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Exploring the effects of selenium treatment on the nutritional quality of tomato fruit

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ABSTRACT

In this work, the effects of selenium (Se) on the nutritional quality of tomato fruit were investigated. The results showed that application of 1 mg L^{-1} sodium selenate foliar spray increased Se content in the fruit without affecting other concentrations of other metals. Se treatment elevated the contents of soluble sugars (glucose and fructose), amino acids, and bioactive compounds, such as flavanoids, glutathione, vitamin C, and vitamin E, in pink tomato fruit. Proteomic analysis using isobaric tags for relative and absolute quantification was performed on tomato fruit at the commercial harvest stage. When comparing Se-enriched tomato fruit with the control group, we identified an aggregate of 269 differentially expressed proteins. Proteins involved in carbohydrate metabolism, amino acids metabolism, and secondary metabolism were highly affected by Se treatment. The results help elucidate the mechanism of Se treatment on improved nutritional quality of tomato fruit.

1. Introduction

Tomato is one of the most widely produced and consumed fruits worldwide. It is rich in antioxidant vitamins, and potassium, and it is free of cholesterol, which makes the tomato an important dietary component (Perveen et al., 2015). To improve the nutritional quality of tomato fruit, a number of strategies have been attempted, such as appropriate macroelement supplementation (Fanasca et al., 2006), fertilization with low nitrogen content fertilizers (Bénard et al., 2009), careful control of temperature and irradiance conditions (Gautier et al., 2008), and genetic engineering approaches (Davuluri et al., 2005).

Selenium (Se), in agriculture applications, has been studied for decades. In plants, low concentrations of Se can enhance plant growth (Turakainen, Hartikainen, & Seppänen, 2004), improve plant yield (Hu, Xu, & Pang, 2003), and counteract oxidative stress induced by diverse abiotic factors (Feng, Wei, & Tu, 2013) or pathogen infection (Zhu, Chen, Zhang, & Li, 2016). In some crops, Se also improves plant quality. According to a previous study, the addition of selenate to nutrient solutions improved the quality and the shelf life of lettuce and chicory (Malorgio, Diaz, Ferrante, Mensuali, & Pezzarossa, 2009). In another work, spraying Se on peach and pear fruit delayed fruit softening and enhanced the shelf life (Pezzarossa, Remorini, Gentile, & Massai, 2012). Our previous work on tomato fruit showed that a foliar spray of 1 mg L^{-1} sodium selenate postponed fruit ripening and maintained fruit quality by reducing the production of ethylene and reactive oxygen species (ROS) (Zhu, Chen, Shi, & Zhang, 2017). These studies implicate the role of Se in regulating the antioxidant system and maintaining cell structure stability. In recent years, several studies have indicated that Se also has beneficial effects on the nutritional quality of higher plants. For example, the application of Se increased the overall amino acid and vitamin C content in green tea leaves (Hu et al., 2003). Additionally, Se treatment enhanced the sensory quality of broccoli by increasing the release of volatile compounds (Lv et al., 2017). More specifically, Se fertilization affected the myrosinase-glucosinolate system and the antioxidant properties in broccoli heads and sprouts (Mahn, 2017; Tian, Xu, Liu, Xie, & Pan, 2016). Furthermore, Se-enriched broccoli showed antiproliferative activity, especially in seedlings (Bachiega et al., 2016). In tomato fruit, the phenolic acid and flavonoid contents were altered by foliar spray with selenate (Schiavon et al., 2013). To date, few studies on improving the nutritional quality of fruit by Se pretreatment have been conducted, and the mechanism involved remains unknown.

In addition to the beneficial effects on various crops, the human health benefits of dietary Se have become popular in scientific research. Rayman (2012) reported that an adequate supplementation of 50–200 μg of Se per day reduces the risk of several human diseases, including autoimmune thyroid disease, cardiovascular disease, type-2

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diabetes, viral infections, and cancer. However, Se deficiency occurs in many areas around the world, especially in China, Africa, India, and Eastern Europe (Finley, 2005). Se-enriched crops serve as a good dietary resource for selenium supplementation due to their high bioaccessibility and bioavailability (Thiry, Ruttens, De Temmerman, Schneider, & Pussemier, 2012). Therefore, the application of Se treatments in crops shows great potential for improving fruit quality and Se supplementation in humans.

Isobaric tags for relative and absolute quantification (iTRAQ) is a new quantification platform in proteomics that uses isotope labeling coupled with two-dimensional liquid chromatography and tandem mass spectrometry (LC-MS/MS). Owing to its high sensitivity and accuracy, iTRAQ has been applied in proteomics analysis of tomato fruit (Pan et al., 2014; Wang et al., 2014). In the current study, we investigated the impact of Se on the nutritional quality of tomato fruit, and iTRAQ-based quantitative proteomics analysis was implemented to identify Se responsive proteins in order to identify the mechanism of Se treatment on tomato fruit.

2. Materials and methods

2.1. Plant material and treatments

Tomato plants (*Solanum lycopersicon*, cv. Provence) (n = 10) from a greenhouse in Shandong, China, were subjected to foliar spray application of 1 mg Se L^{-1} as sodium selenate (Na_2SeO_4) at the onset of flowering. This Se concentration was selected based on our preliminary trials and previous study (Zhu et al., 2016). Corresponding plants (n = 10) treated with water served as a control group. Tomato fruits were harvested at commercial maturity (pink stage) from Se treated and control plants, and fruits were quickly transported to the laboratory. A minimum of 120 fruits were harvested from each of the Se-treated and control plants. Half of the fruits were used for fruit quality determination, and the other half were used for proteomic analysis. They were frozen in liquid nitrogen and kept at −80 °C until they were needed.

2.2. Determination of mineral content

Mineral content was determined according to our previous method (Zhu et al., 2016). Dried fruit tissue (100 mg) per treatment was digested in HNO_3 (65%) and H_2O_2 (30%), and the final solution was analyzed via inductively coupled plasma mass spectrometry (ICP-MS).

2.3. Quantification of sugars, organic acids, and amino acids

Sugars (glucose and fructose), and organic acids (malic acid and citric acid) were quantified as described by Zhu, Liu, Li, and Tian (2013), Wu, Génard, Lescourret, Gomez, and Li (2002). Briefly, fruit tissue (3 g) was extracted with ultrapure water and centrifuged at 4 °C. The supernatant was refined with an Agilent SEP-C18 cartridge and a 0.45 μm membrane filter. Sugars and organic acids were quantified with a Dionex P680 HPLC system. The separation of sugars was performed on a Transgenomic CARB Sep Coregel 87C column at 85 °C. The mobile phase used double-distilled water with a flow rate of 0.6 mL min^{-1}, and a Shodex RI-101 refractive index detector was employed to track the eluted sugars.

We employed an Inertsil ODS-3 column at 40 °C to perform organic acids analysis. The mobile phase used 0.02 M of KH_2PO_4 with a flow rate of 0.8 mL min^{-1}. We used a Dionex PDA-100 detector at 210 nm to identify the organic acids. We approximated the concentrations of sugars and organic acids by observing the peak areas based on the external standard solution calibrations. We acquired the sugars and organic acids standards from Sigma Chemical Co.).

The amino acids were analyzed as described by Mazumder, Morvan, Thakur, and Ray (2004). The fruit sample (0.1 g) was hydrolyzed with 6 M of HCl at 110 °C for 24 h in a vacuum-sealed tube. The extracted amino acids were determined using a Hitachi L-8900 amino acid analyzer.

2.4. Bioactive substance determination

Total flavonoids were determined by a colorimetric method described by Kim, Jeong, and Lee (2003). Absorbance was measured at 510 nm, and the content of total flavonoids was calculated according to a rutin standard curve.

A glutathione assay kit (Beyotime, China) was used to determine glutathione (GSH) content. Absorbance was measured at 412 nm.

Lycopene content was measured by a colorimetric method described by Choi and Huber (2008). Absorbance was measured at 503 nm, and lycopene content was calculated using the molar extinction coefficient of 17.2 L mol^{-1} m^{-1}.

Vitamin analyses were performed at the Analysis Laboratory of Beijing Nutrition Resources Institute. Vitamin E content was determined by HPLC, following Chinese Standard GB/T 5009.82-2003. Total vitamin E concentration is the sum of α-vitamin E, β-vitamin E and γ-vitamin E. Vitamin C content was determined with fluorometric method that follows Chinese standards GB/T 5009.86-2003. Vitamin B3 (niacin) concentration was determined with the microbial technique, that follows Chinese standards GB/T 5009.89-2003.

2.5. Protein extraction and digestion

We ground 10 g of frozen tissue taken from 20 fruit into powder and suspended in 30 mL of extraction buffer (0.05 M of Tris-HCl pH 8.0, 2 mM of EDTA, 1 mM of PMSF, 0.1 M of KCl, 0.7 M sucrose). Then, 30 mL of Tris-saturated phenol was placed into the extract. The mixture was homogenized and centrifuged at 15,000*g* for 10 min. The upper phase was discarded, and the extraction procedure was performed again. Four volumes of saturated ammonium (in methanol) were placed in with the extract. The mixture was incubated overnight and then centrifuged at 15,000*g* for 10 min. The precipitated protein pellet was successfully acquired and dissolved in lysis buffer (8 M of urea, 50 mM of triethylammonium bicarbonate (TEAB), pH 8.5, and 2% CHAPS) with constant shaking for 1 h. The protein supernatant was gathered following centrifugation at 15,000*g* for 10 min, and the protein concentration was determined with a Protein Assay Kit (Bio-rad, Hercules, CA, USA).

A total of 100 μg protein was reduced by dithiothreitol (DTT) for an ultimate concentration of 10 mM, which was incubated at 56 °C for 1 h. Then, 55 mM iodoacetamide was added, and the mixture was incubated for 1 h in the dark. Samples were diluted in 50 mM TEAB containing 10 mM DTT. Finally, protein was digested with trypsin (Promega, Madison, WI, USA) at 37 °C overnight.

2.6. iTRAQ labeling and SCX fractionation

Peptides were labeled with iTRAQ-8plex reagents (Applied Biosystems, CA, USA) according to the manufacturer's protocol. Samples were labeled with reagents as follows: Se 1 (113 tag), Se 2 (114 tag), Se 3 (115 tag), Control 1 (116 tag), Control 2 (117 tag), Control 3 (118 tag). The peptides were incubated for 2 h at room temperature. Then, the peptides were combined and vacuum-dried. All labeled peptide mixtures were dissolved in buffer (10 mM of KH_2PO_4, 25% ACN, pH 2.6) accompanied by centrifugation at 15,000*g* for 10 min. The peptides were loaded onto a strong cation exchange (SCX) chromatography column (Phenomenex Luna SCX, 4.6 mm in diameter, 250 mm in length, 100 Å, 5 μm particle size, USA). The eluted peptides were separated into 16 fractions and desalted with a Strata-X C18 column (Phenomenex, USA).