transform cotyledons and hypocotyls of tomato. The transformants were selected on Murashige and Skoog [28] medium supplemented with 50 mg L^{-1} kanamycin. Two independent SP lines, A14-6 and A19-3, two independent OP lines, S-18 and S-28, and nontransformed (NT) control plants were further used for the analysis. Plants used in these experiments were from the F_7–F_8 generations. A14-6 possesses two linked T-DNA copies [29]. A19-3 also shows a single-locus inheritance of the antisense transgene. Both S-18 and S-28 carry at least two T-DNA copies [8]. These plants were grown in a laboratory culture room with a 13 h photoperiod at a photosynthetic photon flux density (PPFD) of 200–300 μmol m^{-2} s^{-1} at plant height, and 26–30 °C day/night temperature. Under these conditions, A14-6 and A19-3 had ca. 1.5–7.3-fold decreased PPO activity, whereas S-18 and S-28 contained 2.0–5.7-fold increased PPO activity. However, total protein contents of all five tomato genotypes were not significantly different (data not shown).

2.2. Insect feeding assay

Common cutworm larvae were collected from a marigold field, a sunflower field and a grape vineyard on the Suranaree University of Technology Farm and reared on artificial diet until pupation. The performance of common cutworm larvae when feeding on 6–10-week-old tomato genotypes varying in PPO activity levels was evaluated. Leaf position was counted from the top of the plants with the top-most (youngest) leaf node as node 1. One pair of male and female adults was mated and the resulting eggs were divided equally onto detached leaves from nodes 4 and 8 of five tomato genotypes, NT, A14-6, A19-3, S-18 and S-28. Detached leaves were placed in 16 cm × 22 cm plastic boxes, one genotype per box. Leaves were changed every 2 days, or sooner if they were almost completely eaten. Approximately 6 days after the eggs hatched, the mortality and weight gain of first instars were recorded. After molting to the second instar, larvae were transferred to individual 6-cm-diameter round plastic containers (one larva per container) containing one or more detached leaves, the leaf areas of which were measured using a leaf area meter. Molting was checked every 12 h, and the mortality and weight gain of second instars were recorded. The third and fourth instars were treated similarly and similar parameters were recorded. Fresh leaf material was added every 2 days, and larvae were never food limited until the experiment was terminated at the end of the fourth instar.

Five replications were used for each treatment. Each replication consisted of five larvae, each in its own plastic container, fed leaves from 5 to 10 plants grown near each other for the entire experiment. Thus, a total of 25 larvae were used per treatment per experiment. For statistical analysis, mean weights and leaf areas consumed were calculated for the five larvae in each replication and these mean weights and areas for the five replications were used in the analyses. Leaf areas were measured when leaves were changed and after each molt, and the leaf areas consumed were calculated from the original leaf areas minus the leaf areas remaining after feeding. Simple growth rates were calculated by dividing weight gain by the duration of the feeding period in days. Cumulative percent mortality was calculated by summing percent mortality at each stage. A repeated-measures ANOVA was conducted using PROC MIXED in SAS [30,31] to evaluate the effects of plant genotype, leaf age (node 4 or 8), larval instar, and the interactions of these fixed effects on larval simple growth rates and leaf areas consumed. Replicate was considered a random effect in these analyses.

To further evaluate the effects of PPO on larval performance through pupation, a similar experiment was conducted using three representative tomato genotypes, NT, A14-6 and S-28. The mortality, weight gain and leaf areas consumed were recorded after each molt through pupation, and simple growth