Scientia Horticulturae 129 (2011) 183–188

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Defense response of tomato fruit at different maturity stages to salicylic acid and ethephon

Yu-Ying Wang[a], Bo-Qiang Li[a], Guo-Zheng Qin[a], Li Li[b], Shi-Ping Tian[a,*]

[a] Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Nanxincun 20, Xiangshan, Beijing 100093, PR China
[b] Department of Plant Breeding and Genetics, Cornell University, Tower Road, Ithaca, NY 14853, USA

ARTICLE INFO

Article history:
Received 20 December 2010
Received in revised form 12 March 2011
Accepted 14 March 2011

Keywords:
Tomato fruit
Defense response
Maturity stage
Salicylic acid
Ethephon

ABSTRACT

In order to elucidate whether fruit maturity stage influence the induced resistance of exogenous elicitors in tomato and the involved mechanisms, we investigated the defense responses of tomato fruits against *Botrytis cinerea*, ethylene production and internal quality following treatments of fruit with salicylic acid (SA) or ethephon (ET) at mature green (MG) and breaker (BR). SA significantly suppressed decay and disease incidence in tomato fruits at both MG and BR stages, along with higher expression level of *PR1* gene after 2 days of treatment. All fruits treated by SA had lower contents of ethylene and lycopene. The ET-treated fruit at both maturity stages showed lower disease incidence and higher level of *PR2* and *PR3* expression compared with the control fruit. ET treatment significantly enhanced ethylene and lycopene contents, and accelerated fruit ripening. Our results suggest that SA and ET induced disease resistance in fruits by mediating the expression of different pathogenesis-related genes and have different effects on fruit ripening, which in turn influences the disease resistance of tomato fruits.

1. Introduction

Fungal diseases during fruit storage cause the huge economic losses. Among many strategies for control of postharvest diseases in fruit, activation of inducible defense systems was particularly attractive (Terry and Joyce, 2004; Tian et al., 2006). In recent years many elicitors, such as salicylic acid, silicon, brassinosteroids and oxalic acid, have been proved to effectively induce fruit resistance to fungal pathogens (Zeng et al., 2006; Qin and Tian, 2005; Zhu et al., 2010; Wang et al., 2009). However, limited information such as the time to apply elicitors restrains their practical application. A better understanding of the mechanisms through which the induced resistance is activated in fruit at different maturities is essential for the use of elicitors in controlling fungal diseases during fruit storage.

Unlike plant vegetative tissue, fruit undergoes a mature and ripening process, resulting in great biochemical and physiological changes, including sugar/acid ratio and fruit softening. Such changes consequently provide nutrients for pathogens and activate the fungal pathogenicity factors (Prusky, 1996). In climacteric fruit, these processes are often coupled with the evolution of ethylene hormone, which induces expression of the ripening-related genes and increases susceptibility of fruit to pathogen (Giovannoni, 2001). Apart from its role in fruit ripening, ethylene also activates the expression of plant defense-related proteins which might be associated with fruit resistance to pathogens (van Loon et al., 2006a). The existence of ethylene during fruit ripening increases the complexity of exogenous elicitors in inducing resistance. In tomato, postharvest treatment with different elicitors such as methyl jasmonate (MeJA), ultra-light C (UV-C) and chitosan were confirmed to be effective in controlling decays caused by *Botrytis cinerea* (Yu et al., 2009; Charles et al., 2009; Badawy and Rabea, 2009). Most of these researches have focused on fruits at green stage of maturity probably because ethylene production at this stage is of low level and ripening qualities is not completely formed. In fact, however, to acquire good qualities in commercial, tomato fruit are usually picked at higher ripening degree than mature-green stage. Therefore, it is important to evaluate the effect of elicitors on fruit defense response at other stages of maturity.

Salicylic acid (SA) plays a central role in plant resistance (Sticher et al., 1997). Exogenous application of SA increases resistance to fungal pathogen in sweet cherry (Yao and Tian, 2005) and grape berries (Derckel et al., 1998). Such an increased resistance was

Abbreviations: SA, salicylic acid; ET, ethephon; MG, mature green; BR, breaker; PR, pathogenesis-related; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; SSC, soluble solids content.

* Corresponding author at: Institute of Botany, Chinese Academy of Sciences, Nanxincun 20, Xiangshan, Beijing 100093, PR China. Tel.: +86 10 62836559; fax: +86 10 82594675.
E-mail address: tsp@ibcas.ac.cn (S.-P. Tian).

0304-4238/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2011.03.021

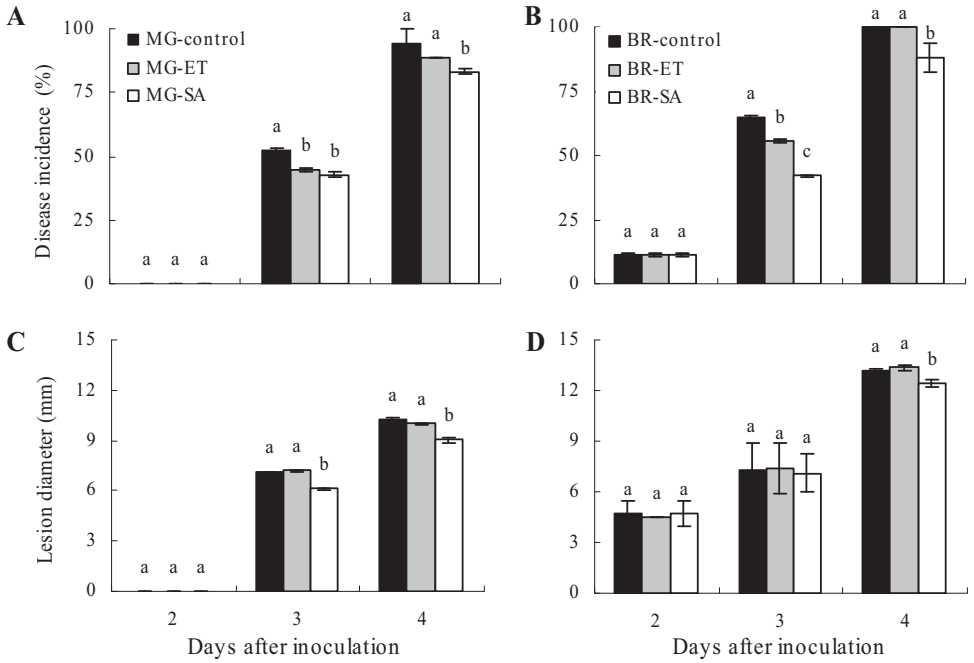

Fig. 1. Effects of salicylic acid (SA) and ethephon (ET) treatment on disease incidence and lesion diameter caused by *B. cinerea* in tomato fruits at mature green stage (MG) (A and C) and breaker stage (BR) (B and D), respectively. Tomato fruits were treated with 0.5 mmol L^{-1} ET or 5 mmol L^{-1} SA or water (control), inoculated with *B. cinerea*, and then stored at 20 °C with 90% RH for 4 days. Vertical bars represent standard errors of means and different letters above bars are significantly different at $p < 0.05$ according to Duncan's multiple range tests.

found to be correlated with enhanced expression or/and activities of glucanase and chitinase (Yao and Tian, 2005; Derckel et al., 1998). 2-Chloroethylphosphonic acid (ethephon; ET) is often used as a substitute for ethylene in triggering the ethylene signaling pathway (van Kan et al., 1995). Treatment with ET induces *glucanase* and *chitinase* expression in plant (van Kan et al., 1995). However, the effects of ET or ethylene on disease development are still unclear (Barkai-Golan et al., 2008), and few studies investigate their role in disease resistance during fruit postharvest. In this study, we used SA and ET as exogenous elicitors to treat tomato fruit at different maturity stages. We examined the resistance of tomato fruit against *B. cinerea*; the expressions of *PR1*, *PR2*, and *PR3*; and their effects on ethylene production and fruit qualities to understand whether fruit maturity stage influences the defense response of fruit to SA and ethylene.

2. Materials and methods

2.1. Fruits and treatments

Tomato (*Lycopersicon esculentum* L. cv. Fenhong) fruits were harvested at two maturity stages according to the description by Mitcham et al. (1989): mature green (MG) (fully expanded but unripe fruit with mature seed) and breaker (BR) (first visible sign of carotenoid accumulation on bottom). Fruits were selected based on the uniform size and no physical injuries or infections. Prior to use, fruits were surface-disinfected with 2% (v/v) sodium hypochlorite for 2 min, rinsed with tap water, and air-dried. Then, fruits at each stage were immersed in different solutions (5 mmol L^{-1} SA, 0.05 mmol L^{-1} ET, and distilled water) for 15 min. After drying in air, each group of fruits was further divided into two parts. One was for the inoculation experiment; the other was directly placed into plastic boxes with approx. 90% relative humidity (RH), stored at 20 °C, and sampled from fruit pericarp at various time intervals after treatments.

2.2. Pathogen and inoculation

B. cinerea was originally isolated from infected tomato fruit showing a typical gray mold symptom. The isolate was purified by a single spore isolation technique and identified based on cultural and morphological characteristics and cultured on potato dextrose agar (PDA) plates at 25 °C for 7 days. Spores were obtained by flooding the surface of the culture with sterile distilled water containing 0.05% Tween-80 (v/v). Spore suspension was filtered through four layers of sterile cheesecloth to remove any hyphal fragments. The suspension concentration was adjusted to 1×10^5 spores mL^{-1} using a hemocytometer.

Tomato fruits were treated with either SA or ET as described above. After treatment for 24 h, fruits were wounded (4 mm depth and 3 mm width) with a sterile nail and inoculated with one drop (10 μL) of the spore suspension on the surface of each wound and stored at 20 °C with 90% RH. Disease incidence and lesion diameter were measured daily according to the following formulas until all control fruits decayed. There were 3 replicates in each treatment with 15 fruits, and the experiment was repeated twice.

$$\text{Disease incidence}\,(\%) = \frac{\sum \text{Number of decayed tomato fruit}}{\sum \text{Total number of treated fruit}} \times 100$$

$$\text{Lesion diameter}\,(\text{mm}) = \frac{\sum \text{Lesion diameter of decayed tomato fruit}}{\sum \text{Total number of treated fruit}}$$

2.3. RNA isolation and semi-quantitative RT-PCR analysis

RNA isolation and semi-quantitative RT PCR analysis were performed as described previously by Moore et al. (2005) and Spencer and Christensen (1999), respectively. The glyceraldehyde 3-phosphate dehydrogenase gene (GAPDH) (gi: U93208) was used as the internal control to normalize cDNA templates. PCR conditions used were: 94 °C for 3 min, 33 cycles of 94 °C for 30 s, 57 °C for