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Effects of brassinosteroids on quality attributes and ethylene synthesis in postharvest tomato fruit

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ABSTRACT

Effects of brassinosteroids (BRs) on postharvest ripening of tomato fruit were studied in this work. Mature green tomato fruit were harvested and treated with brassinolide (BL, the most active brassinosteroid) or brassinazole (BRZ, a brassinosteroid biosynthesis inhibitor). Following treatment, fruit were stored at 20 °C with 85% RH for 20 days. Fruit quality, respiration rate, ethylene production, lycopene content, chlorophyll content and the expression of ethylene and lycopene biosynthesis related genes, including *golden 2-like* (*LeGLK2*), *phytoene synthase 1* (*LePSY1*), *ripening-related ACC synthase 2* (*LeACS2*), *ripening-related ACC synthase 4* (*LeACS4*), *1-aminocyclopropane-1-carboxylate oxidase 1* (*LeACO1*) and *1-aminocyclopropane-1-carboxylate oxidase 4* (*LeACO4*) were measured. The results showed that during fruit ripening, the application of brassinolide was effective in inducing tomato fruit ripening, increasing soluble sugars, ascorbic acid, lycopene contents, respiration rate and ethylene production, but significantly decreasing chlorophyll content compared with the control. Furthermore, the expression of *LeACS2*, *LeACS4*, *LeACO1*, *LeACO4* and *LePSY1* was increased by brassinolide treatment, while the expression of *LeGLK2* was reduced. However, fruit treated with brassinazole showed the opposite effects, where tomato fruit ripening was delayed. These findings suggest that brassinosteroids are involved in the development of fruit quality attributes and ethylene-mediated fruit ripening of tomato.

1. Introduction

Ethylene is the major hormone that initiates and controls climacteric fruit ripening, and its biosynthesis has been studied extensively in plant tissues (Srivastava and Handa, 2005; Argueso et al., 2007). There are two systems of ethylene production in plants. System-1 represents basal ethylene in unripe fruit and is regulated in an auto-inhibitory manner, whereas system-2 operates during climacteric fruit ripening and flower senescence and is autocatalytic (Barry and Giovannoni, 2007; Yokotani et al., 2009). Studies have shown that the suppression of ethylene production results in a strong ripening inhibition by knocking down 1-aminocyclopropane-1-carboxylate (ACC) oxidase (ACO) and ACC synthase (ACS) (Hamilton et al., 1990; Oeller et al., 1991). Conversely, application of exogenous ethylene to climacteric fruit at the mature stage stimulates system-2 ethylene biosynthesis, which will accelerate ripening (Nakatsuka et al., 1998).

Brassinosteroids (BRs) are plant steroid hormones known mainly for their effects on cell expansion and a wide range of developmental and physiological processes that occur ubiquitously in plants (Aghdam et al., 2012; Wang et al., 2012; Guo et al., 2013). Extensive studies using genetic, molecular and proteomic approaches have identified most of the major brassinosteroids signaling components, which have been assembled into a series of signal transduction cascades (Kim and Wang, 2010). Research over the past two decades has revealed that brassinosteroids are essential for plant development and regulate a range of physiological processes, such as stem elongation, root growth, leaf epinasty, vascular differentiation and reproductive development (Brosa, 1999; Sasse, 2003). The potential of brassinosteroids to regulate fruit ripening has also been investigated. Application of brassinosteroids to tomato fruit pericarp discs elevated levels of lycopene and lowered chlorophyll levels. Fruit ripening induced by brassinosteroids was associated with increasing in ethylene production (Vidya Vardhini and Rao, 2002). Liu et al. (2014) found that brassinosteroid response transcription factor brassinazole resistant 1 (BZR1)

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mutants could enhance carotenoid accumulation in tomato fruit. However, little is known about the relationship between brassinosteroids and ethylene during tomato fruit ripening. Also, the mechanisms involved in regulation of chlorophyll, lycopene and ethylene of postharvest fruit by brassinosteroids have not been elucidated in detail. Therefore, the aim of this study was to investigate the effects of brassinosteroids on postharvest fruit quality attributes and ethylene synthesis in tomato fruit and the possible mechanisms involved.

2. Materials and methods

2.1. Plant material and chemicals

Tomato fruit (*Solanumly copersicum* L. cv. Yuanbao) were harvested at the mature green stage from an orchard in the Huayang district, Sichuan, China, and immediately transported to the laboratory after harvest. 15–20 fruit of uniform size, maturity and free from visual blemishes and diseases were selected and randomly divided into 7 lots for each assay, which were repeated three times.

Brassinolide (BL, the most active brassinosteroid), brassinazole (BRZ, a brassinosteroid biosynthesis inhibitor), ethephon (ET) and 1-methylcyclopropene (1-MCP) were purchased from Sigma.

2.2. Treatments

The first lot tomato fruit were treated for 12 h by immersion in solutions of 3 μmol L^{-1} brassinolide diluted with distilled water and then air-dried and placed at 25 ± 1 °C. The second lot of fruit were treated for 12 h by immersion in solutions of 5 μmol L^{-1} brassinosteroid diluted with distilled water, and then air-dried and placed at 25 ± 1 °C. For ethylene treatment, one lot fruit was incubated in 500 μL L^{-1} ethephon solution in a closed container at room temperature for 12 h, and then air-dried and placed at 25 ± 1 °C. For the 1-MCP treatment, one lot fruit was placed in 20 L containers and exposed to 0.5 μL L^{-1} 1-MCP gas (SmartFresh™, 0.14% a.i., Rohm and Haas, Philadelphia, PA, USA) for 12 h at room temperature. For the ethephon + brassinazole treatment, one lot fruit was incubated in 500 μL L^{-1} ethephon solution in a closed container at room temperature for 12 h then air-dried and immediately treated with 5 μmol L^{-1} brassinazole solutions for another 12 h and then air-dried and placed at 25 ± 1 °C. For the 1-MCP + brassinolide treatment one lot fruit was placed in 20 L containers and exposed to 0.5 μL L^{-1} 1-MCP gas (SmartFresh™, 0.14% a.i., Rohm and Haas, Philadelphia, PA, USA) for 12 h at room temperature then immersed them in 3 μmol L^{-1} brassinolide solution for another 12 h. The control fruit were treated with distilled water and then air-dried and placed at 25 ± 1 °C. After treatment, tomato fruit were placed in boxes and stored at 23 ± 1 °C with approximately 90% RH for 23 days. Three replicates each of 30 fruit were used for each treatment.

2.3. Measurement of fruit quality parameters

Firmness, soluble sugars content, titratable acidity (TA) and ascorbic acid (AsA) content of the fruit were determined.

Fruit firmness of each individual tomato was measured at three points of the equatorial region by using the FT327 fruit pressure tester (Breuzzi Company, Milano, Italy). The probe penetrated the sample with a uniform force to a depth of 10 mm. The three measurements were averaged for each fruit and expressed in kg/cm. Weight loss was determined by weighing fruit at the start of the experiment and at various intervals during storage. Total soluble sugars of tomato fruit peel and pulp was extracted according to Ozaki et al. (2009). Titratable acidity was determined using the method of Ranggana (1977) by measuring the amount of 0.1 N NaOH. AsA contents of the fruit were measured according to the method of Kampfenkel et al. (1995). Each treatment contained three replicates

2.4. Measurement of total chlorophyll and lycopene content

To measure tomato total chlorophyll contents, about 5 g of fruit peel was extracted in 80% acetone and measured according to Lichtenthaler and Wellburn (1983). The absorbance of samples was read at the wavelength of 645 and 663 nm using a spectrophotometer (TU1800 spectrophotometer, P-general Limited Company, Beijing, China). Total chlorophyll content was estimated as mg/g fresh weight (FW).

Lycopene content was analyzed by the method of Marković et al. (2006). Approximately 5 g samples of fresh tomato peel and pulp was carefully weighed into a 200 mL flask wrapped with aluminum foil to keep out light. The samples of fresh tomatoes were homogenized in a blender. A 100 mL mixture of hexane–acetone–ethanol, 2:1:1 (v/v) was added to the flask and agitated continuously for 10 min on a magnetic stirrer plate. After that, 15 mL of water was added followed by another 5 min of agitation. The solution was separated into distinct polar and nonpolar layers. The hexane solution containing lycopene was filtered through 0.2-mm filter paper; and the filtrate was then diluted with a mixture of hexane–acetone–ethanol (2:1:1, v/v). The residue on the filter paper was colorless, indicating rapid and complete extraction of lycopene. Lycopene concentration was estimated by measuring the absorbance of the hexane solution containing lycopene at 472 nm on a spectrophotometer.

2.5. Measurement of respiration rate

Respiratory oxygen consumption was measured using Clark-type electrodes (Hansatech, King's Lynn, UK) as previously described (Xu et al., 2012). Approximately 0.05 g fruit peel were weighted and cut into small pieces, then pretreated with 5 mL deionized water for 15 min in order to eliminate wound-induced respiration. Measurements were done at 25 °C in a final volume of 2 mL phosphate buffer (pH 6.8), and the cuvette was tightly closed to prevent diffusion of oxygen from the air. Inhibitors of the cytochrome pathway (1 mM KCN) and the alternative pathway (0.5 mM nPG) were used. Total respiration (V_t) is defined as O_2 uptake rate by tomato peel without any inhibitor. The capacity of the alternative pathway (V_{alt}) is defined as O_2 uptake rate in the presence of 1 mM KCN. Residual respiration (V_{res}) is defined as O_2 uptake in the presence of both 1 mM KCN and 0.5 mM nPG. Cytochrome pathway capacity (V_{cyt}) was calculated by the formula: $V_{cyt} = V_t - V_{alt} - V_{res}$.

2.6. Ethylene production

For ethylene production, tomato fruit were placed in a 10 cm × 10 cm closed container for 2 h at 25 ± 1 °C and 85% RH. Then, a 1 mL sample of gas from each container headspace was injected into a FID gas chromatograph (Agilent 6890 Series GC System, Salem, MA) equipped with an activated alumina SS column. The carrier gas (helium) flow rate was 0.5 mL/s. The detector and injector were operated at 100 °C, and the oven was at 50 °C. The ethylene production is expressed as mL/kg/h.

2.7. RNA extraction and qRT-PCR for gene expression analysis

Total RNA was extracted from tomato fruit according to Xu et al. (2012). RNA contents were calculated by measuring the absorbance value taken at 260 nm. First-strand cDNA was reverse transcribed from DNase I-treated RNA with oligo (dT) as the primer. All gene