Fruits 75(2), 55–70 | ISSN 0248-1294 print, 1625-967X online | https://doi.org/10.17660/th2020/75.2.1 | © ISHS 2020

Original article

High MT-sHSP23.6 expression and moderate water deficit influence the antioxidant system in 'Micro-Tom' tomato fruit under hypoxia

G. Niemeyer Reissig[1,a], D.A. Posso[2], J. Borella[3], R. Vieira Dutra da Silveira[2], C.V. Rombaldi[1] and M.A. Bacarin[2]

[1] Department of Agroindustrial Food Science and Technology, Eliseu Maciel Agronomy College, Federal University of Pelotas, Brazil

[2] Department of Botany, Biology Institute, Federal University of Pelotas, Brazil

[3] Department of Crop Science, Institute of Agronomy, Federal Rural University of Rio de Janeiro, Brazil

Summary

Introduction – **Pre-harvest abiotic factors can influence post-harvest responses during storage. sHSPs are important to maintain cellular homeostasis and may influence the antioxidant system. We investigated the influence of moderate water deficit in the antioxidant system of 'Micro-Tom' tomato fruit with different MT-sHSP23.6 expression levels under hypoxia storage.** *Materials and methods* – **Wild type and high MT-sHSP23.6 expression 'Micro-Tom' tomato fruit were used. The fruits were harvested at the breaker stage from plants under normal irrigation or under water deficit. After the harvest, the fruits were subjected to hypoxia. Color tone, antioxidant enzymes, reactive oxygen species, non-enzymatic antioxidant compounds, and antioxidant activity were evaluated.** *Results and discussion* – **At the end of the storage, the Sense genotype that expressed higher MT-sHSP23.6 level under water deficit had redder color tone. Transformed genotype showed the highest activity under hypoxia for all antioxidant enzymes analyzed, both for irrigated and water deficit treatments. Sense genotype under water deficit in the post-hypoxia period showed the highest lycopene content. At the end of the storage, the total phenols content was higher and also the water deficit treatments had the highest content of total phenols. On the harvest day, treatments under water deficit had the highest content of L-ascorbic acid. Additionally, at the end of the storage, irrigated treatments showed the highest content of L-ascorbic acid. We have found that at the end of the storage period, tomato under water deficit treatment had higher radical scavenging activity, as well as high total phenols and lycopene content.** *Conclusion* – **Water deficit and expression level of MT-sHSP23.6 influenced the components of the antioxidant system.**

Keywords

antioxidant enzymes, antioxidant metabolites, irrigation suspension, low oxygen storage, post-harvest physiology, sHSP

Significance of this study

What is already known on this subject?

- The MT-sHSP23.6 protein is important for plant cellular homeostasis and it may influence plant antioxidant system.

What are the new findings?

- The results demonstrated the influence of MT-sHSP23.6 protein on the antioxidant system of tomato fruits subjected to mild water deficit and hypoxia.

What is the expected impact on horticulture?

- Knowing the role of small heat shock proteins, such as MT-sHSP23.6, is an important part of understanding plant plasticity mechanisms.

Introduction

Tomato (*Solanum lycopersicum* L.) is a horticultural crop of great importance worldwide because it is consumed by many people, both natural and processed (Slimestad and Verheul, 2009). During cultivation and post-harvest storage, tomato plants may face several biotic and abiotic stress factors that may lead to loss of productivity and/or quality. However, if these factors are applied intentionally (moderated and controlled), this can lead to an accumulation of compounds that increase the quality of the fruit. Also, it can increase the plant's tolerance and prepare it for subsequent stress (Capanoglu, 2010; Pedreschi and Lurie, 2015; Ripoll *et al*., 2014).

Similarly to the other living organisms with different levels of complexity, tomato plants contain a group of proteins that play a fundamental role in the maintenance of its protein cellular homeostasis. They also act as molecular chaperones under normal conditions, or when subjected to some stressors, and are known as heat shock proteins (HSPs) (Demidchik, 2015; Fu, 2014; Rodziewicz *et al*., 2014).

Stress factors such as water deficit and hypoxia can lead to stress response, known as oxidative damage, which can damage the components of the cell and cause its dysfunction. Oxidative stress is caused by overproduction and accumulation of reactive oxygen species (ROS). Owing to a disorder in normal cell physiology, it leads to an imbalance between the production and detoxification of ROS (Demidchik, 2015; Morales and Munné-Bosch, 2016). However, water deficit can also be used as a pre-treatment to increase plant tolerance

[a] Corresponding author: gabriela.niemeyer.reissig@gmail.com; Tel.: + 55 53 981167941.

to future stress. Different types of abiotic stresses share the same responses; thus, by using different stresses (through cross-tolerance) it is possible to perform pre-acclimatization of plants (Capanoglu, 2010; Ripoll *et al*., 2014).

Plants have evolved strategies to deal with oxidative stress, such as the synthesis of antioxidant enzymes that remove ROS. Some examples of antioxidant enzymes are catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and guaiacol peroxidase (GPOD). In addition to the enzymatic antioxidant system, the plants produce specialized metabolites that perform various functions in the cells and may have antioxidant capacity. Examples of these compounds are tocopherols, L-ascorbic acid, phenolic compounds, and carotenoids (Demidchik, 2015; Mittler, 2017).

In circumstances where redox unbalance occurs in the cells, the HSPs proteins and the antioxidant system complement each other to increase the plant's tolerance. While antioxidants (enzymatic and non-enzymatic) promote the detoxification of ROS in the cell, HSPs act to prevent structural damage to cellular proteins (Timperio *et al*., 2008). Previous studies with tomato plants overexpressing a mitochondrial small HSP (MT-sHSP23.6) showed an increment in tomato plant's plasticity (Huther *et al*., 2018). They also demonstrated that these plants are able to restore their photosynthetic parameters after heat stress (Huther *et al*., 2013). However, no evaluation of fruit during ripening and with an abiotic pre-harvest treatment has been carried out until now.

We acknowledged that HSPs proteins, and especially MT-sHSP23.6, are involved in the development of plant tolerance to the most diverse environmental stress factors. Thus, we hypothesized that the greater expression of the gene encoding this protein (when the plants are grown under pre-harvest water deficit and post-harvest hypoxia, or a combination of these abiotic factors) will: 1) promote a greater activity of antioxidant enzymes, and 2) lead to a higher accumulation of non-enzymatic antioxidant compounds in 'Micro-Tom' tomatoes at the reproductive stage.

The aim of this study was to investigate the influence of a pre-harvest moderate water deficit and post-harvest hypoxia on the antioxidant system of two tomato-plant genotypes with different MT-sHSP23.6 expression levels.

Materials and methods

Plant growth and treatments

Seeds of two tomato genotypes of cultivar 'Micro-Tom' were used. The first genotype was the wild-type (WT) and the second one was that with the high expression of MT-sHSP23.6 protein (Sense). The transformation stability and physiological behavior of tomato plants under normal cultivation conditions have been described previously (Huther *et al*., 2013; Huther *et al*., 2018).

The seeds of each genotype were germinated in Gerbox® with blotting paper moistened with distilled water. They remained in the germinating chamber (25 °C, photoperiod of 12 h) for a period of 10 days. After this period, the seedlings were transplanted into 0.5-L plastic pots filled with commercial organic substrate. The tomato plants were grown in a greenhouse at the Federal University of Pelotas, Capão do Leão Campus (geographical coordinates: 31°52'32"S, 52°21'24"W, altitude 13 m).

After the transplantation, the plants were irrigated on alternate days (0.05 L). Additionally, 0.015 L of nutrient solution (Hoagland and Arnon, 1938) was applied three times a week. The irrigation and the nutrient solution were suspended for eight days (induction of water deficit) until the fruits were still green (84 days after germination). During the applied water deficit, the irrigated plants were watered normally but they have not received a nutrient solution. The average temperature in the greenhouse during the experimental period was 25 ± 5 °C. The irradiance with natural light was 8×10^{-4} mol photons m^{-2} s^{-1}. During the irrigation suspension, stomatal conductance and soil moisture were monitored.

The watering and the adding of nutrient solution were restored when the soil moisture reached about 3% and the conductance was approximately 50% lower than that of the control (which corresponded to eight days of treatment). After two days of irrigation reestablishment, the fruit reached the breaker stage (approximately 90 days after sowing the seeds). At this time they were harvested and then subjected to hypoxic conditions.

Twelve fruit of the hypoxic treatment from the two genotypes were harvested, stored in transparent plastic pots (0.5 L) and kept in the dark at 23 °C. The plastic pots had an aeration control system consisting of an inlet and outlet for the gas flow. The hypoxic conditions were generated by the introduction of nitrogen gas and they were scanned daily (0.098 MPa for 10 min). This procedure was performed during the three days of hypoxia. After the three days of hypoxia, the samples were exposed to normal atmospheric condition for five days. Three fruit were collected immediately before the storage (0 days) and after the hypoxia treatment. After the returning to normal atmospheric condition, three fruit were collected on the first and fifth days, corresponding to 4 and 8 days after the beginning of the post-harvest treatment, respectively. At each sampling point, a color analysis was performed on the fruit epicarp, which was immediately stored at -86 °C for further biochemical analysis.

Color tone analysis

The color tone changes during fruit storage were evaluated using the Minolta CR-300 colorimeter with the parameters L^* (luminosity), a^* and b^*. Three fruit from each biological repetition were randomly chosen. In an attempt to better represent the coloration, three readings were taken from different parts of each fruit. The results were expressed in hue angle (h°) by the equation:

$$h° = [\text{arc tangent } (b^*/ a^*)].$$

Enzymatic antioxidant system

Enzyme activity was measured on samples from 3 fruit of each biological replicate (± 200 mg), which were homogenized with 5% (w:v) polyvinylpolypyrrolidone (PVPP) and 100 mM potassium phosphate buffer, pH 6.0 containing 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 20 mM sodium ascorbate. The homogenate was centrifuged at 12,000 *g* for 20 min at 4 °C and the supernatant obtained was used as crude enzyme extract (Azevedo *et al*., 2006). In order to express the enzymatic activity as a specific activity, the content of the total soluble proteins was determined from the same extract (Bradford, 1976).

Superoxide dismutase (SOD – EC 1.15.1.1) activity was tested by monitoring the inhibition of the nitro blue-tetrazolium (NBT) coloration at 560 nm (Giannopolitis and Ries, 1977). The results were expressed in U kg^{-1}. The concentrations are expressed on a protein mass basis.

Catalase (CAT – EC 1.11.1.6) activity was determined as described by Beers and Sizer (1952). It is based on the oxidation rate of hydrogen peroxide. Its activity was monitored

by the decrease in absorbance at 240 nm (ε_{240nm} = 39.4 × 10^3 M^{-1} cm^{-1}) for two min at a reading interval of 10 s. The results were expressed in mmol min^{-1} kg^{-1} H_2O_2 on a protein mass basis.

Ascorbate peroxidase (APX – EC 1.11.1.11) activity was analyzed according to Nakano and Asada's (1981) method using sodium ascorbate as a substrate. The activity was monitored by the ascorbate oxidation rate for 2 min with absorbance reading in the range of 10 s at 290 nm (ε_{290nm} = 2.80 × 10^3 M^{-1} cm^{-1}). The results were expressed in mmol min^{-1} kg^{-1} AsA on a protein mass basis.

Guaiacol peroxidase (GPOD 1.11.1.7) activity was analyzed as described by Azevedo *et al.* (2006) by monitoring the production rate of tetraguaiacol at 470 nm (ε_{470nm} = 26.6 × 10^3 M^{-1} cm^{-1}) in a spectrophotometer for 2 min in a 10 s interval. The results were expressed as mmol min^{-1} kg^{-1} H_2O_2 on a protein mass basis.

Non-enzymatic antioxidants

Total phenols were determined spectrophotometrically according to the methodology proposed by Singleton and Rossi (1965). Approximately, 250 mg of tomato fruit sample was weighed and diluted in methanol. The quantification of the total phenols was performed through a calibration curve obtained by reading the absorbances of gallic acid standards. The absorbance was read at 725 nm and the results were expressed in g kg^{-1} gallic acid equivalent on a fresh weight basis.

Carotenoids β-carotene and lycopene were determined by spectrophotometry according to the methodology proposed by Nagata and Yamashita (1992). The absorbances were read at 453, 505, 645 and 663 nm. The following equations were used to calculate the concentration of β-carotene and lycopene: β-carotene (g L^{-1}) = $0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}$; lycopene (g L^{-1}) = $-0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$. The results were expressed in g kg^{-1} β-carotene/lycopene on a fresh weight basis.

L-ascorbic acid was determined according to the method of Oliveira (2010). L-ascorbic acid was quantified spectrophotometrically at 520 nm using a calibration curve obtained from L-ascorbic acid standards. The results were expressed as g kg^{-1} L-ascorbic acid on a fresh weight basis.

Hydrogen peroxide and superoxide content

The hydrogen peroxide content was determined using the method described by Velikova *et al.* (2000). The H_2O_2 content was measured on a standard curve prepared with known concentrations of hydrogen peroxide. The absorbance was read at 390 nm and the results expressed in µmol kg^{-1} H_2O_2 on a fresh weight basis.

The superoxide anion was determined using the methodology of Elstner and Heupel (1976). The absorbance was measured at 530 nm. The superoxide anion content was calculated using a standard curve of sodium nitrite ($NaNO_2$). The results were expressed in µmol kg^{-1} $O_2^{\bullet-}$ on a fresh weight basis.

Radical scavenging activity

Radical scavenging activity by the capture of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) was performed by a spectrophotometer according to the method proposed by Brand-Williams *et al.* (1995). The same extract obtained for determination of total phenolic compounds was used. The absorbance was read at 517 nm.

Following the method of Rufino *et al.* (2007), using a spectrophotometer, we did a radical scavenging activity – the capture of ABTS radical (2,2′-Azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]). We used the same extract obtained for determination of total phenolic compounds. The absorbance was read at 734 nm and both results were expressed as radical scavenging activity (%) of DPPH and ABTS.

Experimental design and statistical analysis

Each treatment consisted of four biological replicates (three fruits per replicate) for each genotype of 'Micro-Tom' tomato. All variables analyzed were evaluated in triplicate. The experimental data were analyzed as a completely randomized design in a factorial scheme, where one factor was "Genotype" (2 levels) and the other was "Water deficit" (2 levels). Analysis of variance (ANOVA) was performed. When the interaction was significant, the interaction deployment was performed to compare the means by the Tukey test. Genotypes were compared within each level of water deficit. The levels of water deficit were compared within each level of genotypes. The significance level was $P \leq 0.05$. The data were also subjected to Pearson's correlation coefficient analysis. The analyses were performed with R 3.5.1 (2018).

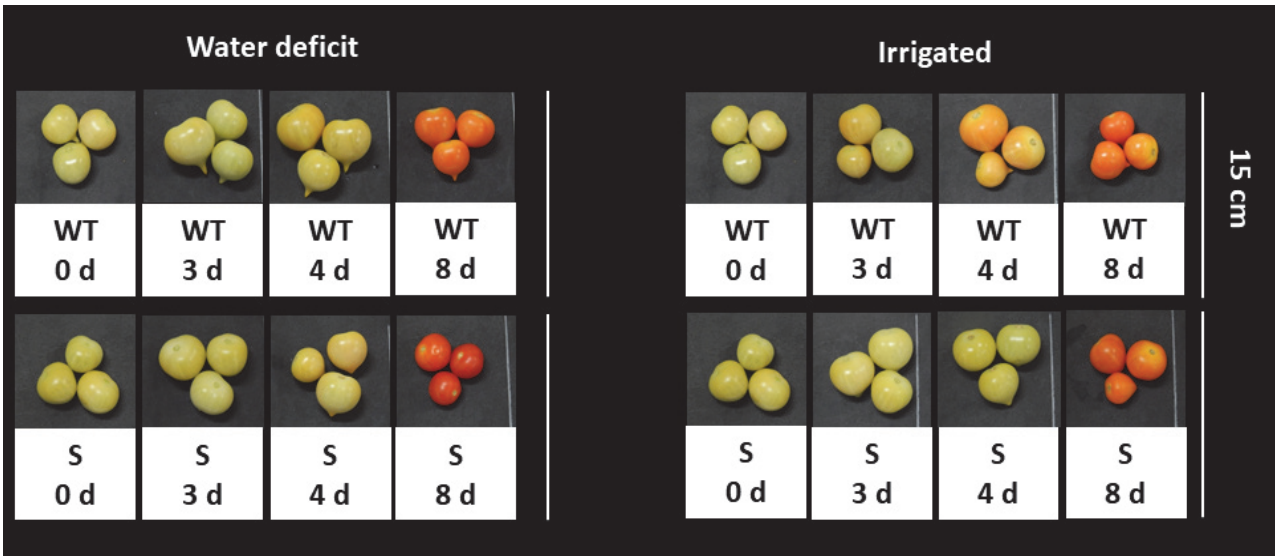

Figure 1. Changes in 'Micro-Tom' tomato fruit color subjected to pre-harvest irrigation, water deficit, and post-harvest hypoxia storage. WT: wild type; S: high MT-sHSP23.6 expression.

Results

A significant interaction was found between all evaluated variables (see Supplementary Information 4) for which the interaction unfolding was made. Without considering the genotype, it was not clear whether the effect of water deficit on the antioxidant system was stronger or weaker than that of the irrigation. It was not also clear if the enzymatic activity, phytochemicals content, or antioxidant activity was greater in a given genotype, as responses will depend on the treatment.

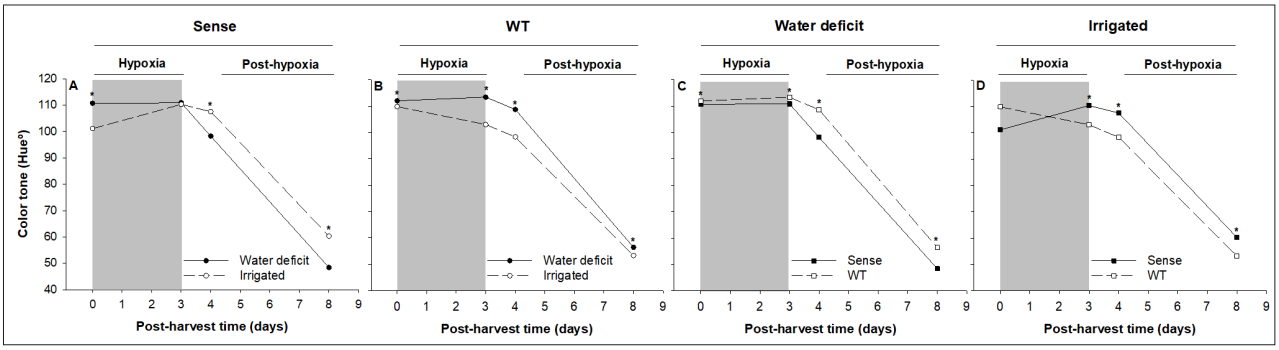

FIGURE 2. Color tone (°Hue) of 'Micro-Tom' tomato subjected to pre-harvest irrigation, water deficit, and post-harvest hypoxia storage (A, B, C, and D). Multiple comparisons between genotypes within each level of irrigation and irrigation within each level of genotype. Values represent the mean ($n = 4$). WT: wild type; Sense: high MT-sHSP23.6 expression.

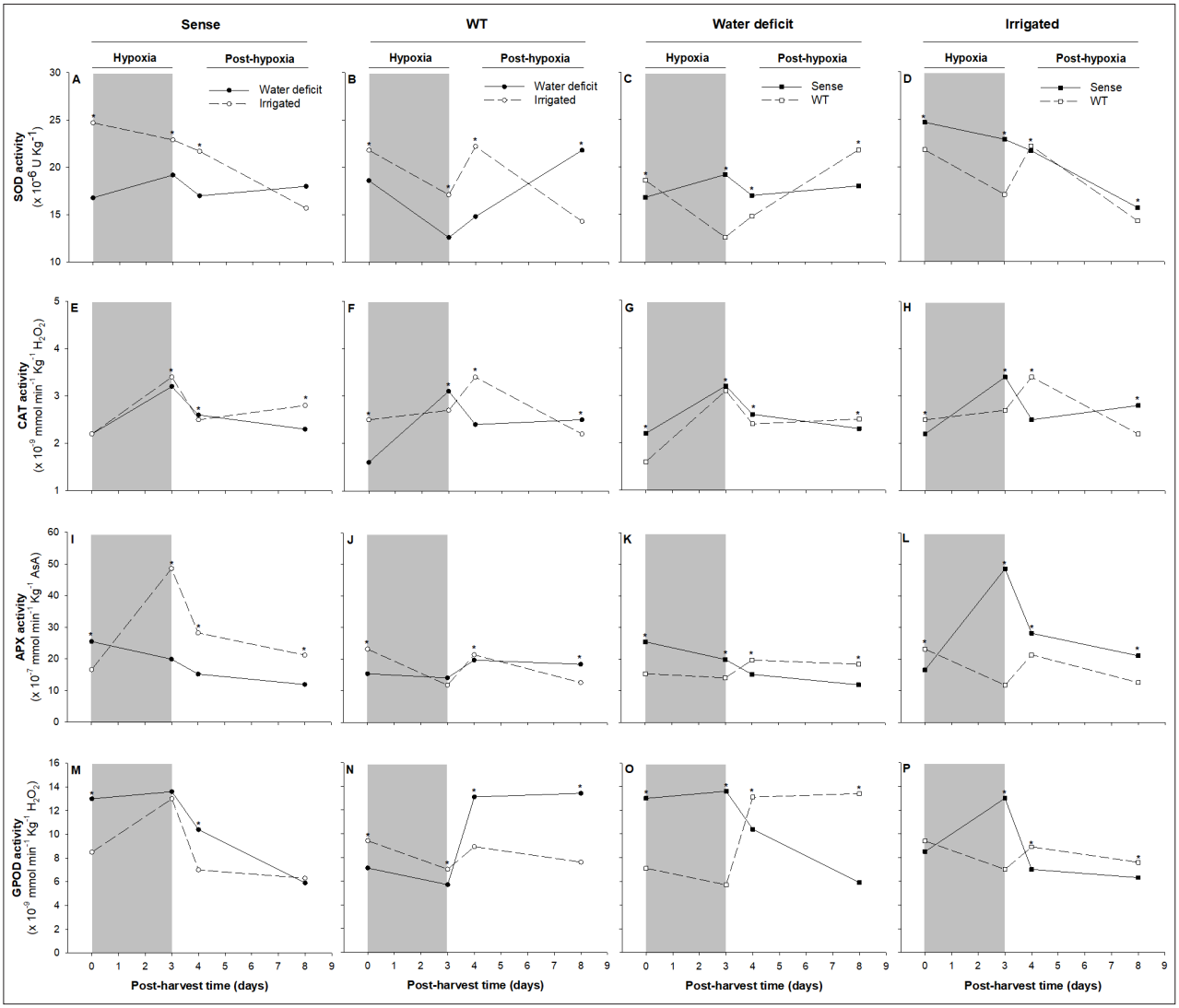

FIGURE 3. Antioxidant enzymes activity of 'Micro-Tom' tomato subjected to pre-harvest irrigation, water deficit, and post-harvest hypoxia storage. Multiple comparisons between genotypes within each level of irrigation and irrigation within each level of genotype. Superoxide dismutase (SOD) activity (A, B, C, and D); Catalase (CAT) activity (E, F, G, and H); Ascorbate peroxidase (APX) activity (I, J, K, and L); Guaiacol peroxidase (GPOD) activity (M, N, O, and P). Values represent the mean ($n = 4$). WT: wild type; Sense: high MT-sHSP23.6 expression.

Ripening evolution

Considering the Sense genotype (Figure 2A), we found that the color tone of water deficit treatment tended to be more reddish (Hue angle closer to 0°) after the hypoxic period. The opposite was found for WT genotype, where the color tone of irrigated treatment tended to be more reddish after the hypoxia period (Figure 2B). A similar trend appears when comparing genotypes between each irrigation level. The Sense genotype showed reddish color under water deficit (Figure 2C), but under irrigation (Figure 2D), WT genotype had the reddish color tone.

Enzymatic antioxidant system

The SOD activity was higher in the irrigated treatments until the fourth day of storage. Both (Sense and WT) genotypes demonstrated high activity (Figures 3A and 3B), but comparatively, Sense had higher activity. The water deficit treatment had a higher SOD activity on the last day of the experiment. The WT genotype under water deficit showed higher activity on the first and the last day of the storage (Figure 3C). The irrigated Sense genotype showed a similar trend (Figure 3D). The Sense genotype had a higher activity on the third day of storage for both irrigated and water deficient treatments.

The irrigated Sense genotype had a higher CAT activity on the third and eighth days of the storage, but water deficient treatment had higher activity only on the fourth day (Figure 3E). The irrigated WT genotype showed higher activity on the first and third days (Figure 3F). The Sense genotype under water deficit had higher activity until the fourth storage day (Figure 3G) but the irrigated (Figure 3H) Sense genotype showed higher activity only on the third and eighth days.

With the exception of the first day, the irrigated Sense genotype (Figure 3I) had a higher APX activity. On the other hand, the irrigated WT (Figure 3J) showed a higher APX activity on the first and third days. The Sense genotype grown-up under water deficit (Figure 3K) showed the higher activity on the first and third days of the storage, while the irrigated (Figure 3L) Sense genotype exceeded WT's activity during the whole period with the exception of the first day.

The Sense genotype grown-up under water deficit (Figure 3M) showed higher GPOD activity on the first and fourth days. The irrigated WT genotype (Figure 3N) had higher GPOD activity during hypoxia than did the water-deficient

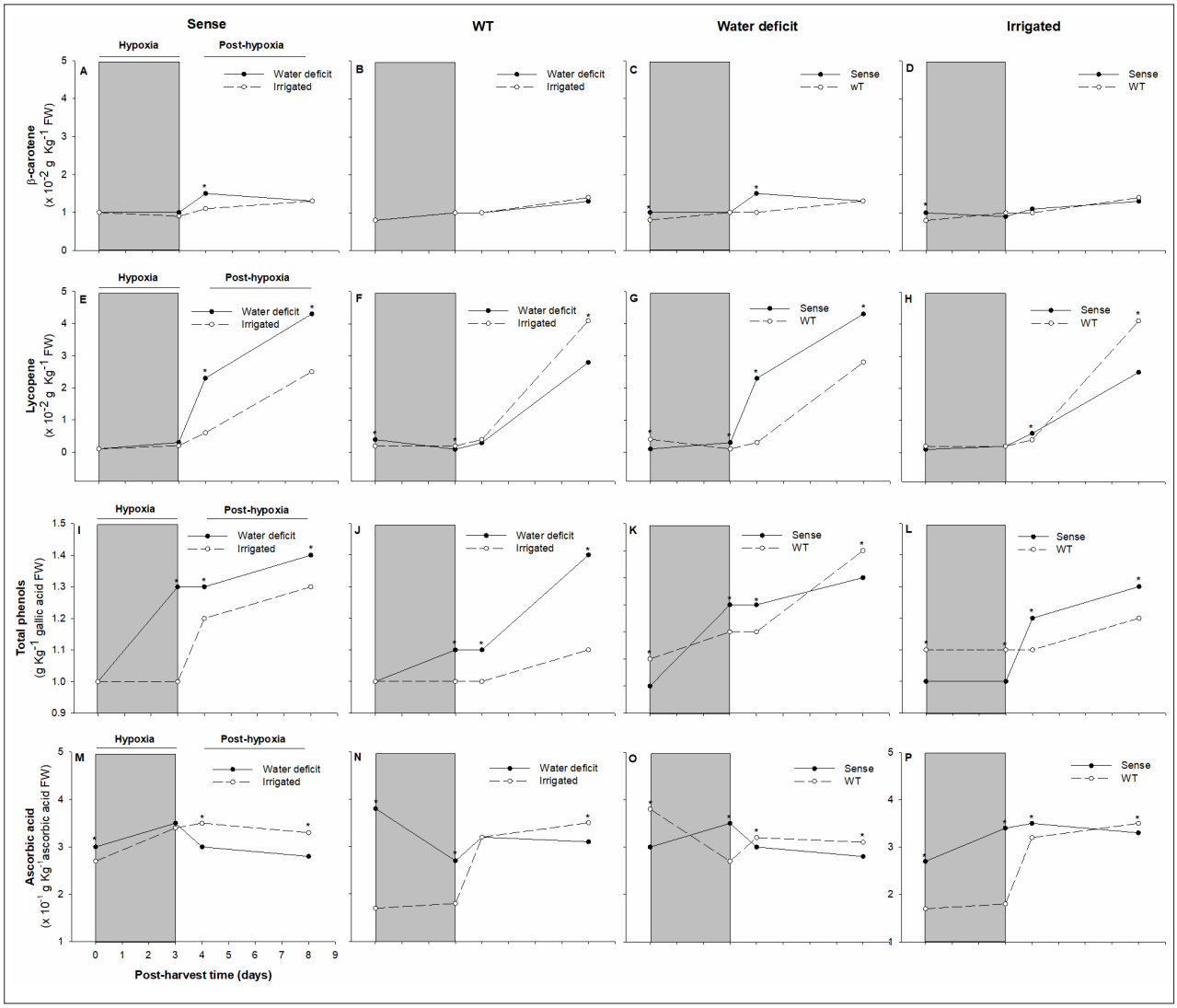

Figure 4. Non-enzymatic antioxidant system of 'Micro-Tom' tomato subjected to pre-harvest irrigation, water deficit, and post-harvest hypoxia storage. Multiple comparisons between genotypes within each level of irrigation and irrigation within each level of genotype. β-carotene (A, B, C, and D); Lycopene (E, F, G, and H); Total phenols (I, J, K, and L); L-ascorbic acid (M, N, O, and P). Values represent the mean ($n = 4$). WT: wild type; Sense: high MT-sHSP23.6 expression.

one. Additionally, the post-hypoxia water deficient treatment showed a higher GPOD activity. On the other hand, drought-stressed Sense genotype (Figure 3O) had a higher GPOD activity on the first two days of the storage, while WT had its peak on the last two days. The irrigated (Figure 3P) Sense genotype had a higher activity in the hypoxia period, but WT in the post-hypoxia period.

Non-enzymatic antioxidant system

Drought-stressed Sense genotype (Figure 4A) showed the higher concentration β-carotene only on the fourth storage day. There was no significant difference ($P \leq 0.05$) between WT genotype under water deficit and irrigated conditions (Figure 4B). Considering the irrigation levels, drought-stressed (Figure 4C) Sense genotype showed higher β-carotene content than that of WT genotype on the first and fourth storage day. When irrigated (Figure 4D), the two genotypes showed a significant difference ($P \leq 0.05$) only on the first day. The Sense genotype was the one with the greater β-carotene content.

Drought-stressed Sense genotype (Figure 4E) showed the higher lycopene content in the post-hypoxia period compared to the irrigated one. On the contrary, drought-stressed WT genotype (Figure 4F) showed higher lycopene content on the first day compared to the irrigated treatment; the latter, in turn, had higher lycopene content than the water deficit treatment on the fourth and eighth days. Turning back to irrigation levels, it is obvious that drought-stressed (Figure 4G) WT genotype shows higher lycopene content than did the Sense genotype only on the first storage day. However, when irrigated (Figure 4H), the Sense genotype had greater lycopene content than did the WT genotype on the fourth day, but the opposite was observed on the eighth day.

Drought-stressed Sense genotype showed higher total phenols content than did the WT genotype in almost all storage days with the exception of the first one (Figures 4I and 4J). In relation to irrigation levels, drought-stressed (Figure 4K) Sense genotype had higher total phenol content than did the WT genotype on the third and fourth days, while WT showed higher levels on the first and last days. When irrigated (Figure 4L), WT genotype showed higher phenol content than did the Sense genotype during hypoxia, but the opposite was documented in post-hypoxia conditions.

The Sense genotype under water deficit had higher L-ascorbic acid content than did the irrigated treatment on the first day; however, the irrigated treatment had higher content post-hypoxia (Figure 4M). Drought-stressed WT genotype showed higher content of L-ascorbic acid during hypoxia than did the irrigated one. The irrigated genotype showed higher content on the last day (Figure 4N). Under different irrigation levels, drought-stressed (Figure 4O) Sense genotype showed higher content of L-ascorbic acid than did the WT genotype only on the third storage day; however, the opposite was documented during the rest of the observed period. With the exception of the last day, the irrigated (Figure 4P) Sense genotype had the higher concentration of L-ascorbic acid during hypoxia period, but in the post-hypoxia period, WT genotype showed significantly higher concentrations of L-ascorbic acid.

Reactive oxygen species

The Sense genotype under water deficit (Figure 5A) had a higher content of hydrogen peroxide only in the first storage day, while irrigated one had higher content on the third and eighth days. The WT genotype (Figure 5B) showed almost the same trend, with the exception of the fourth storage day, when drought-stressed treatment had higher H_2O_2 content than did the irrigated treatment. WT and Sense genotypes were compared within each irrigation level. There was no significant difference ($P \leq 0.05$) between the genotypes under water deficit (Figure 5C). The irrigated Sense genotype showed higher H_2O_2 content than did the WT genotype on the first and fourth storage days, but the opposite was observed on the last storage day (Figure 5D).

Drought-stressed Sense genotype (Figure 5E) showed higher superoxide anion content than did the irrigated one on the third and fourth days, while the opposite was true on the first and last days. WT genotype under water deficit (Fig-

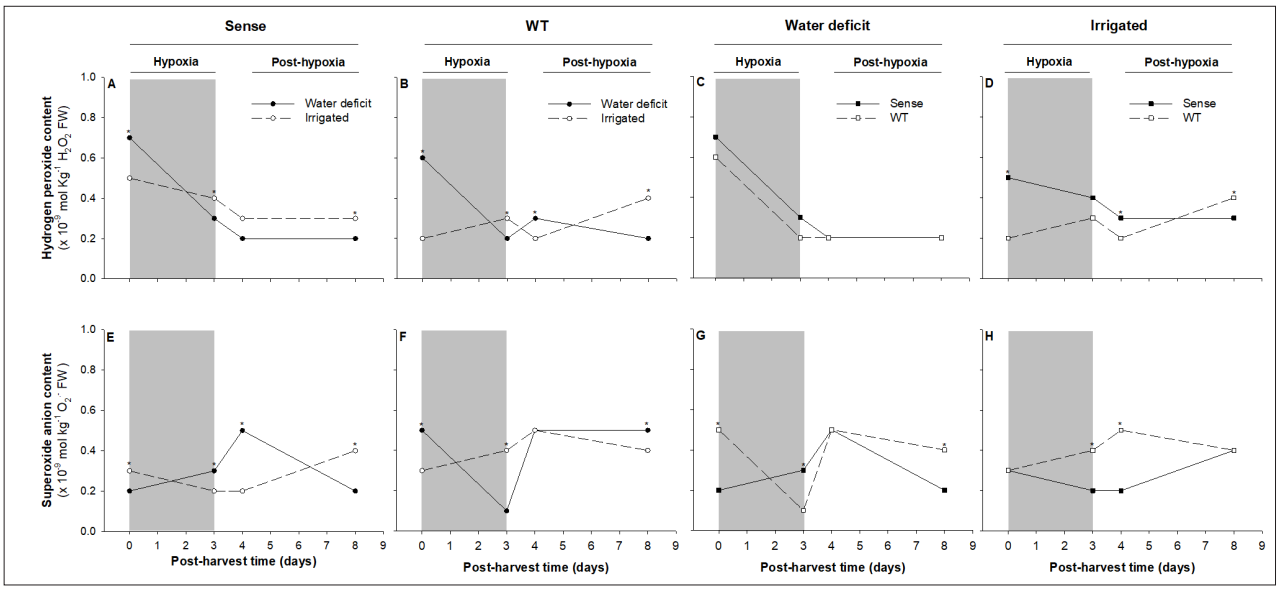

Figure 5. Superoxide anion and hydrogen peroxide content of 'Micro-Tom' tomato subjected to pre-harvest irrigation, water deficit, and post-harvest hypoxia storage. Multiple comparisons between genotypes within each level of irrigation and irrigation within each level of genotype. Superoxide anion (A, B, C, and D); Hydrogen peroxide (E, F, G, and H). Values represent the mean ($n = 4$). WT: wild type; Sense: high MT-sHSP23.6 expression.

ure 5F) showed higher content of superoxide anions than did the irrigated one on the first and eighth days. The irrigated WT genotype showed higher content on the third day. When WT and Sense genotypes were compared within each irrigation level, drought-stressed Sense genotype exceeded WT genotype only on the third storage day. Under irrigation, a significant difference ($P \leq 0.05$) in superoxide anion content between the genotypes was found only on the third and fourth days. WT genotype had significantly higher superoxide anion content.

Antioxidant activity

The Sense genotype grown-up under water deficit (Figure 6A) showed a higher percentage of DPPH radical inhibition in the post-hypoxia period, while the irrigated one showed a higher percentage on the third storage day. WT genotype (Figure 6B) grown-up under water deficit showed a higher percentage of radical inhibition than did the irrigated one for all storage periods evaluated. WT and Sense genotypes were compared within each irrigation level. Drought-stressed (Figure 6C) WT genotype showed a higher percentage of radical inhibition than did the Sense genotype for all storage periods evaluated. Under irrigation (Figure 6D), there was no significant difference ($P \leq 0.05$) between the genotypes on the first day. However, on the other storage days Sense genotype showed a higher percentage of radical inhibition.

The Sense genotype under water deficit showed a higher percentage of ABTS radical inhibition than did the irrigated one in almost all storage days, with the exception of the third storage day, when the irrigated one showed higher radical inhibition levels (Figure 6E). WT genotype (Figure 6F) showed a higher percentage of radical inhibition than did the irrigated one on the first, third, and, eighth storage days. WT and Sense genotypes were compared within each irrigation level. Drought-stressed (Figure 6G) WT genotype had a higher percentage of radical inhibition than did Sense genotype only on the last storage day. Sense genotype had a higher radical inhibition on the other days. Under irrigation, we observed the same trend (Figure 6H).

Discussion

The color tone is a good feature to follow 'Micro-Tom' tomato fruit ripening evolution. The Sense genotype under water deficit had higher ripening evolution. According to some studies (Arias *et al.*, 2000; Chen *et al.*, 2014), the red color of a ripe tomato is mainly associated with lycopene content. Water deficit can promote the accumulation of tomato fruit ethylene content, which in turn may increase lycopene content (Chen *et al.*, 2014). However, water deficit will not always generate a greater accumulation of compounds such as carotenoids (Ripoll *et al.*, 2014). The water deficit can also decrease the fruit's yield (lower humidity and size) which can raise apparently the concentration of phytochemicals (Chen *et al.*, 2014). Drought-stressed WT genotype did not show the same response. Related to the irrigated WT genotype, we observed higher values of lycopene on the last day of storage, indicating that the transformation of a plant from the same species to a greater expression of MT-sHSP23.6 causes a difference in the accumulation of these compounds. The overexpression of MT-sHSP23.6 associated with water deficit increased lycopene accumulation.

We observed that under the hypoxia period the transformed genotype (Sense) both irrigated and with moderated water deficit had the highest activity for all analyzed enzymes. This showed that the overexpression of MT-sHSP23.6 influences the enzymatic antioxidant system during low oxygen storage. Interestingly, we observed that the treatments under water deficit rarely showed a higher enzyme activity on the harvest day. Generally, when plants are subjected to an abiotic factor that can cause stress they increase the activity of enzymes related to the ROS scavenging (Nora *et al.*, 2012; Reissig *et al.*, 2018). Probably, the simulated water-deficient conditions were not sufficient to create an increase in the activity of these enzymes being the genotype a factor that influences the most. With the exception of CAT activity, the genotype with the high MT-sHSP23.6 expression (Sense) had the highest enzyme activity on the harvest day. This means that it is difficult to establish steady covariations for all enzymes in relation to a stressor agent.

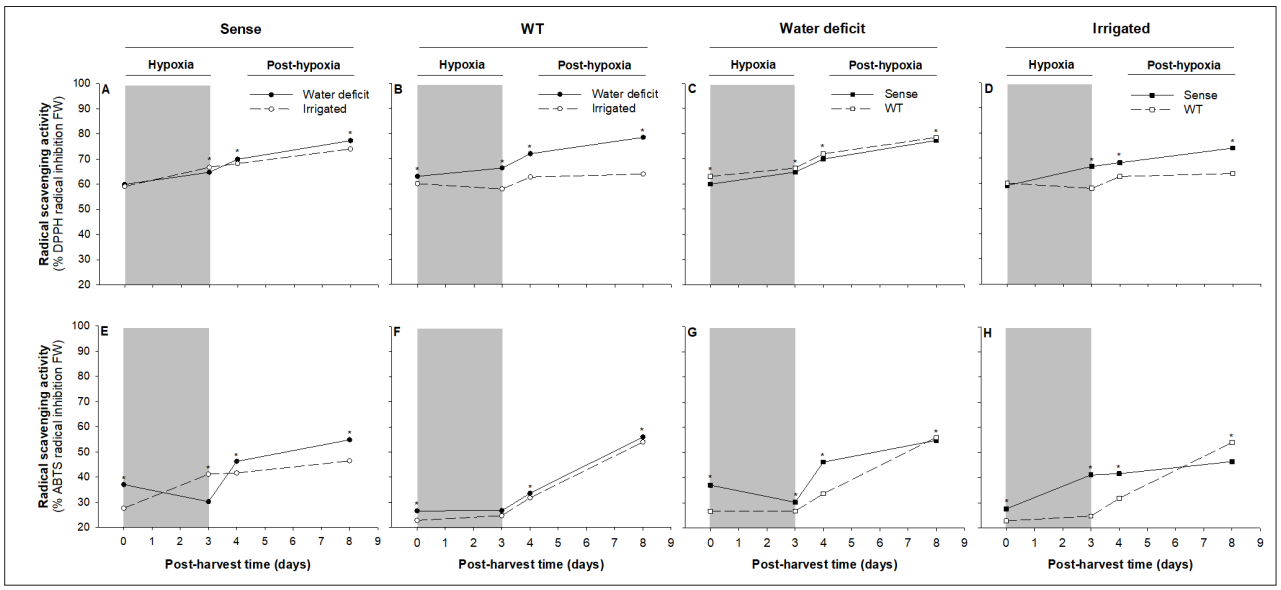

Figure 6. Antioxidant activity of 'Micro-Tom' tomato subjected to pre-harvest irrigation, water deficit, and post-harvest hypoxia storage. Multiple comparisons between genotypes within each level of irrigation and irrigation within each level of genotype. ABTS radical inhibition (A, B, C, and D); DPPH radical inhibition (E, F, G, and H). Values represent the mean ($n = 4$). WT: wild type; Sense: high MT-sHSP23.6 expression.

The fourth storage day had the highest superoxide anion concentration for almost all treatments. Reestablishing of normal oxygen conditions promotes a respiration increase leading to a higher accumulation of anion superoxide that originates from the electron escape from the electron transport chain (Perotti *et al*., 2014). However, irrigated Sense genotype showed the highest content on the eight day. The high expression of MT-sHSP23.6, and probably its highest concentration, decreases the formation of ROS in the resumption of oxygen conditions. Either by the antioxidant activity of the small HSPs or by the protection given to other proteins due to their action as a molecular chaperone (Aghdam *et al*., 2015; Reissig *et al*., 2018). On the last storage day, we observed that the genotype and the water deficit influenced the superoxide anion content. What we have found was that the Sense genotype had markedly the lowest content.

We did not find a significant correlation between the enzymes and ROS. As an exception was the APX ($r = -0.94$ with H_2O_2) and GPOD ($r = -0.88$ with H_2O_2) within the irrigated WT genotype and CAT ($r = -0.85$) within the same genotype under water deficit. In addition to the enzymatic antioxidant system, plants have different mechanisms to deal with the excess of ROS and free radicals. Small HSPs may have antioxidant activity (Aghdam *et al*., 2015), as well as alternative oxidase, mitochondrial uncoupling protein, L-ascorbic acid, carotenoids, and phenols (Rodziewicz *et al*., 2014).

Pre-harvest abiotic factors, such as water deficit, can influence the phenotype at harvest. When used moderately, it might enhance fruit tolerance to different stresses through up-regulation of genes and pathways (Pedreschi and Lurie, 2015; Toivonen and Hodges, 2011). Water deficit is known to increase the accumulation of several non-enzymatic antioxidants in the fruit. Applied moderately and in specific developmental stages, it may improve fruit quality. During fruit ripening, moderate water deficit may increase the content of potentially bioactive phytochemicals, which are very important for human health (González-Chavira *et al*., 2018; Ripoll *et al*., 2014).

β-carotene and lycopene had the highest content in the post-hypoxia period, especially the genotype with high MT-sHSP23.6 expression under water deficit. The sHSP proteins may act as molecular chaperones. Studies with Orange (OR) and ClpB3 chaperones showed that these proteins protect enzymes from the carotenoid biosynthetic pathway, deoxyxylulose 5-phosphate synthase, and phytoene synthase. They also promote the differentiation of chromoplasts and prevent carotenoid degradation (D'Andrea *et al*., 2018).

During hypoxia and post-hypoxia period, water deficit treatments had a higher accumulation of total phenols. Thus, water deficit may influence carotenoid, phenol, and L-ascorbic acid accumulation in different ways. There are at least two mechanisms that may occur and interact. One of them is caused by a reduction in primary metabolites that are a precursor of secondary metabolite biosynthesis. This is caused by a reduction in leaf stomatal conductance, which consequently leads to a decrease in net photosynthesis. Another mechanism is the stress/oxidative signaling, where ROS is responsible to affect directly and indirectly the biosynthesis of phenols, carotenoids, and L-ascorbic acid (Fanciullino *et al*., 2014; González-Chavira *et al*., 2018; Ripoll *et al*., 2016).

L-ascorbic acid seems to be the most prominent antioxidant component analyzed on the harvest day and is directly influenced by water deficit. It is well known that together with other antioxidant components, L-ascorbic acid plays an important role in plant protection from oxidative damage caused by abiotic stress factors (Toivonen and Hodges, 2011). The L-ascorbic acid acts as a sequestrant of ROS, forming compounds with a lower reactivity, such as ascorbyl radical and dehydroascorbic acid. Also, together with APX, it participates in the ascorbate-glutathione cycle H_2O_2-scavenging pathway (Davey *et al*., 2000). During hypoxia period, the high MT-sHSP23.6 expression influenced the response. Both irrigated and water-deficient treatments had higher L-ascorbic acid content when compared to the WT genotype.

We found a higher antioxidant activity in water deficit treatments at the end of storage. They also had a higher content of phenols and lycopene. Pearson's correlation analysis showed a high correlation for these components. WT genotype had $r = 0.96$ for DPPH and total phenols content. ABTS and lycopene content also showed a strong correlation ($r = 0.97$). The transformed genotype (Sense) had $r = 0.93$ for DPPH and total phenols content, as well as $r = 0.96$ for ABTS and lycopene content. These results demonstrate the biological activity of phenols and carotenoids as antioxidants that help plant cells to maintain their redox balance.

Conclusions

High MT-sHSP23.6 expression and pre-harvest water deficit influence the responses in the antioxidant system of tomato fruit subjected to low-oxygen storage. The activity of antioxidant enzymes is markedly influenced by the high expression of MT-sHSP23.6 during the hypoxia storage period. The water deficit has a predominant influence in the non-enzymatic antioxidant system. It was demonstrated by the higher levels of total phenols, L-ascorbic acid, and antioxidant activity measured by DPPH and ABTS radical scavenging methods.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasil (CAPES), Finance Code 001. Authors are also grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support provided.

References

Aghdam, M.S., Sevillano, L., Flores, F.B., and Bodbodak, S. (2015). The contribution of biotechnology to improving post-harvest chilling tolerance in fruits and vegetables using heat-shock proteins. J. Agr. Sci. *153*, 7–24. https://doi.org/10.1017/S0021859613000804.

Arias, R., Lee, T.C., Logendra, L., and Janes, H. (2000). Correlation of lycopene measured by HPLC with the *L**, *a**, *b** color readings of a hydroponic tomato and the relationship of maturity with color and lycopene content. J. Agric. Food Chem. *48*, 1697–1702. https://doi.org/10.1021/jf990974e.

Azevedo, A.D., Prisco, J.T., Enéas-Filho, J., Abreu, C.E.B., and Gomes-Filho, E. (2006). Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environm. Exp. Bot. *56*, 87–94. https://doi.org/10.1016/j.envexpbot.2005.01.008.

Beers Jr., R.F., and Sizer, I.W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. *195*, 133–140.

Bradford, M.M. (1976). Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. *72*, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Brand-Williams, W., Cuvelier, M.E., and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol. *28*,

25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.

Capanoglu, E. (2010). The potential of priming in food production. Trends Food Sci. Technol. *21*, 399–407. https://doi.org/10.1016/j.tifs.2010.05.001.

Chen, J., Kang, S., Du, T., Guo, P., Qiu, R., Chen, R., and Gu, F. (2014). Modeling relations of tomato yield and fruit quality with water deficit at different growth stages under greenhouse condition. Agr. Water Mgt. *146*, 131–148. https://doi.org/10.1016/j.agwat.2014.07.026.

D'Andrea, L., Simon-Moya, M., Llorente, B., Llamas, E., Marro, M., Loza-Alvarez, P., Li, L., and Rodriguez-Concepcion, M. (2018). Interference with Clp protease impairs carotenoid accumulation during tomato fruit ripening. J. Exp. Bot. *69*, 1557–1568. https://doi.org/10.1093/jxb/erx491.

Davey, M.W., Montagu, M.V., Inzé, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D., and Fletcher, J. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. J. Sci. Food Agric. *80*, 825–860. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<825::AID-JSFA598>3.0.CO;2-6.

Demidchik, V. (2015). Mechanisms of oxidative stress in plants: From classical chemistry to cell biology. Environm. Exp. Bot. *109*, 212–228. https://doi.org/10.1016/j.envexpbot.2014.06.021.

Elstner, E.F., and Heupel, A. (1976). Inhibition of nitrite formation from hydroxylammonium-chloride: a simple assay for superoxide dismutase. Anal. Biochem. *70*, 616–620. https://doi.org/10.1016/0003-2697(76)90488-7.

Fanciullino, A.L., Bidel, L.P.R., and Urban, L. (2014). Carotenoid responses to environmental stimuli: integrating redox and carbon controls into a fruit model. Plant Cell Environm. *37*, 273–289. https://doi.org/10.1111/pce.12153.

Fu, X. (2014). Chaperone function and mechanism of small heat-shock proteins. Acta Biochim. Biophys. Sin. *46*, 347–356. https://doi.org/10.1093/abbs/gmt152.

Giannopolitis, C.N., and Ries, S.K. (1977). Superoxide dismutases. I. Occurrence in higher plants. Plant Physiol. *59*, 309–314. https://doi.org/10.1104/pp.59.2.309.

González-Chavira, M.M., Herrera-Hernández, M.G., Guzmán-Maldonado, H., and Pons-Hernandéz, J.L. (2018). Controlled water deficit as abiotic stress factor for enhancing the phytochemical content and adding-value of crops. Sci. Hortic. (Amsterdam) *234*, 354–360. https://doi.org/10.1016/j.scienta.2018.02.049.

Hoagland, D.R., and Arnon, D.I. (1938). The water culture method for growing plants without soil. Calif. Agric. Exp. Stn. *347*, 1–39.

Huther, C.M., Ramm, A., Rombaldi, C.V., and Bacarin, M.A. (2013). Physiological response to heat stress of tomato 'Micro-Tom' plants expressing high and low levels of mitochondrial sHSP23.6 protein. Plant Growth Regul. *70*, 175–185. https://doi.org/10.1007/s10725-013-9790-y.

Huther, C.M., Martinazzo, E.G., Schock, A.A., Rombaldi, C.V., and Bacarin, M.A. (2018). Production components in transformed and untransformed 'MicroTom' tomato plants. Rev. Cienc. Agron. *49*, 85–92. https://doi.org/10.5935/1806-6690.20180010.

Mittler, R. (2017). ROS are good. Trends Plant Sci. *22*, 11–19. https://doi.org/10.1016/j.tplants.2016.08.002.

Morales, M., and Munné-Bosch, S. (2016). Oxidative stress: A master regulator of plant trade-offs? Trends Plant Sci. *21*, 996–999. https://doi.org/10.1016/j.tplants.2016.09.002.

Nagata, Y., and Yamashita, I. (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. J. Jpn. Soc. Food Sci. *39*, 925–928. https://doi.org/10.3136/nskkk1962.39.925.

Nakano, Y., and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol. *22*, 867–880.

Nora, L., Dalmazo, G.O., Nora, F.R., and Rombaldi, C.V. (2012). Controlled water stress to improve fruit and vegetable postharvest quality. In Water Stress, M.D. Ismail, R. Mofizur, and H. Hiroshi, eds. (Rijeka: InTech Open Science), p. 59–72. https://doi.org/10.5772/30182.

Oliveira, L.A. (2010). Manual de laboratório: análises físico-químicas de frutas e mandioca. (Cruz das Almas: Embrapa Mandioca e Fruticultura), 248 pp.

Pedreschi, R., and Lurie, S. (2015). Advances and current challenges in understanding postharvest abiotic stresses in perishables. Postharv. Biol. Technol. *107*, 77–89. https://doi.org/10.1016/j.postharvbio.2015.05.004.

Perotti, V.A., Moreno, A.S, and Podestá, F.E. (2014). Physiological aspects of fruit ripening: The mitochondrial connection. Mitochondrion *17*, 1–6. https://doi.org/10.1016/j.mito.2014.04.010.

Reissig, G.N., Posso, D.A., Borella, Jr., da Silveira, R.V.D., Rombaldi, C.V., and Bacarin, M.A. (2018). High MT-sHSP23.6 expression increases antioxidant system in 'Micro-Tom' tomato fruits during post-harvest hypoxia. Sci. Hortic. (Amsterdam) *242*, 127–136. https://doi.org/10.1016/j.scienta.2018.07.035.

Ripoll, J., Urban, L., Staudt, M., Lopez-Lauri, F., Bidel, L.P.R., and Bertin, N. (2014). Water shortage and quality of fleshy fruits – Making the most of the unavoidable. J. Exp. Bot. *65*, 4097–4117. https://doi.org/10.1093/jxb/eru197.

Ripoll, J., Urban, L., Brunel, B., and Bertin, N. (2016). Water deficit effects on tomato quality depend on fruit developmental stage and genotype. J. Plant Physiol. *190*, 26–35. https://doi.org/10.1016/j.jplph.2015.10.006.

Rodziewicz, P., Swarcewicz, B., Chmielewska, K., Wojakowska, A., and Stobiecki, M. (2014). Influence of abiotic stresses on plant proteome and metabolome changes. Acta Physiol. Plant. *36*, 1–19. https://doi.org/10.1007/s11738-013-1402-y.

Rufino, M.S.M., Alves, R.E., Brito, E.S., Morais, S.M., Sampaio, C.G., Pérez-Jiménez, J., and Saura-Calixto, F.D. (2007). Metodologia científica: Determinação da atividade antioxidante total em frutas pela captura do radical livre ABTS.+. (Fortaleza: Ceará, Embrapa, Comunicado Técnico).

Singleton, V.L., and Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Amer. J. Enol. Viticult. *16*, 144–158.

Slimestad, R., and Verheul, M. (2009). Review of flavonoids and other phenolics from fruit of different tomato (*Lycopersicon esculentum* Mill.). J. Sci. Food Agric. *89*, 1255–1270. https://doi.org/10.1002/jsfa.3605.

Timperio, A.M., Egidi, M.G., and Zolla, L. (2008). Proteomics applied on plant abiotic stress: Role of heat shock proteins (HSP). J. Proteomics *71*, 391–411. https://doi.org/10.1016/j.jprot.2008.07.005.

Toivonen, P., and Hodges, M. (2011). Abiotic stress in harvested fruits and vegetables. In Abiotic Stress in Plants – Mechanisms, Adaptations, A. Shunker, ed. (Croatia: InTech), p. 39–58.

Velikova, V., Yordanov, I., and Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. Plant Sci. *151*, 59–66. https://doi.org/10.1016/S0168-9452(99)00197-1.

Received: Feb. 2, 2019
Accepted: Nov. 15, 2019

Supplemental Information – Table S1. Averages of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPOD), hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\bullet-}$), from genotypes with different MT-sHSP23.6 expression levels (WT and Sense). Multiple comparisons between genotypes within each level of irrigation and irrigation within each level of genotype.

Days	Gen.	SOD*		CAT**		APX***		GPOD****		H_2O_2*****		$O_2^{\bullet-}$******	
		WD	Irrig.	WD	Irrig.	WD	Irrig.	WD	Irrig.	WD	Irrig.	WD	Irrig.
0	Sense	16.8×10^{-6} bB	24.7×10^{-6} aA	2.2×10^{-9} aA	2.2×10^{-9} bA	25.5×10^{-7} aA	16.6×10^{-7} bB	13.0×10^{-9} aA	8.5×10^{-9} aB	0.7×10^{-9} aA	0.5×10^{-9} aB	0.2×10^{-9} bB	0.3×10^{-9} aA
	WT	18.6×10^{-6} aB	21.8×10^{-6} bA	1.6×10^{-9} bB	2.5×10^{-9} aA	15.4×10^{-7} bB	23.2×10^{-7} aA	7.1×10^{-9} bB	9.4×10^{-9} aA	0.6×10^{-9} aA	0.2×10^{-9} bB	0.5×10^{-9} aA	0.3×10^{-9} aB
3	Sense	19.2×10^{-6} aB	22.9×10^{-6} aA	3.2×10^{-9} aB	3.4×10^{-9} aA	19.9×10^{-7} aB	48.6×10^{-7} aA	13.6×10^{-9} aA	13.0×10^{-9} aA	0.3×10^{-9} aB	0.4×10^{-9} aA	0.3×10^{-9} aA	0.2×10^{-9} bB
	WT	12.6×10^{-6} bB	17.1×10^{-6} bA	3.1×10^{-9} bA	2.7×10^{-9} bB	14.1×10^{-7} bA	11.8×10^{-7} bA	5.7×10^{-9} bB	7.0×10^{-9} bA	0.2×10^{-9} aB	0.3×10^{-9} aA	0.1×10^{-9} bB	0.4×10^{-9} aA
4	Sense	17.0×10^{-6} aB	21.7×10^{-6} bA	2.6×10^{-9} aA	2.5×10^{-9} bB	15.2×10^{-7} bB	28.2×10^{-7} aA	10.4×10^{-9} bA	7.0×10^{-9} bB	0.2×10^{-9} aA	0.3×10^{-9} aA	0.5×10^{-9} aA	0.2×10^{-9} bB
	WT	14.8×10^{-6} bB	22.2×10^{-6} aA	2.4×10^{-9} bB	3.4×10^{-9} aA	19.7×10^{-7} aB	21.4×10^{-7} bA	13.1×10^{-9} aA	8.9×10^{-9} aB	0.3×10^{-9} aA	0.2×10^{-9} bB	0.5×10^{-9} aA	0.5×10^{-9} aA
8	Sense	18.0×10^{-6} bA	15.7×10^{-6} aA	2.3×10^{-9} bB	2.8×10^{-9} aA	11.9×10^{-7} bB	21.2×10^{-7} aA	5.9×10^{-9} bA	6.3×10^{-9} bA	0.2×10^{-9} aB	0.3×10^{-9} bA	0.2×10^{-9} bB	0.4×10^{-9} aA
	WT	21.8×10^{-6} aA	14.3×10^{-6} bB	2.5×10^{-9} aA	2.2×10^{-9} bB	18.4×10^{-7} aA	12.6×10^{-7} bB	13.4×10^{-9} aA	7.6×10^{-9} aB	0.2×10^{-9} aB	0.4×10^{-9} aA	0.5×10^{-9} aA	0.4×10^{-9} aB

Values followed by the same lower-case letters in the column (among genotypes) and capitals in the line (among irrigation level) do not differ significantly from each other, according to Tukey's test at 5% probability. * Expressed as U kg^{-1}. ** Expressed as mmol min^{-1} kg^{-1} H_2O_2. *** Expressed as mmol min^{-1} kg^{-1} AsA. **** Expressed as mmol min^{-1} kg^{-1} H_2O_2. ***** Expressed as mol kg^{-1} H_2O_2. ****** Expressed as mol kg^{-1} $O_2^{\bullet-}$.

Supplemental Information – Table S2. Averages of Hue angle, β-carotene, lycopene, total phenols and ascorbic acid, from genotypes with different MT-sHSP23.6 expression levels (WT and Sense). Multiple comparisons between genotypes within each level of irrigation and irrigation within each level of genotype.

Days	Gen.	Hue angle*		β-carotene**		Lycopene***		Total phenols****		Ascorbic acid*****	
		WD	Irrig.	WD	Irrig.	WD	Irrig.	WD	Irrig.	WD	Irrig.
0	Sense	110.7 bA	101.1 aB	1.0×10^{-2} aA	1.0×10^{-2} aA	0.1×10^{-2} bA	0.1×10^{-2} aA	1.0 bA	1.0 bA	3.0×10^{-1} bA	2.7×10^{-1} aB
	WT	112.0 aA	109.8 aB	0.8×10^{-2} bA	0.8×10^{-2} bA	0.4×10^{-2} aA	0.2×10^{-2} aB	1.1 aA	1.1 aA	3.8×10^{-1} aA	1.7×10^{-1} bB
3	Sense	110.9 bA	110.3 aA	1.0×10^{-2} aA	0.9×10^{-2} aA	0.3×10^{-2} aA	0.2×10^{-2} aA	1.3 aA	1.0 bB	3.5×10^{-1} aA	3.4×10^{-1} aA
	WT	113.4 aA	103 bB	1.0×10^{-2} aA	1.0×10^{-2} aA	0.1×10^{-2} bB	0.2×10^{-2} aA	1.2 bA	1.1 aB	2.7×10^{-1} bA	1.8×10^{-1} bB
4	Sense	98.2 bB	107.5 aA	1.5×10^{-2} aA	1.1×10^{-2} aB	2.3×10^{-2} aA	0.6×10^{-2} aB	1.3 aA	1.2 aB	3.0×10^{-1} bB	3.5×10^{-1} aA
	WT	108.7 aA	98.3 bB	1.0×10^{-2} bA	1.0×10^{-2} aA	0.3×10^{-2} bA	0.4×10^{-2} bA	1.2 bA	1.1 bB	3.2×10^{-1} aA	3.2×10^{-1} bA
8	Sense	48.4 bB	60.3 aA	1.3×10^{-2} aA	1.3×10^{-2} aA	4.3×10^{-2} aA	2.5×10^{-2} bB	1.4 bA	1.3 aB	2.8×10^{-1} bB	3.3×10^{-1} bA
	WT	56.4 aA	53.3 bB	1.3×10^{-2} aA	1.4×10^{-2} aA	2.8×10^{-2} bB	4.1×10^{-2} aA	1.5 aA	1.2 bB	3.1×10^{-1} aB	3.5×10^{-1} aA

Values followed by the same lower-case letters in the column (among genotypes) and capitals in the line (among irrigation level) do not differ significantly from each other, according to Tukey's test at 5% probability. * Expressed as Hue angle (h°). ** Expressed as g kg^{-1} β-carotene. *** Expressed as g kg^{-1} lycopene. **** Expressed as g kg^{-1} gallic acid. ***** Expressed as g kg^{-1} ascorbic acid.

Supplemental Information – Table S3. Averages of antioxidant activity of the DPPH and ABTS radical method, of genotypes with different MT-sHSP23.6 expression levels (WT and Sense). Multiple comparisons between genotypes within each level of irrigation and irrigation within each level of genotype.

Days	Gen.	DPPH*		ABTS**	
		WD	Irrig.	WD	Irrig.
0	Sense	59.9 bA	59.1 aA	37.0 aA	27.6 aB
	WT	63.0 aA	60.1 aB	26.6 bA	22.8 bB
3	Sense	64.7 bB	66.7 aA	30.2 aB	41.1 aA
	WT	66.3 aA	58.0 bB	26.7 bA	24.7 bA
4	Sense	69.9 bA	68.2 aB	46.2 aA	41.6 aB
	WT	72.0 aA	62.7 bB	33.6 bA	31.8 bB
8	Sense	77.3 bA	74.0 aB	54.8 bA	46.4 bB
	WT	78.5 aA	63.9 bB	56.0 aA	54.0 aB

Values followed by the same lower-case letters in the column (among genotypes) and capitals in the line (among irrigation level) do not differ significantly from each other, according to Tukey's test at 5% probability. * Expressed as % of radical scavenging activity.

Supplemental Information – Table S4.

Table S4 – 1. Evaluation at 0 days.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
1. ANOVA of SOD (superoxide dismutase) activity.					
Treatment	1	1.186	1.186	3.4679	0.08723
Deficit	1	123.193	123.193	360.2882	$2.566e^{-10}$ ***
Treatment:Deficit	1	22.741	22.741	66.5085	$3.087e^{-06}$ ***
Residuals	12	4.103	0.342		
2. ANOVA of CAT (catalase) activity.					
Treatment	1	0.10524	0.10524	27.995	0.0001911 ***
Deficit	1	0.85046	0.85046	226.234	3.767e-09 ***
Treatment:Deficit	1	0.69779	0.69779	185.624	1.161e-08 ***
Residuals	12	0.04511	0.00376		
3. ANOVA of APX (ascorbate peroxidase) activity.					
Treatment	1	11.917	11.917	15.5166	0.001966 **
Deficit	1	1.271	1.271	1.6555	0.222482
Treatment:Deficit	1	281.569	281.569	366.6184	2.318e-10 ***
Residuals	12	9.216	0.768		
4. ANOVA of GPOD (guaiacol peroxidase) activity.					
Treatment	1	24.867	24.867	31.7302	0.0001102 ***
Deficit	1	5.333	5.333	6.8042	0.0228655
Treatment:Deficit	1	45.408	45.408	57.9392	6.233e-06 ***
Residuals	12	9.405	0.784		
5. ANOVA of H_2O_2 (hydrogen peroxide) content.					
Treatment	1	0.16744	0.16744	47.337	1.698e-05 ***
Deficit	1	0.34627	0.34627	97.893	4.018e-07 ***
Treatment:Deficit	1	0.06789	0.06789	19.192	0.0008948 ***
Residuals	12	0.04245	0.00354		
6. ANOVA of O_2°- (superoxide anion) content.					
Treatment	1	0.048929	0.048929	1662.8	3.062e-14 ***
Deficit	1	0.003843	0.003843	130.6	8.318e-08 ***
Treatment:Deficit	1	0.043952	0.043952	1493.6	5.803e-14 ***
Residuals	12	0.000353	0.000029		
7. ANOVA of color tone (Hue angle).					
Treatment	1	0.9131	0.9131	1.9304	0.189956
Deficit	1	7.4084	7.4084	15.6617	0.001902 **
Treatment:Deficit	1	2.7287	2.7287	5.7685	0.033411 *
Residuals	12	5.6763	0.4730		

Table S4 – 1. Evaluation at 0 days.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
8. ANOVA of lycopene content.					
Treatment	1	0.120873	0.120873	78.561	1.297e-06 ***
Deficit	1	0.083598	0.083598	54.334	8.604e-06 ***
Treatment:Deficit	1	0.057259	0.057259	37.215	5.333e-05 ***
Residuals	12	0.018463	0.001539		
9. ANOVA of β-carotene content.					
Treatment	1	0.090245	0.090245	18.9227	0.0009448 ***
Deficit	1	0.000007	0.000007	0.0015	0.9699140
Treatment:Deficit	1	0.000939	0.000939	0.1968	0.6651895
Residuals	12	0.057230	0.004769		
10. ANOVA of total phenols content.					
Treatment	1	124.143	124.143	177.537	1.494e-08 ***
Deficit	1	38.240	38.240	54.687	8.330e-06 ***
Treatment:Deficit	1	58.256	58.256	83.312	9.514e-07 ***
Residuals	12	8.391	0.699		
11. ANOVA of ascorbic acid content.					
Treatment	1	9.01	9.01	16.353	0.001628 **
Deficit	1	566.29	566.29	1027.825	5.355e-13 ***
Treatment:Deficit	1	313.78	313.78	569.513	1.758e-11 ***
Residuals	12	6.61	0.55		
12. ANOVA of DPPH radical scavenging activity.					
Treatment	1	16.5191	16.5191	45.510	2.055e-05 ***
Deficit	1	13.8215	13.8215	38.078	4.794e-05 ***
Treatment:Deficit	1	4.4689	4.4689	12.312	0.004311 **
Residuals	12	4.3558	0.3630		
13. ANOVA of ABTS radical scavenging activity.					
Treatment	1	232.280	232.280	404.163	1.313e-10 ***
Deficit	1	173.972	173.972	302.707	7.050e-10 ***
Treatment:Deficit	1	30.223	30.223	52.587	1.012e-05 ***
Residuals	12	6.897	0.575		

Signification of the codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Table S4 – 2. Evaluation at 3 days.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
1. ANOVA of SOD (superoxide dismutase) activity.					
Treatment	1	151.972	151.972	251.2954	2.063e-09 ***
Deficit	1	67.864	67.864	112.2174	1.915e-07 ***
Treatment:Deficit	1	0.740	0.740	1.2238	0.2903
Residuals	12	7.257	0.605		
2. ANOVA of CAT (catalase) activity.					
Treatment	1	0.61399	0.61399	366.657	2.317e-10 ***
Deficit	1	0.05242	0.05242	31.304	0.0001171 ***
Treatment:Deficit	1	0.34658	0.34658	206.966	6.260e-09 ***
Residuals	12	0.02009	0.00167		
3. ANOVA of APX (ascorbate peroxidase) activity.					
Treatment	1	1806.40	1806.40	6091.1	<2.2e-16 ***
Deficit	1	699.74	699.74	2359.5	3.795e-15 ***
Treatment:Deficit	1	958.59	958.59	3232.3	5.786e-16 ***
Residuals	12	3.56	0.30		

Table S4 – 2. Evaluation at 3 days.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
4. ANOVA of GPOD (guaiacol peroxidase) activity.					
Treatment	1	196.299	196.299	573.8004	1.682e-11 ***
Deficit	1	0.439	0.439	1.2838	0.279327
Treatment:Deficit	1	3.689	3.689	10.7835	0.006534 **
Residuals	12	4.105	0.342		
5. ANOVA of H_2O_2 (hydrogen peroxide) content.					
Treatment	1	0.014944	0.014944	4.1368	0.06468 .
Deficit	1	0.032965	0.032965	9.1254	0.01065 *
Treatment:Deficit	1	0.007185	0.007185	1.9891	0.18383
Residuals	12	0.043349	0.003612		
6. ANOVA of O_2°- (superoxide anion) content.					
Treatment	1	0.000005	0.000005	0.086	0.7743
Deficit	1	0.034054	0.034054	623.706	1.029e-11 ***
Treatment:Deficit	1	0.062665	0.062665	1147.702	2.781e-13 ***
Residuals	12	0.000655	0.000055		
7. ANOVA of color tone (Hue angle).					
Treatment	1	15.947	15.947	34.188	7.874e-05 ***
Deficit	1	104.524	104.524	224.090	3.978e-09 ***
Treatment:Deficit	1	81.257	81.257	174.208	1.663e-08 ***
Residuals	12	5.597	0.466		
8. ANOVA of lycopene content.					
Treatment	1	0.009815	0.009815	2.3579	0.15058
Deficit	1	0.002830	0.002830	0.6799	0.42570
Treatment:Deficit	1	0.037621	0.037621	9.0378	0.01094 *
Residuals	12	0.049951	0.004163		
9. ANOVA of β-carotene content.					
Treatment	1	0.003297	0.003297	1.0453	0.32676
Deficit	1	0.000889	0.000889	0.2817	0.60525
Treatment:Deficit	1	0.012253	0.012253	3.8851	0.07222 .
Residuals	12	0.037847	0.0031540		
10. ANOVA of total phenols content.					
Treatment	1	11.593	11.593	42.036	3.008e-05 ***
Deficit	1	179.211	179.211	649.805	8.080e-12 ***
Treatment:Deficit	1	25.680	25.680	93.115	5.258e-07 ***
Residuals	12	3.310	0.276		
11. ANOVA of ascorbic acid content.					
Treatment	1	568.69	568.69	973.99	7.370e-13 ***
Deficit	1	91.85	91.85	157.31	2.951e-08 ***
Treatment:Deficit	1	64.70	64.70	110.81	2.051e-07 ***
Residuals	12	7.01	0.58		
12. ANOVA of DPPH radical scavenging activity.					
Treatment	1	50.517	50.517	110.760	2.056e-07 ***
Deficit	1	40.888	40.888	89.648	6.442e-07 ***
Treatment:Deficit	1	106.191	106.191	232.825	3.196e-09 ***
Residuals	12	5.473	0.456		
13. ANOVA of ABTS radical scavenging activity.					
Treatment	1	398.16	398.16	1028.45	5.336e-13 ***
Deficit	1	78.35	78.35	202.37	7.113e-09 ***
Treatment:Deficit	1	165.29	165.29	426.95	9.530e-11 ***
Residuals	12	4.65	0.39		

Signification of the codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Table S4 – 3. Evaluation at 4 days.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
1. ANOVA of SOD (superoxide dismutase) activity.					
Treatment	1	2.840	2.840	7.1865	0.020009 *
Deficit	1	147.210	147.210	372.5081	2.113e-10 ***
Treatment:Deficit	1	7.264	7.264	18.3813	0.001055 **
Residuals	12	4.742	0.395		
2. ANOVA of CAT (catalase) activity.					
Treatment	1	0.38006	0.38006	119.45	1.360e-07 ***
Deficit	1	0.78828	0.78828	247.74	2.238e-09 ***
Treatment:Deficit	1	1.30097	1.30097	408.88	1.227e-10 ***
Residuals	12	0.03818	0.00318		
3. ANOVA of APX (ascorbate peroxidase) activity.					
Treatment	1	5.099	5.099	11.834	0.004894 **
Deficit	1	215.219	215.219	499.465	3.801e-11 ***
Treatment:Deficit	1	127.064	127.064	294.883	8.203e-10 ***
Residuals	12	5.171	0.431		
4. ANOVA of GPOD (guaiacol peroxidase) activity.					
Treatment	1	20.711	20.711	26.6119	0.0002376 ***
Deficit	1	58.500	58.500	75.1686	1.635e-06 ***
Treatment:Deficit	1	0.600	0.600	0.7712	0.3970844
Residuals	12	9.339	0.778		
5. ANOVA of H_2O_2 (hydrogen peroxide) content.					
Treatment	1	0.0133685	0.0133685	18.5358	0.001022 **
Deficit	1	0.0003378	0.0003378	0.4684	0.506741
Treatment:Deficit	1	0.0075242	0.0075242	10.4326	0.007220 **
Residuals	12	0.0086547	0.0007212		
6. ANOVA of O_2°- (superoxide anion) content.					
Treatment	1	0.100553	0.100553	1361.4	1.007e-13 ***
Deficit	1	0.104155	0.104155	1410.2	8.171e-14 ***
Treatment:Deficit	1	0.096746	0.096746	1309.9	1.268e-13 ***
Residuals	12	0.000886	0.000074		
7. ANOVA of color tone (Hue angle).					
Treatment	1	1.84	1.84	3.4990	0.08598 .
Deficit	1	1.25	1.25	2.3736	0.14935
Treatment:Deficit	1	387.62	387.62	735.9116	3.875e-12 ***
Residuals	12	6.32	0.53		
8. ANOVA of lycopene content.					
Treatment	1	4.8173	4.8173	1475.57	6.238e-14 ***
Deficit	1	2.6899	2.6899	823.94	1.986e-12 ***
Treatment:Deficit	1	2.8600	2.8600	876.04	1.381e-12 ***
Residuals	12	0.0392	0.0033		
9. ANOVA of β-carotene content.					
Treatment	1	0.291630	0.291630	42.447	2.872e-05 ***
Deficit	1	0.104537	0.104537	15.216	0.002107 **
Treatment:Deficit	1	0.230749	0.230749	33.586	8.535e-05 ***
Residuals	12	0.082444	0.00687		
10. ANOVA of total phenols content.					
Treatment	1	880.22	880.22	1564.82	4.397e-14 ***
Deficit	1	397.01	397.01	705.80	4.961e-12 ***
Treatment:Deficit	1	18.35	18.35	32.62	9.737e-05 ***
Residuals	12	6.75	0.56		

Table S4 – 3. Evaluation at 4 days.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
11. ANOVA of ascorbic acid content.					
Treatment	1	1.0752	1.0752	1.9691	0.1858831
Deficit	1	17.4905	17.4905	32.0326	0.0001056 ***
Treatment:Deficit	1	23.2826	23.2826	42.6404	2.81e-05 ***
Residuals	12	6.5523	0.5460		
12. ANOVA of DPPH radical scavenging activity.					
Treatment	1	11.579	11.579	52.944	9.788e-06 ***
Deficit	1	121.190	121.190	554.117	2.065e-11 ***
Treatment:Deficit	1	57.028	57.028	260.748	1.668e-09 ***
Residuals	12	2.625	0.219		
13. ANOVA of ABTS radical scavenging activity.					
Treatment	1	501.56	501.56	1006.360	6.070e-13 ***
Deficit	1	41.24	41.24	82.754	9.858e-07 ***
Treatment:Deficit	1	7.94	7.94	15.938	0.001786 **
Residuals	12	5.98	0.50		

Signification of the codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Table S4 – 4. Evaluation at 8 days.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
1. ANOVA of SOD (superoxide dismutase) activity.					
Treatment	1	5.659	5.659	13.102	0.003516 **
Deficit	1	96.669	96.669	223.803	4.007e-09 ***
Treatment:Deficit	1	26.876	26.876	62.222	4.342e-06 ***
Residuals	12	5.183	0.432		
2. ANOVA of CAT (catalase) activity.					
Treatment	1	0.16871	0.16871	148.637	4.052e-08 ***
Deficit	1	0.03504	0.03504	30.876	0.0001245 ***
Treatment:Deficit	1	0.61319	0.61319	540.246	2.397e-11 ***
Residuals	12	0.01362	0.00114		
3. ANOVA of APX (ascorbate peroxidase) activity.					
Treatment	1	4.092	4.092	8.6112	0.0124994 *
Deficit	1	12.342	12.342	25.9726	0.0002634 ***
Treatment:Deficit	1	226.377	226.377	476.3969	5.016e-11 ***
Residuals	12	5.702	0.475		
4. ANOVA of GPOD (guaiacol peroxidase) activity.					
Treatment	1	77.673	77.673	138.577	5.987e-08 ***
Deficit	1	29.560	29.560	52.738	9.980e-06 ***
Treatment:Deficit	1	38.706	38.706	69.057	2.542e-06 ***
Residuals	12	6.726	0.561		
5. ANOVA of H_2O_2 (hydrogen peroxide) content.					
Treatment	1	0.004018	0.004018	3.5916	0.08241 .
Deficit	1	0.039779	0.039779	35.5541	6.583e-05 ***
Treatment:Deficit	1	0.005441	0.005441	4.8629	0.04769 *
Residuals	12	0.013426	0.001119		
6. ANOVA of O_2°- (superoxide anion) content.					
Treatment	1	0.049604	0.049604	592.41	1.394e-11 ***
Deficit	1	0.022763	0.022763	271.85	1.312e-09 ***
Treatment:Deficit	1	0.042946	0.042946	512.90	3.253e-11 ***
Residuals	12	0.001005	0.000084		

Table S4 – 4. Evaluation at 8 days.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
7. ANOVA of color tone (Hue angle).					
Treatment	1	1.154	1.154	2.3261	0.1531
Deficit	1	77.397	77.397	156.0505	3.087e-08 ***
Treatment:Deficit	1	221.819	221.819	447.2382	7.263e-11 ***
Residuals	12	5.952	0.496		
8. ANOVA of lycopene content.					
Treatment	1	0.0024	0.0024	0.6467	0.4369
Deficit	1	0.2096	0.2096	57.4043	6.531e-06 ***
Treatment:Deficit	1	9.9038	9.9038	2,712.5574	1.650e-15 ***
Residuals	12	0.0438	0.0037		
9. ANOVA of β-carotene content.					
Treatment	1	0.002269	0.002269	0.8250	0.38161
Deficit	1	0.003555	0.003555	1.2923	0.27781
Treatment:Deficit	1	0.010996	0.010996	3.9975	0.06873 .
Residuals	12	0.033008	0.0027507		
10. ANOVA of total phenols content.					
Treatment	1	78.39	78.39	126.55	9.900e-08 ***
Deficit	1	823.90	823.90	1329.98	1.158e-13 ***
Treatment:Deficit	1	146.20	146.20	236.01	2.957e-09 ***
Residuals	12	7.43	0.62		
11. ANOVA of ascorbic acid content.					
Treatment	1	24.024	24.024	40.8336	3.453e-05 ***
Deficit	1	72.398	72.398	123.0553	1.155e-07 ***
Treatment:Deficit	1	1.546	1.546	2.6278	0.131
Residuals	12	7.060	0.588		
12. ANOVA of DPPH radical scavenging activity.					
Treatment	1	78.71	78.71	157.52	2.929e-08 ***
Deficit	1	322.81	322.81	646.07	8.360e-12 ***
Treatment:Deficit	1	126.95	126.95	254.07	1.936e-09 ***
Residuals	12	6.00	0.50		
13. ANOVA of ABTS radical scavenging activity.					
Treatment	1	76.055	76.055	207.79	6.121e-09 ***
Deficit	1	108.299	108.299	295.88	8.044e-10 ***
Treatment:Deficit	1	40.608	40.608	110.94	2.038e-07 ***
Residuals	12	4.392	0.366		

Signification of the codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.