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Selenium delays tomato fruit ripening by inhibiting ethylene biosynthesis and enhancing the antioxidant defense system

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ABSTRACT

The antioxidant activity of selenium (Se) detoxifies reactive oxygen species (ROS) in plants and animals. In the present study, we elucidated the mechanism underlying Se induced fruit development and ripening. Our study showed that foliar pretreatment with 1 mg L^{-1} sodium selenate effectively delayed fruit ripening and maintained fruit quality. Gene expression studies revealed that the repression of ethylene biosynthetic genes 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase decreased ethylene production and respiration rate. Moreover, Se treatment probably boosted the antioxidant defense system to reduce ROS generation and membrane damage. The enhanced antioxidative effect was attributed to higher glutathione content and increased activity of enzymes such as glutathione peroxidase and glutathione reductase. The upregulation of respiratory burst oxidase homologue genes in tomato fruit may also contribute to the enhanced antioxidative effect. Selenium treatment represents a promising strategy for delaying ripening and extending the shelf life of tomato fruit.

1. Introduction

Selenium (Se) is a trace element essential for both plants and animals, but is toxic at high concentrations. In human beings, this element is a key component of 25 selenoproteins including glutathione peroxidase, iodothyronine deiodinases, and thioredoxin reductases, which are involved in several major regulative and protective redox mechanisms (Rayman, 2012). Low concentrations of Se have positive, antioxidant effects on plants such as enhanced plant growth (Hartikainen, Xue, & Piironen, 2000), improved cold tolerance (Hawrylak-Nowak, Matraszek, & Szymanska, 2010), drought tolerance (Hasanuzzaman & Fujita, 2011) and tolerance to UV-B radiation (Yao, Chu, & Ba, 2010) and aluminum toxicity (Cartes, Jara, Pinilla, Rosas, & Mora, 2010). Nevertheless, the mechanism underlying Se-enhanced abiotic stress resistance in plants is not well known. Se may act via regulating reactive oxygen species (ROS) and maintaining cellular structure and function under unfavourable conditions (Feng, Wei, & Tu, 2013).

Senescence is a genetically regulated oxidative process and is characterized by a general decline in physiological function (del Río et al., 1998). Se enhances the antioxidative capacity of senescing lettuce to induce growth (Xue, Hartikainen, & Piironen, 2001) and postpones senescence by reinforcing ROS scavenging systems in soybean (Djanaguiraman, Devi, Shanker, Sheeba, & Bangarusamy, 2005). Se enhances the shelf life and quality of both lettuce and chicory by lowering phenylalanine ammonia lyase (PAL) activity and ethylene production (Malorgio, Diaz, Ferrante, Mensuali, & Pezzarossa, 2009). The effects of Se on fruit physiology have become a hotspot for research in recent years. Se accumulation as a result of foliar application in leaves and fruits of peach and pear delays softening of fruits and increases their shelf-life (Pezzarossa, Remorini, Gentile, & Massai, 2012). Moreover, Se decreases the rate of ethylene biosynthesis, which delays fruit ripening in tomato (Pezzarossa, Rosellini, Borghesi, Tonutti, & Malorgio, 2014). Our earlier study on tomato fruit revealed that a foliar spray of Se can reduce postharvest decay and improve fruit quality (Zhu, Chen, Zhang, & Li, 2016). However, whether Se can affect the fruit ripening process from mature green to red ripe stage during postharvest storage remains unknown. Moreover, the molecular and biochemical mechanisms involved need to be further studied.

Ethylene controls the climacteric ripening pattern in tomato fruits (Carrari & Fernie, 2006). The genes *LeACS2*, *LeACS4*, and *LeACO1* from the relevant gene families primarily function in ethylene production (Wang, Li, & Ecker, 2002). Moreover, ROS can also regulate the fruit senescence process (Qin, Wang, Liu, Li, & Tian, 2009), which indicates that their intracellular levels should be optimal. We hypothesized that the antioxidative properties of Se were pivotal in delaying fruit ripening in post-harvest storage. Thus, the objective of the current study was to study the effect of Se on fruit ripening in tomato in post-harvest storage conditions.

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Secondly, we assessed the possible mechanism of Se action based on changes in the ROS level, key enzymes involved in oxidative metabolism, and ethylene biosynthesis genes.

2. Materials and methods

2.1. Plant material and treatments

Tomato plants (*Solanum lycopersicon*, cv. Provence) (n = 28) from a greenhouse located in Shandong, China, were fertilized with 1 mg Se L^{-1} as sodium selenate (Na_2SeO_4) at the onset of flowering (Zhu et al., 2016). Corresponding plants (n = 28) not treated with Se were used as controls. The experiments were conducted once in 2013 and twice in 2014. Tomato fruits (at the mature green stage) were harvested from Se treated and untreated (control) plants and quickly brought to the laboratory. The fruits were stored at 25 °C for 20 d in a covered plastic food tray. Since ripening does not occur at the same time for all fruits on a plant, we harvested the fruits at three independent, mature green stages. We harvested 150 fruits from each of the Se-treated and control groups every five days. The fruits were flash frozen in liquid nitrogen and stored at −80 °C until further use.

2.2. Ethylene production and respiration rate of fruit

To measure ethylene and respiration in tomato fruits, we selected six fruits (1 kg) per treatment and placed them in a 5 L air-tight jar at 25 °C. A syringe was used to extract 1 mL sample from the headspace, which was then injected into an Agilent 7820A gas chromatograph (Santa Clara, CA, USA), equipped with a stainless steel Supelco Porapak-Q column and a flame ionization detector for ethylene determination.

Respiration was measured as CO_2 production. Fruit were sealed in a jar and the air was passed through the jar. The respiration rate was measured using an infrared analyzer (GXH-3051, Beijing, China) connected to the effluent air. Three replications for each treatment were performed.

2.3. Weight loss and vitamin C

Three replicates (10 fruits per replicate) per treatment were used to determine fruit weight loss using the following formula:

$$\text{Weight loss} = (W_i - W_f)/Wi \times 100\%,$$

where W_i is the initial sample weight and W_f the final sample weight. The results were expressed as percentage weight loss.

Vitamin C content was measured as described by Kampfenkel, Van Montagu, and Inzé (1995) with a slight modification. Briefly, 2 g of fruit tissue was homogenized in 5 mL chilled metaphosphoric acid (60 mM) followed by centrifugation at 12,000×*g* for 30 min. The reduction of Fe^{3+} to Fe^{2+} by reduced vitamin C and the complex of Fe^{2+} and 2,2-dipyridyl resulted in colour development, which was detected at 525 nm. The oxidized vitamin C was reduced by preincubating the sample with dithiothreitol (DTT). Excess DTT was removed with *N*-ethylmaleimide to determine the total vitamin C content, which was determined using the standard curve of vitamin C.

2.4. Reactive oxygen species (ROS) assay and cell membrane permeability

The quantification of H_2O_2 was based on a ferrous oxidation/xylenol orange assay. hydrogen peroxide assay kit (Beyotime, China) was used to determine H_2O_2 at 560 nm. A standard curve of H_2O_2 was used for calibration, and the H_2O_2 content was expressed as μmol g^{-1}. The rate of superoxide production was measured by monitoring the nitrite formation from hydroxylamine in the presence of O_2^- as per the method of Song et al. (2009). Corrections were made for O_2^- production in the absence of hydroxylamine hydrochloride. The results were expressed as μmol g^{-1} min^{-1}.

Membrane permeability was measured as a function of electrolyte leakage, which was estimated using an EC 215 conductivity meter (Hanna Instruments, Padova, Italy) as described by Wang, Tian, and Xu (2005). Fruit discs were rinsed with deionized water and incubated in distilled water at 25 °C for 3 h to determine the initial electrolyte leakage (E_i). The tubes were then boiled in water for 20 min, cooled to room temperature, and the final conductivity (E_f) was measured. The following formula was used to calculate the relative electrolyte leakage:

$$\text{Relative electrolyte leakage} = (E_i/E_f) \times 100\%.$$

Three replicates were maintained per treatment, and each experiment was repeated three times.

2.5. Reduced glutathione and antioxidant enzymes

Ten grams of tissue (fruit flesh) was collected from 10 fruits. Three biological and three technical replicates were maintained per treatment. The DTNB-GSSG reductase recycling assay was used to measure the reduced glutathione (GSH) content (Loggini, Scartazza, Brugnoli, & Navari-Izzo, 1999). Neutralized samples and samples pretreated with 2-vinyl pyridine were used to determine total glutathione and oxidized glutathione (GSSG). The total glutathione and GSSG were calculated using a standard curve. GSH was determined by subtracting GSSG from the total glutathione content. The determined GSH content was expressed as μmol g^{-1}.

A cellular glutathione peroxidase assay kit (Beyotime, China) was used to determine glutathione peroxidase (GPX) activity (assayed by coupling to NADPH oxidation in the presence of excess glutathione reductase). GPX activity was expressed as U mg^{-1} protein, where one unit represents the nmol of NADPH oxidized per minute at 25 °C.

Glutathione reductase (GR) activity was assayed by monitoring glutathione-dependent oxidation of NADPH at 340 nm using the glutathione reductase assay kit (Beyotime, China) as per the manufacturer's instructions. NADPH oxidation corrections were made in the absence of GSSG and the results were expressed as U mg^{-1} protein, where one unit represented the nmol of NADPH oxidized per minute at 25 °C.

Protein content was determined using a BCA protein assay kit (Pierce, Thermo Scientific, USA) with bovine serum albumin as standard.

2.6. Gene expression analysis

Ten fruits were sampled for RNA isolation using the method of Moore, Payton, Wright, Tanksley, and Giovannoni (2005). RNA was reverse-transcribed using a PrimeScript™ RT reagent kit with gDNA Eraser (TaKaRa, Dalian, China) as per the manufacturer's instructions. An Illumina Eco real-time PCR system was used for qPCR (Illumina, Gene Company Limited) using SYBR *Premix Ex Taq*™ II (Tli RNaseH Plus) (TaKaRa). The primers for the target genes and the reference genes were designed according to previous studies (Table 1) (Dong et al., 2013; Ebrahimi, Abdullah, Aziz, & Namasivayan, 2015; Fujisawa et al., 2014; Li et al., 2015; Wang, Feng, & Chen, 2009). Three replicates were maintained per reaction and a negative control was used for every run. PCR was performed using the following cycle: 95 °C for 30 s, followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s. Relative expression levels were