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Protective structures and manganese amendments effects on antioxidant activity in pepper fruit

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ABSTRACT

Sweet pepper (*Capsicum annuum* L.) is an excellent source of bioactive nutrients. Manganese is a crucial micro-element for plant nutrition, has a role in photosynthesis, and is a cofactor in antioxidant enzymes. Pepper fruit grown under a protective environment are nevertheless exposed to high temperatures during the summer that cause oxidation-related heat damage to the pericarp beneath the fruit skin. We demonstrate that high temperatures significantly modify total antioxidant activity in peppers at different ripening stages. Pepper cultivars differ in their antioxidant activity, which was correlated with their ability to cope with high temperature damages. Manganese applied to pepper plants modifies antioxidant activity, but different cultivars responded differently to the manganese application. In summary, pepper fruit grown under temperature stress will possess higher antioxidant activity than those grown at lower temperatures and will therefore be of higher nutritional value for human consumption. Agronomic practices such as growing conditions or manganese supply may alter, in a cultivar-specific manner, the antioxidant activity and the nutritional values of the crop.

1. Introduction

Sweet pepper (*Capsicum annuum* L.), an important high-value vegetable cash crop cultivated in the Mediterranean basin, is grown under protective structures (greenhouse, net-house, high and low tunnels) that are critical to the successful production of fresh vegetables (Gruda, 2005). In fact, plants grown in any simple protective structure (e.g., greenhouse, net-house, or tunnels) are exposed to elevated daytime temperatures that can be as high as 45 °C and are typically higher than those outside the greenhouse. The main limiting factors in pepper production under such high temperatures include the reduction of reproductive organ viability and of pollination and a consequent decrease in fruit set and quality (Aloni et al., 2001; Erickson and Markhart, 2002; Polowick and Sawhney, 1985; Rylski and Spigelman, 1982). In work with detached young pepper fruit, Silber et al. (2009) found that exposure to high temperature increased the concentration of hydrogen peroxide (H_2O_2) and probably of other oxygen radicals in the fruit apoplastic fluid, changes that may also cause heat damage (pale spots) (Silber et al., 2009).

Sweet pepper is an excellent source of bioactive nutrients such as ascorbic acid, provitamin A (carotenoids), phenolic compounds, and potassium (Navarro et al., 2006; Sun et al., 2007). Natural antioxidants recognized as beneficial in preventing a broad range of cancers and cardiovascular diseases (Byers and Perry, 1992). Effective free radical scavengers, these antioxidant compounds may also protect against age-related macular degeneration and cataracts (Howard et al., 2000). Pepper fruit antioxidant content changes during fruit ripening and is affected by abiotic stress such as salinity (Howard et al., 2000; Navarro et al., 2006).

Manganese (Mn), an essential nutrient in most, if not all, organisms (Pittman, 2005), is particularly important in photosynthetic organisms such as plants, where a cluster of Mn atoms is required for the light induced oxidation of water that occurs in photosystem II. In addition, Mn is also a required cofactor for a variety of enzymes such as Mn^{2+}-dependent superoxide dismutase (MnSOD) (Armstrong, 2008; Pittman, 2005). Past research on Mn supplementation in soilless pepper cultivation showed that Mn reduced the incidence of crop heat damage (Silber et al., 2009). In this work, it was found that H_2O_2 content, SOD and ascorbic acid oxidase activities in detached young pepper fruit supplemented with Mn were modified in the fruit apoplastic tissue.

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The objective of this work was to elucidate the role of Mn in pepper fruit quality under different protective structures. We hypothesized that Mn modifies the total antioxidant content, and in doing so, it may improve the ability of the pepper plant to cope with the damage associated with the increase temperatures under protective environment conditions.

2. Materials and methods

2.1. Experiment description and manganese treatments

On May 1, 2012, 4-week-old pepper plants were transplanted into a greenhouse or a net-house located at the Besor Experimental Station in Israel (31°16′ N, 34°23′ E) where they were grown until November 2012. Both greenhouse and net-house were 250 m^2 in size, wall height were 3.8 m and cover with 50 mess net, total height of both structure was 6 m at the highest point of the roof.

The three cultivars identified as numbers 7227 and 7199 (*C. annuum* L. 'Dalias' and 'Rita', respectively, Zeraim Gedera, Revadim, Israel) and as *C. annuum* L. 'Romans' (Soli, Kiriat Malachi, Israel), are commonly grown in this region and were selected due to their differential susceptibilities to high temperature damage. Previous experiments indicated that the three cultivars have high, moderate and low susceptibility to high temperature damage, respectively. Plants were irrigated using a drip system consisting of lateral pipes situated parallel to each pepper row.

Temperature data were collected over the course of the entire growing season from each greenhouse using data loggers (HOBO U23 Pro V2, Onset Computer Corporation, Inc., Bourne, MA, USA).

2.1.1. Manganese treatment and analysis

Manganese treatment was begun when the first flower appeared and continued until the end of the experiment. Mn from concentrated liquid Mn-EDTA chelate (Mn 18.5 g L^{-1}, Fertilizers & Chemicals Ltd., Haifa, Israel) was injected into the irrigation solution using an online proportional pump. The supplementary Mn treatments results in Mn concentration of 2.5 mg L^{-1} in the irrigation solution as compare to 0.3 mg L^{-1} with no Mn addition. To determine plant Mn uptake among the different treatments and cultivars, Mn contents in the leaves and fruit were determined before the beginning of fruit harvest.

2.1.2. Manganese analysis

Total Mn was determined in diagnostic leaves (the youngest fully expanded leaf, usually leaf number 4–5 from the apical meristem), 10 of which were collected from each plot. The leaves were rinsed with de-ionized water, dried at 70 °C and ground. Similarly, four mature green fruit were harvested from each plot for Mn determination. The dry tissues were ground to pass through a 20-mesh sieve, and 100-mg samples were wet ashed with $HClO_4$–HNO_3. Leaf and fruit Mn contents were determined by atomic absorption spectrophotometer (AAnalyst 200, Perkin-Elmer, Akron, OH, USA).

To elucidate possible direct Mn antioxidant activity in the pepper fruit extract (see Section 2.2), a sub-sample of the extract was taken and centrifuged at 7000 rpm for 10 min and then at 13,000 rpm for 10 min to remove debris. Clear extract was acidified with concentrated H_2SO_4 (3% final acid concentration) for sugar removal, and Mn concentration in the extract was determined using an atomic absorption spectrophotometer as described above.

2.2. Antioxidant content analysis

Sample preparation: 2 g of fresh pepper samples were homogenized in 100 mM of phosphate buffer saline, pH = 7.4. Samples were immediately frozen and kept at −20 °C until analyzed within 90 days. In preparation for analyses, samples were thawed and then centrifuged at 10,000 rpm for 5 min. The supernatant was used for analysis.

2.2.1. Cyclic voltammetry (CV)

Solutions of pepper fruit extracts were placed in a CV cell equipped with a working electrode (3.2 mm in diameter, glassy carbon), a reference electrode (Ag/AgCl), and an auxiliary electrode (platinum wire). The potential was applied linearly to the working electrode at a constant rate (100 mV/s) toward the positive potential (i.e., evaluation of reducing equivalents). An electrochemical working station (CH Instruments Inc., 610B, Austin, TX, USA) was used. During CV operation, a potential current curve was recorded. In general, the anodic potential represents the identity of the reducing equivalent or of the group of reducing equivalents and is expressed in volts. The current measured at each peak potential ($E1$ and $E2$) correlates with the reducing equivalent concentration according to the Randles–Sevcik equation [$Ip = 2.69 \times 10^5\, n^{3/2} A D^{1/2} C n^{1/2}$] (Bard and Faulkner, 1980). Method theory and details was reviewed elsewhere (Kohen et al., 1999).

2.2.2. Differential pulse voltammetry (DPV)

DPV analysis was done using the same electrochemical working station and electrode as above. Activation of the working electrode potential was done at a scan rate of 40 mV/s, pulse amplitude of 50 mV, sample width of 17 ms, pulse width of 50 ms, and a pulse period of 200 ms. The output of the DPV experiments was a potential–current curve (Kohen et al., 1999).

2.2.3. Ferric reducing antioxidant power (FRAP) assay

The method measures the ferric reducing ability of plasma (FRAP). At low pH, when a ferric-tripyriltriazine (Fe^{III}-TPTZ) complex is reduced to ferrous (Fe^{II}) form, an intense blue color with an absorption maximum at 593 nm develops. The antioxidant capacity of each fruit extract as describe for CV and DPV was estimated according to the procedure described by Benzie and Strain (1996). FRAP values were calculated relative to FRAP values of an ascorbic acid calibration curve.

2.3. Determination of heat damage

High temperatures may cause damage to mature fruit that will reduce fruit quality to the extent that it is not suitable for export. Heat spots were observed on mature red fruit at the beginning of harvest season of fruit grown under high temperature conditions. Thirty fruit from each plot were used to determine the severity of heat damage on a three-step scale (to measure the incidence of symptoms) from low to intermediate to high. The severity of heat spot damage was determined based on the percentage of fruit's surface area covered with heat spots and the size of each individual spot on the fruit. Data are presented as the percentage of fruit exhibiting each severity level of damage from the total fruit of the specific sample.

2.4. Statistics

Results are reported as the means from five different blocks of the same cultivars. Each analytical method was done with at least three triplicates. Statistical analysis was performed using ANOVA test and LSMeans Student's t post-test at $p < 0.05$, using GraphPad InStat version 3.05 (GraphPad Software, San Diego, CA, USA). Manganese content data were analyzed using two-way ANOVA with JMP 10.0 software (SAS Institute Inc., Cary, NC, USA). Default significance levels were set at $a = 0.05$.