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Impact of moderate water deficit at the fruit development stage of tomato (*Solanum lycopersicum* L.): effects on plant growth, physiology, fruit yield and quality and expression of carotenoid biosynthesis genes

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Abstract

Climate change has an increasingly negative impact on the availability of water for agriculture. Therefore, enhancing water use efficiency under poor irrigation conditions has become a major objective. Here, we investigate the effects of moderate water deficit on tomato plant growth, physiology, fruit yield and quality and expression of carotenoid biosynthesis genes. The treatments control (irrigated) and water deficit were applied from the fruit fixation stage forward and the observations were recorded when the fruits were mature. The results revealed adaptive responses to moderate water deficit, in which plants showed a reduction in leaf water potential, leaf gas exchange and growth variables but an increase in intrinsic and instantaneous water use efficiencies. These adaptive responses were also observed in the activities of antioxidant enzymes in leaves and fruits, which did not show significant differences between treatments. Analysis of abscisic acid (ABA)-regulated carotenoid biosynthesis genes in fruits showed that most of them were either negatively or negligibly regulated by water deficit, except for the carotene isomerases, and their responses were altered by changes in carotenoid metabolites. Principal component analysis (PCA) revealed that *9-cis-epoxycarotenoid dioxygenase* 5 (*LeNCED*), *phytoene synthase* (*PSY*) and *beta-carotene hydroxylase 1* (*CRTR-B1*) and *2* (*CRTR-B2*) were the genes that most contributed to the variations observed in the expression patterns between treatments. Overall, these findings contribute to a further understanding of tomato plant and fruit responses to moderate water deficit.

Keywords Carotenes · Drought tolerance · Fruit maturation · Fruit quality · Fruit yield · Water stress

Introduction

Water resources have been increasingly impacted by global climate change. Therefore, studies on irrigation and the rational use of water are critical to reduce consumption and improve the efficiency of water use in agriculture (Chaves and Oliveira 2004). Water deficit alters the water balance of cells and tissues, which negatively affects many biological processes (Dai 2013; Siddiqui et al. 2015; Zhou et al. 2015; Zandalinas et al. 2018).

The acclimatization of plants to water deficit depends on a series of events that cause adaptive changes in plant growth and physiological-biochemical processes, including modifications of plant structure, growth rate, osmotic potential and antioxidant defenses (Anjum et al. 2011). Abiotic stresses such as drought increase the generation of free reactive oxygen species that attack biological structures, damage DNA, and stimulate amino acid and protein oxidation and lipid peroxidation (Jaleel et al. 2009). Plant responses to water deficit may allow a balance between growth and defense mechanisms, but this is still poorly understood (Claeys and Inzé 2013).

The narrow genetic variations in crops and the complex nature of drought-tolerance traits make the improvement

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of drought-tolerant varieties challenging (Hill et al. 2013; Langridge and Reynolds 2015). The observed susceptibility to water deficit calls for the improvement of more tolerant varieties that can cope with lower water availability without compromising crop yield and quality (Mickelbart et al. 2015). In this context, genetic diversity can be considered as a valuable genetic resource to improve crop productivity under unfavourable environments (Conti et al. 2019).

Drought tolerance relies on the plant's ability to capture water through the roots and to prevent water loss from leaves. Since the favorable effects of each of these processes are difficult to separate, the strategy to improve drought tolerance is related to the increase of water uptake by the root system (Yamaguchi and Sharp 2010; Slovak et al. 2016).

To obtain the necessary knowledge for the development of varieties with greater tolerance to water deficit, it is important to associate physiological analyses with the identification of the main genes involved in drought tolerance (Zhu et al. 2016). In this context, transcriptome analysis is an important approach to identify genes and understand the molecular mechanisms associated with drought tolerance (Fan et al. 2015; Sprenger et al. 2016).

An important regulator of plant response to water deficit is abscisic acid (ABA), which is also involved in regulating fruit maturation; however, a comprehensive investigation of its influence on maturation processes is still lacking. As ABA induces the transcription of genes involved in pigment metabolism, including carotenoids (Taiz et al. 2017), studies demonstrate that the *PSY* gene, a precursor of the carotenoid biosynthesis pathway, plays a role in stress-induced ABA formation, thus suggesting that the first committed step in carotenogenesis is a limiting step in ABA biosynthesis (Cidade et al. 2012).

Tomato is an economically relevant vegetable crop worldwide (Perveen et al. 2015; Raja et al. 2020) whose domestication caused a greater susceptibility to water deficit (Kissoudis et al. 2016; Mareri et al. 2016), as is the case of cv. Santa Clara (Oliveira et al. 2021) as compared to the wild species *Solanum pennelli* (Bolger et al. 2014). Pervez et al. (2009) showed that cultivated tomato seedlings are sensitive to drought, compromising the productivity of an entire harvesting season. Other studies reported that water deficit significantly affected fruit production of cultivated tomato, however, increasing irrigation status to a specific level did not positively influence production, which remained stable (Wahb-Allah et al. 2011; SHARMA et al. 2015). In addition to yield, water deficit affects the fruit volume, diameter and composition in terms of the content of total soluble solids (SSC) and carotenoids (Sivakumar and Srividhya 2016). Regarding the content of carotenoids, the effects of water deficit range from negative to zero or positive, depending on the stress intensity and duration, genotype and stage of development of the plant and fruit (Ripoll et al. 2014, 2016).

All of these parameters are useful indicators of drought tolerance. In tomato, the mechanisms of drought tolerance are still not well characterized and only a small number of genes associated with this trait have been identified (Iovieno et al. 2016; Arms et al. 2017).

The present study aimed to investigate the effects of moderate water deficit, applied at the fruit development stage, on plant growth, physiology, yield and quality of fruits, as well as on the expression of carotenoid biosynthesis genes in fruits of tomato (*Solanum lycopersicum* L.).

Materials and methods

Plant material and experimental conditions

The experiment was conducted under greenhouse conditions at the Universidade Estadual de Santa Cruz, located in the municipality of Ilhéus, Bahia (14°47′00" S, 39°02′00" W), from November 14, 2018, to March 8, 2019, with average temperatures in the period ranging from 24 to 27 °C. Seedlings of tomato (*Solanum lycopersicum L.*) cv. Santa Clara was propagated by the seminal route and transplanted when they reached the stage of 4–6 true leaves in 11 L pots containing a mixture of soil and washed sand in a 2:1 ratio, and cultivated under adequate conditions of water availability and nutrients until the beginning of treatments. At the fruit fixation stage, which occurred 42 days after transplanting, the plants were subjected to two water treatments as described by Kusvuran and Dasgan (2017), with the following adaptations: (i) control, with irrigation at 100% of the substrate field capacity (FC; cm^{-3} cm^{-3}) when the water content decreased to 75% of the FC and (ii) moderate water deficit, with irrigation of 40% of the total irrigation volume applied in the control treatment. The plants in the water deficit treatment were irrigated whenever the control treatment plants were irrigated. The water treatments were extended until the fruit maturation stage, totaling 72 days. Twenty-five plants were used per treatment and the decrease in water content in the containers was monitored by gravimetry.

The near-isogenic lines (NILs) in cv. Micro-Tom carrying the mutations *yellow flesh* (*r*), *Beta-carotene* (*B*) and *old-gold crimsom* (*og*) (Carvalho et al. 2011; Sestari et al. 2014) were used to further assess the impact of water deficit on the regulation of carotenoid metabolism. The *r* allele eliminates fruit carotenoids by disrupting the activity of the fruit-specific phytoene synthase (PSY1), whereas the *B* and *og* alleles produce fruits with high contents of, respectively, beta-carotene or lycopene, by enhancing or disrupting, respectively, the activity of the fruit-specific lycopene beta-cyclase (Cyc-B). 'Micro-Tom' plants were subjected to the same experimental conditions as described above for 'Santa Clara'.

Physiological and plant growth analyses

The predawn leaf water potential (Ψw) was determined using a Model 1000 Pressure Chamber (PMS Instrument Company, USA) on the second or third fully expanded and mature leaf from the plant apex during the fruit harvesting period in five replications per treatment. Measurements were made when the fruits reached the breaker stage (B), breaker + 7 days (B + 7), and breaker + 14 days (B + 14), from 1:00 am to 3:00 am. Relative leaf water content (RWC) was determined according to procedures described by Smart and Bingham (1974), from ten leaf discs per repetition collected in parallel to the Ψw measurements. Leaf gas exchanges were evaluated between 8:00 am and 9:00 am with a Li-6400 (Li-Cor Inc., Nebraska, USA) portable photosynthesis meter, under artificial saturating light of 1000 μmol photons m^{-2} s^{-1} and atmospheric CO^2 concentration of 380 ± 10 μmol mol^{-1}, concomitantly with the days of Ψw measurements with the same five repetitions per treatment. Water use efficiency (WUE) was expressed in two ways: instantaneous water use efficiency (A/E), which was obtained by the ratio between the net photosynthetic rate (A) and the transpiration rate (E), and intrinsic water use efficiency (A/gs), which was calculated by the ratio between the net photosynthetic rate (A) and stomatal conductance to water vapor (gs). The carboxylation efficiency (A/Ci) was obtained by the ratio between the net photosynthetic rate (A) and the intercellular concentration of CO_2 (Ci). The a and b chlorophyll indices were determined using a portable Falker ClorofiLOG® 1030.

Plant growth variables were analyzed at the beginning and end of treatments application by measuring stem diameter, total plant height, dry biomass of roots, stems and leaves by drying in a forced air circulation oven at 70 °C until reaching constant weight, number of leaves per plant, leaf area per plant and root volume. The productive efficiency of fruits was calculated by the ratio of the average dry biomass of fruits per plant and the average dry biomass of the fruits of their corresponding treatment. Biomass data were used to calculate leaf area, root volume and number of leaves per plant, mass ratio of roots, stems, leaves, leaf area, leaf area ratio and specific leaf mass, in addition to the relative growth rate (RGR) and net assimilation rate (NAR) according to Hunt's (1990) methodology. Biomass allocation was calculated as the ratio of the dry mass of the respective plant part to the final total dry mass. For these calculations, five plants were collected at the time of transplantation and at the end of the experiment.

Fruit yield and quality phenotyping

Fruits were evaluated in relation to the number of days from transplanting until the fixation stage and from transplanting to the breaker stage. Fruits were collected seven days after the start of the breaker stage (B + 7) and phenotyped for characteristics related to fresh weight, obtained using a precision analytical balance; to equatorial lower and upper polar diameters, measured using a manual caliper; to pH, determined with the aid of a digital pH meter (PHS-3E-BI; Ion, Araucária, Brazil); and to the total soluble solids content (SSC), obtained using an analog refractometer (0 to 32% Brix; Akso, RHB32, São Leopoldo, Brazil).

Antioxidant enzyme activities

Freeze-dried samples of mature leaves and fruits from three biological replicates per treatment collected at stage B + 7 were used for the analysis of the activities of antioxidant enzymes. The leaf and pericarp tissues of these samples were macerated in liquid nitrogen in the presence of 0.7% (w/w) of polyvinylpyrrolidone (PVP) to prevent oxidation of the material. Subsequently, 40 mg of the macerate was homogenized in 800 μl of extraction buffer, specific for each enzymatic assay, and vortexed. Then, the samples were sonicated in a GEX 130 probe ultrasonicator (Cole-Parmer, Vernon Hills, USA) under an amplitude of 70%, 8 five-second pulses, with intervals of 10 s, and subjected to centrifugation at 14,000 rpm for 10 min at 4 °C. Finally, the supernatant was collected to be used in the analysis of superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and phenylalanine ammonia-lyase (PAL) activities, as described by Gonçalves et al. (2016). The enzymatic assays were performed in quadruplicate for each biological sample in the microplate spectrophotometer (SPECTRAMax Paradigm, Molecular Devices, Sunnyvale, USA).

In silico analyses

RNA-Seq data (Illumina, San Diego, USA) obtained by Mou et al. (2015) were used to generate comparative gene expression profiles of the carotenoid biosynthesis genes in fruits of *Solanum lycopersicum L.* treated with ABA and its biosynthesis inhibitor nordihydroguaiaretic acid (NDGA). Fruits were harvested at the mature green (MG) stage and treated with either 25 μl of aqueous exogenous ABA solution (10 mM), aqueous solution of NDGA, or distilled water (control), by injecting the respective solutions into each fruit from the pedicle using a microsyringe (Mou et al. 2015). Fruits were kept in the dark at 20 °C and 90% RH and samples of the 9th day after the treatments were used for RNA sequencing.

RNA extraction, cDNA synthesis and quantitative real-time PCR (RT-qPCR)

Total RNA from B + 7 stage fruits was isolated using the reagent TRIzol®, following the manufacturer's instructions.

The quality and integrity of isolated RNA were evaluated in 1% agarose gel and quantified with the aid of NANOdrop (Thermo Scientific™, 2000/2000c, Wilmington, USA). RNA samples were treated with RNAse-free DNAse I (Invitrogen, California, USA) after the extraction process, following the manufacturer's instructions. Reverse transcription reaction for cDNA synthesis was performed using the Applied biosystems high-capacity kit (Thermo ScientificTM, Carlsbad, USA), as per the manufacturer's instructions.

Gene expression analysis via RT-qPCR was carried out using oligonucleotides specifically designed for the amplification of carotenoid biosynthesis genes based on their available sequences in the Sol Genomics database (https://solgenomics.net/). The reference genes *ACT* (Solyc03g078400), *GAPDH* (Solyc05g014470), *PP2Ac1* (Solyc05g006590) and *RPL2* (Solyc10g006580) were amplified along with the target genes as endogenous controls to normalize expressions between different samples. The best reference gene was selected using the program NormFinder (Andersen et al. 2004) (https://moma.dk/normfinder-software). Table S1 shows the sequences of endogenous target and control genes. For gene expression quantification, the comparative method of C_t: $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen 2001) was used with data from at least three biological replicates that were individually validated using the Ampliqon RealQPlus $2\times$ Master Mix Green Low Rox™ kit. Control reactions devoid of cDNA (NTC) were also used in all analyses.

Statistical analyses

The experiment was carried out in a completely randomized design with 25 replications and two treatments. Initially, data were evaluated in relation to their normal distributions by the Shapiro–Wilk test and homogeneities of variance by Levene's *F* test. Then, statistical comparisons were performed between treatments through analysis of variance (ANOVA) and the Mann–Whitney test for variables that did not meet the assumptions of normality and/or homogeneity, and between the three temporal observations of the variables under study through pairing and solution of the Student's *t* test and Wilcoxon test for variables that were not compliant with the assumptions. Multivariate analyses were performed using Wilks' Lambda test, based on assumptions of normality, homogeneity of covariance-variance matrices using Box's M test, absence of multivariate outliers by calculating the Mahalanobis distance and absence of multicollinearity using Pearson's correlation analysis, and by the Pillai Trace multivariate test when any of these assumptions was violated. Multivariate factor analyses were performed using the principal components method and their adequacy was verified by the Kaiser–Meyer–Olkin (KMO) statistic and the Bartlett sphericity test. The heatmaps of gene expression and FPKM (Fragments Per Kilobase Million) data were plotted using the "ComplexHeatmap" package. All statistical analyses were performed in R software (R Development Core Team) and in IBM SPSS Statistics Software®.

Results

Impact of water deficit on plant water status and photosynthetic aspects

No significant differences for *Ψw*, *A*, *gs*, *Ci/Ca*, *E*, *A/gs* and *A/Ci* were observed among the three evaluation periods (B, B + 7 and B + 14) within the treatments, indicating that their respective values in the different water regimes had been previously established (Table 1). A significant difference among the evaluation periods was only observed for *A/E*. Between treatments, except for *A* and *A/Ci*, each of the gas exchange variables analyzed did not show significant differences in all three evaluation periods (Table 1). Thus, to investigate the effects of water contrasts, subsequent analyses were performed with the temporal means of the variables that showed a difference, at the level of 5%, between treatments.

Water deficit resulted in lower *Ψw* values throughout the evaluation periods when compared to the control treatment, with mean values of − 0.45 to − 0.65 MPa and − 0.28 to − 0.33 MPa, respectively. The *Ψw* differed between treatments, although this difference was not significant in the B stage due to a greater variance of values in plants under water deficit conditions (Fig. 1a). The *RWC* had no significant effect between treatments $(P \leq 0.05)$, ranging from 79 to 82% in the control treatment and from 75 to 79% in the water deficit treatment (Fig. 1b).

The *A*, *gs* and *E* values of plants under water deficit were significantly reduced compared to the control treatment (Table 1). These gas exchange variables were related with *Ψw*, as expected, revealing that the lower the *Ψw*, the lower the values of the gas exchange variables (Fig. 2). In mean values, *A* ranged from 13.05 to 16.73 µmol CO_2 m^{-2} s^{-1} in the control treatment and from 5.04 to 9.52 µmol CO_2 m^{-2} s^{-1} in the water deficit treatment (Fig. 2a). For *gs*, the water potentials provided mean values of 0.14 to 0.52 mol H_2O m^{-2} s^{-1} in control plants and 0.06 to 0.09 mol H_2O m^{-2} s^{-1} in plants under water deficit conditions, showing a smaller variation when compared to the control (Fig. 2b). In relation to *E*, these mean values ranged from 3.13 to 7.95 mmol H_2O m^{-2} s^{-1} in control plants, while in plants under water deficit these values ranged from 1.16 to 2.45 mmol H_2O m^{-2} s^{-1} (Fig. 2c).

Homogeneous groups were formed between the water regimes with the interrelationships of the gas exchange variables and *Ψw*. For *A* with *Ψw*, the multivariate analysis via Pillai Trace revealed a significant simultaneous effect

Table 1 Comparison of means of pairing between temporal observations and water regimes for the variables leaf water potential, leaf gas exchange and water use efficiency in *Solanum lycopersicum* L. cv. Santa Clara

Fruit Stage	Treatment	Ψ_w		A		gs		Ci/Ca		E		A/gs		A/Ci		A/E	
B	Control	-0.38 ± 0.05	Aa	14.9 ± 0.78	Aa	0.31 ± 0.07	Aa	0.74 ± 0.04	Aa	5.5 ± 1.0	Aa	56.7 ± 12.1	Aa	0.05 ± 0.001	Aa	2.9 ± 0.5	Aa
	Water deficit	-0.53 ± 0.16	Aa	6.8 ± 1.02	Ab	0.06 ± 0.01	Ab	0.5 ± 0.07	Ab	1.5 ± 0.2	Ab	121.8 ± 16.7	Ab	0.03 ± 0.007	Ab	4.8 ± 0.5	Ab
B+7	Control	-0.31 ± 0.03	Aa	14.6 ± 2.9	Aa	0.36 ± 0.12	Aa	0.74 ± 0.04	Aa	7.1 ± 1.9	Aa	51.2 ± 10.1	Aa	0.05 ± 0.008	Aa	2.3 ± 0.3	Aa
	Water deficit	-0.49 ± 0.06	Ab	6.4 ± 1.9	Ab	0.08 ± 0.01	Ab	0.65 ± 0.03	Aa	2.3 ± 0.4	Ab	77.1 ± 7.6	Aa	0.03 ± 0.008	Ab	2.7 ± 0.2	Ba
B+14	Control	-0.30 ± 0.06	Aa	13.6 ± 0.81	Aa	0.19 ± 0.17	Aa	0.67 ± 0.05	Aa	3.5 ± 2.1	Aa	73.2 ± 13.1	Aa	0.05 ± 0.009	Aa	3.9 ± 0.6	Aa
	Water deficit	-0.53 ± 0.10	Ab	5.8 ± 1.8	Ab	0.06 ± 0.01	Ab	0.59 ± 0.07	Aa	1.3 ± 0.1	Aa	98.7 ± 16.6	Aa	0.02 ± 0.008	Aa	4.4 ± 0.8	Aa

Ψ_w=leaf water potential in MPa; A=net photosynthetic rate in μmol CO_2 m^{-2} s^{-1}; A/Ci=carboxylation efficiency; A/E=instantaneous water use efficiency; A/gs=intrinsic water use efficiency; Ci/Ca=intercellular concentration in relation to atmospheric CO_2; gs=stomatal conductance to water vapor in mol H_2O m^{-2} s^{-1}; E=transpiration rate in mmol H_2O m^{-2} s^{-1}. Means followed by the same capital letter do not differ between temporal observation and those with lowercase letters do not differ between the water regimes of each temporal observation and by the Mann–Whitney test ($P\leq0.05$) by Student's t test and Wilcoxon test ($P\leq0.05$)
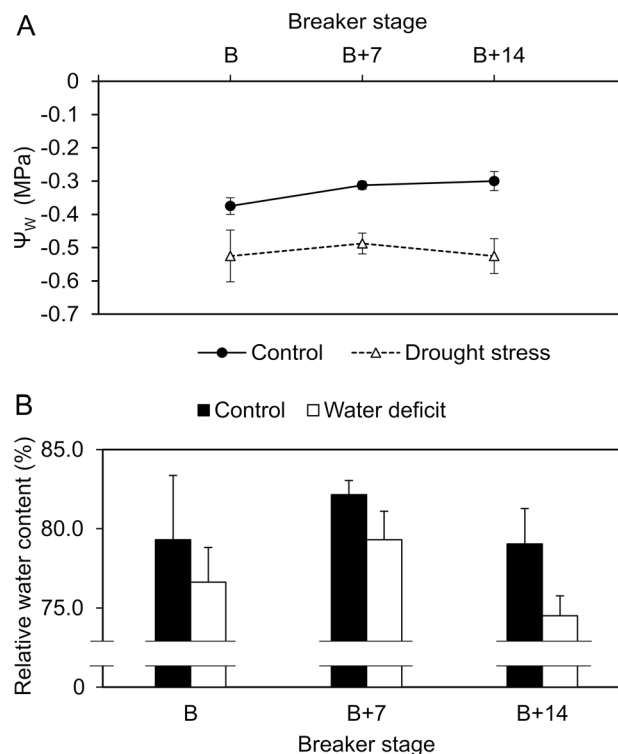
Fig. 1 Predawn leaf water potential and relative leaf water content of *Solanum lycopersicum* L. cv. Santa Clara is subjected to different water regimes. **A** predawn leaf water potential. The points represent the mean of four to five replications and the bars represent the standard error of the mean in each temporal observation. *5% significance by Student's *t* test. **B** Relative leaf water content (%). The columns represent the mean of four replications and the bars represent the standard error of the mean in each temporal observation

($P \leq 0.05$) with the treatments. For gs and E, the correlation with Ψ_w was not significant; however, the effects of treatments on these variables, by the multivariate test by Lambda Wilks, were significant (Fig. 2).
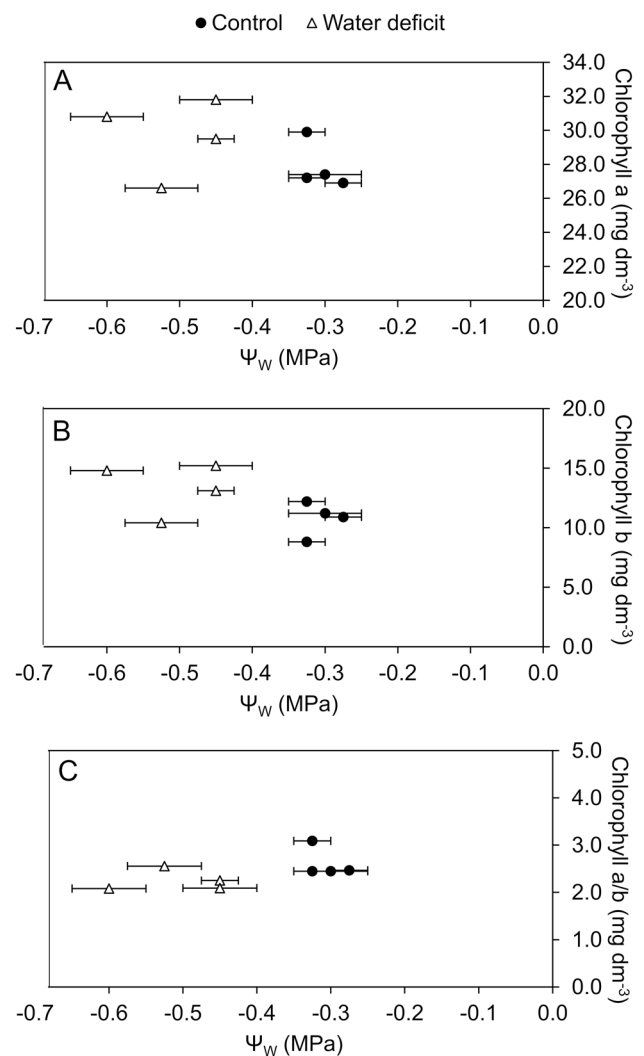
Concerning the leaf chloroplast pigments, the obtained *a* and *b* Falker chlorophyll indices (FCI) were lower in the control treatment compared to the mean values found in the water deficit treatment. In the control treatment, the chlorophyll *a* concentration ranged from 26.9 to 29.9 mg dm^{-3}, while in plants under water deficit conditions they ranged from 26.6 to 31.8 mg dm^{-3} (Fig. 3a). Chlorophyll *b* concentration ranged from 8.8 to 12.2 mg dm^{-3} and from 10.4 to 15.2 mg dm^{-3} for control and water deficit treatments, respectively (Fig. 3b). The chlorophyll *a/b* ratio was higher in the control (2.4 to 3.1 mg dm^{-3}) than in the water deficit treatment (2.1 to 2.6 mg dm^{-3}) (Fig. 3c). Although the concentrations of these pigments were not significantly different between treatments, their simultaneous relationships with Ψ_w show an effect by the Lambda Wilks test.
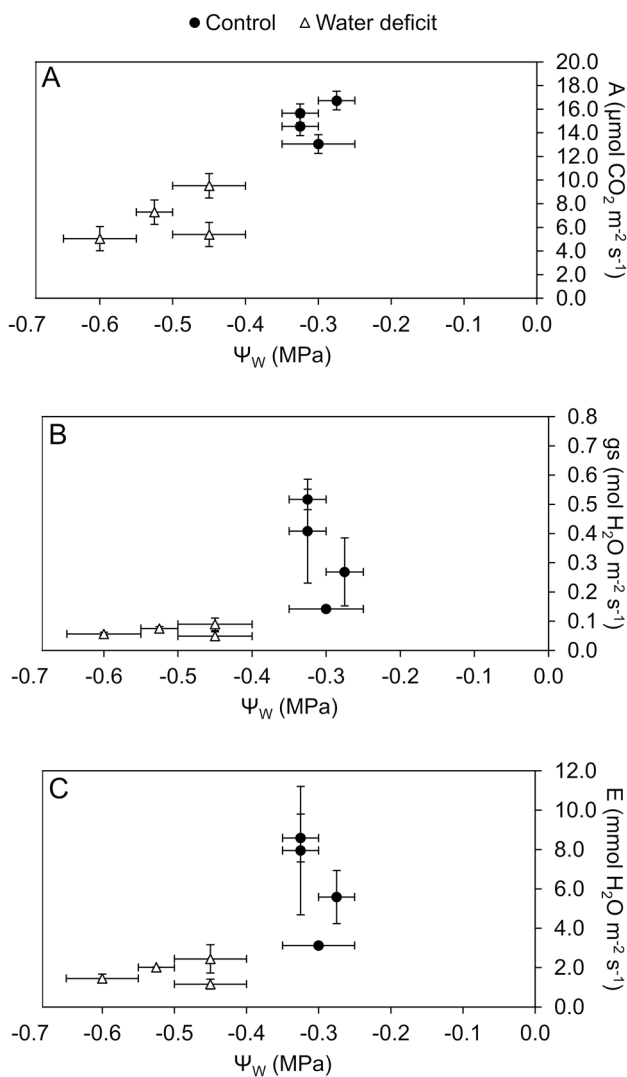
Fig. 2 Gas exchange in *Solanum lycopersicum* L. cv. Santa Clara under different water regimes. **A** Net photosynthetic rate. **B** Stomatal conductance to water vapor. **C** Transpiration rate. The points represent the mean of the plants in the three stages of evaluation (B, B + 7 and B + 14) with four replications per treatment and the bars represent the standard error of the mean

Fig. 3 Chlorophyll *a* and *b* index in *Solanum lycopersicum* L. cv. Santa Clara subjected to different water regimes. **A** Chlorophyll *a* index. **B** Chlorophyll *b* index. **C** Chlorophyll *a/b* ratio. The points represent point estimates of chloroplast pigments and means of temporal measurements of water potentials that differed between treatments at the significance level of 5%

Impact of water deficit on plant growth and fruit-related variables

The stem diameter and total plant height did not show significant differences at the beginning of the treatments (Table 2), indicating that the plants were in similar conditions for conducting the different water regimes. At the end of the analyses, the effects of water deficit on these variables were significantly pronounced. Among the studied variables, the number of leaves and fruits per plant, leaf, stem and total biomass and relative growth rate showed significant differences between treatments.

Although a significant fruit yield reduction per plant was observed in the water deficit treatment, the productive efficiency in this treatment did not differ significantly from the control treatment (Table 2). The leaf, stem and root biomass allocation patterns were similar between treatments, but the statistical similarity was only observed for root allocation (Fig. 4). The leaf biomass was higher in plants under water deficit conditions, while the stem biomass was higher in the control treatment.

Table 3 shows the data obtained for the fruit-related variables. Considering all the studied variables, only fresh biomass, dry biomass, upper polar diameter and equatorial fruit diameter differed significantly between treatments.

Table 2 Descriptive analysis of plant growth and productivity for *Solanum lycopersicum* L. cv. Santa Clara under different water regimes

Variables	N	Control		Water deficit	
Base diameter$_{(initial)}$ (cm)	25	0.87 ± 0.18	a	0.87 ± 0.02	a
Base diameter$_{(final)}$ (cm)	24	1.31 ± 0.04	a	1.07 ± 0.03	b
Height $_{(initial)}$ (m)	25	1.39 ± 0.04	a	1.35 ± 0.04	a
Height $_{(final)}$ (m)	24	1.95 ± 0.07	a	1.76 ± 0.05	b
Leaf number	24	143.58 ± 8.30	a	107.17 ± 6.85	b
Leaf dry biomass (g)	24	50.23 ± 1.98	a	38.97 ± 1.42	b
Stem dry biomass (g)	24	64.28 ± 1.66	a	41.39 ± 1.03	b
Root dry biomass (g)	21	5.87 ± 0.73	a	5.08 ± 0.29	a
Total dry biomass (g)	22	119.63 ± 3.82	a	86.68 ± 2.50	b
Root volume (mL)	21	40.48 ± 3.41	a	33.33 ± 1.70	a
Leaf area (cm^2)	5	4764.83 ± 391.71	a	2787.91 ± 403.57	a
Specific leaf mass (mg cm^{-2})	5	18.75 ± 2.55	a	11.02 ± 2.49	a
Leaf area ratio (cm^2 g^{-1})	5	29.93 ± 3.15	a	39.60 ± 5.49	a
Relative growth rate (g g^{-1} day^{-1})	5	0.065 ± 0.0006	a	0.058 ± 0.0017	b
Net assimilation rate (g m^{-2} day^{-1})	5	15.31 ± 1.38	a	10.55 ± 1.42	a
**Fruit number/plant	25	6.4 ± 0.90	a	1.88 ± 0.34	b
Productive efficiency (g g^{-1})	13	0.92 ± 0.09	a	1.12 ± 0.17	a

N = number of observations. Means followed by the same letter do not differ between water treatments by Student's t test ($P \leq 0.05$) or by the **Mann–Whitney test ($P \leq 0.05$)
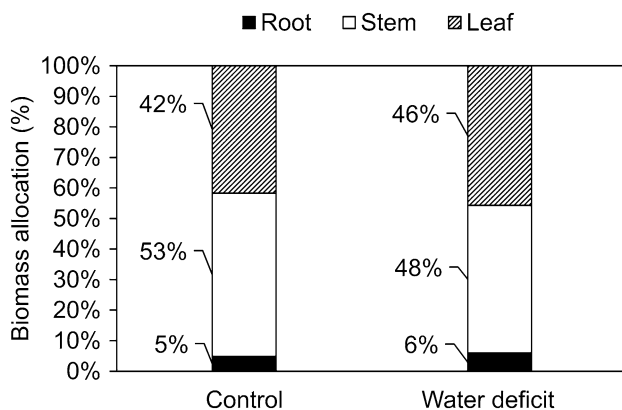
Fig. 4 Biomass allocation in leaves, stems and roots in *Solanum lycopersicum* L. cv. Santa Clara under different water regimes. It was calculated as the ratio of the dry mass of the respective plant part to the final total dry mass. Treatments followed by the same letters do not differ statistically from each other by Student's t test ($P \leq 0.05$)

Exploratory multivariate factor analysis revealed the interdependent relationship of variables that translate the fruit quality under different water regime conditions. Figure 5 shows that the biomass variables have a low correlation with all other variables, in which 27.8% of total variance are shared for the formation of the second factor, while the variables UPD, LPD, ED, pH and SSC share 55.8% of the total variance for the formation of the first factor by principal component analysis. The variable SSC followed by pH had the least influence in determining the first factor. Thus, the diameters, fresh and dry weight biomass of fruits can be used as the only qualitative factors to assess a possible association between water treatments.

Impact of water deficit on antioxidant enzyme activities

Enzyme activities tended to be higher in leaves of plants under water deficit treatment, although significant differences were observed only for APX and PAL (Fig. 6). On the other hand, there were no significant differences between treatments for any of the enzymatic activities evaluated in fruits. SOD and GPX activities were slightly higher in fruits of plants under water deficit conditions, whereas the opposite was observed for the activities of APX and PAL.

Impact of water deficit on the expression of carotenoid biosynthesis genes

According to the RNA-Seq data obtained by Mou et al. (2015), 18 genes of the carotenoid biosynthesis pathway were differentially expressed during the maturation of tomato fruits. Concerning the effects of ABA or NDGA on the carotenoid pathway, these data showed that *PSY*, *CRTR-B1*, *isopentenyl diphosphate isomerase* (*IPI*), *zeta-carotene desaturase* (*ZDS*), *carotene isomerase* (*CRTISO*), *zeta-carotene isomerase* (*ZISO*) and *NCED* were up-regulated in treatment with ABA and down-regulated in treatment with NDGA (Fig. S1). On the other hand, the genes *LeNCED* and *zeaxanthin epoxidase* (*ZEP*) showed higher expression in the NDGA treatment, whereas *neoxanthin synthase*

Table 3 Descriptive analysis of fruit-related variables in *Solanum lycopersicum* L. cv. Santa Clara under different water regimes

Variables	N	Control		N	Water deficit	
Fruit fixation (days)	64	41.98 ± 0.33	a	12	42.5 ± 0.60	a
Breaker (days)	64	45.39 ± 0.89	a	12	46.25 ± 2.29	a
Fresh biomass (g)	69	20.07 ± 1.16	a	14	14.64 ± 2.23	b
Dry biomass (g)	13	0.51 ± 0.05	a	11	0.33 ± 0.06	b
UPD (cm)	69	3.65 ± 0.05	a	14	3.24 ± 0.18	b
LPD (cm)	69	2.56 ± 0.04	a	14	2.32 ± 0.17	a
ED (cm)	69	3.77 ± 0.06	a	14	3.32 ± 0.19	b
pH	74	4.43 ± 0.02	a	13	4.34 ± 0.04	a
SSC (°Brix)	66	4.63 ± 0.08	a	12	5.29 ± 0.32	a

Breaker = number of days from anthesis to beginning of fruit maturation; ED = equatorial diameter; Fruit fixation = number of days from transplantation to anthesis; LPD = lower polar diameter; N = number of observations; SSC = total soluble solids; UPD = upper polar diameter. Means followed by the same letter do not differ between water treatments by Student's t test ($P \leq 0.05$)
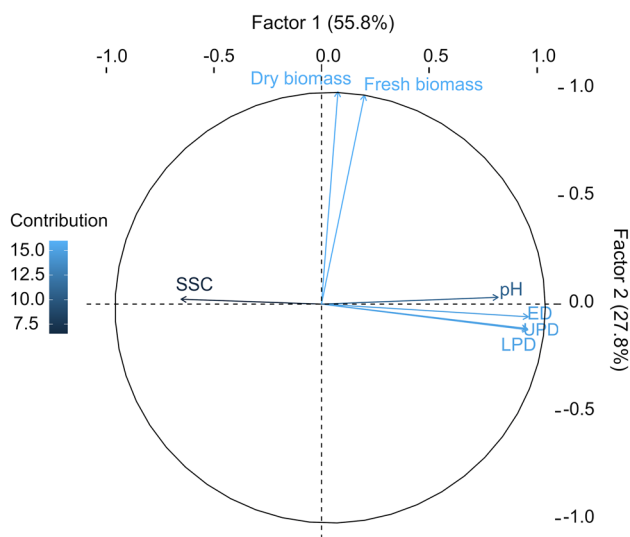
Fig. 5 Principal component analysis performed with Euclidean distance for fruit quality data in *Solanum lycopersicum* L. cv. Santa Clara plants subjected to different water regimes

(*NSY*), *1-deoxy-d-xylulose 5-phosphate synthase* (*DXS*), *geranylgeranyl diphosphate synthase* (*GGPS*) and *CRTR-B2* had higher expression levels in the control treatment. Both *phytoene desaturases* (*PDS*) were inhibited by NDGA, however, their expressions varied between the control and ABA treatments.

The ABA-regulated carotenoid biosynthesis genes were selected for further analysis of their expression patterns under the water deficit conditions established in the present study, through RT-qPCR. Most carotenoid biosynthesis genes were either down-regulated or negligibly affected by water deficit in fruits of 'Santa Clara' (Fig. 7 and Table S2). The only exception was *ZISO*, which was strongly up-regulated. Because these genes belong to the same biosynthetic pathway and to avoid a Type I error with successive comparisons of means, the relationships of the set of genes with different water

regimes were evaluated by means of multivariate analysis. The Lambda Wilks test showed no statistical difference ($P \leq 0.05$) in the effects of water regimes on the set of expression data of the carotenoid biosynthesis genes. In exploring the expression behavior of these genes, principal component analysis showed the construction of two antagonistic factors. The first factor grouped the genes *IPI, PSY, PDS, ZISO, CRTISO, CRTR-B1, CRTR-B2, NCED* and *LeNCED*, which share 68.5% of the total variance, while the second factor grouped the genes *DXS* and *GGPS*, which share 22.7% of the total variance (Fig. S2). The genes *LeNCED* and *PSY*, followed by the genes *CRTR-B2* and *CRTR-B1*, made the greatest contribution to the variations observed in the expression patterns of the carotenoid biosynthesis genes under different water conditions (Fig. S2).

'Micro-Tom' NILs harboring the *r*, *og* and *B* mutant alleles were subjected to the same experimental conditions as 'Santa Clara' to test the hypothesis of whether changes in fruit carotenoid metabolites alter the effects of water deficit on the transcription of the pathway genes. In the WT 'Micro-Tom', most carotenoid biosynthesis genes showed small changes of expression in fruits in response to water deficit, except for *CRTISO* and *CRTR-B2*, which were notably up- and down-regulated, respectively (Fig. 8 and Table S2). On the other hand, different effects of water deficit on the expression of carotenoid biosynthesis genes in fruits were observed depending on the mutant allele. All the carotenoid biosynthesis genes were down-regulated by water deficit in the *r* and *og* mutants, whereas the opposite was observed in the *B* mutant (Fig. 8 and Table S2).

Discussion

To assess how moderate water deficit applied at the fruit development stage can compromise the physiology of cultivated tomato, physiological and plant growth parameters, as well as other parameters related to photosynthesis, were

Fig. 6 Antioxidant enzyme activities in leaves and fruits of *Solanum lycopersicum* L. cv. Santa Clara subjected to different water regimes. **A** Superoxide dismutase (SOD) activity. **B** Ascorbate peroxidase (APX) activity. **C** Guaiacol peroxidase (GPX) activity. **D** Phenylalanine ammonia lyase (PAL) activity. Means followed by the same letters do not differ by Student's *t* test ($P \leq 0.05$)
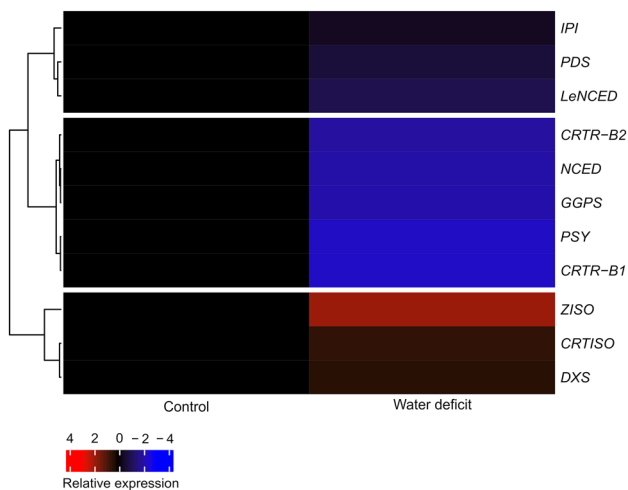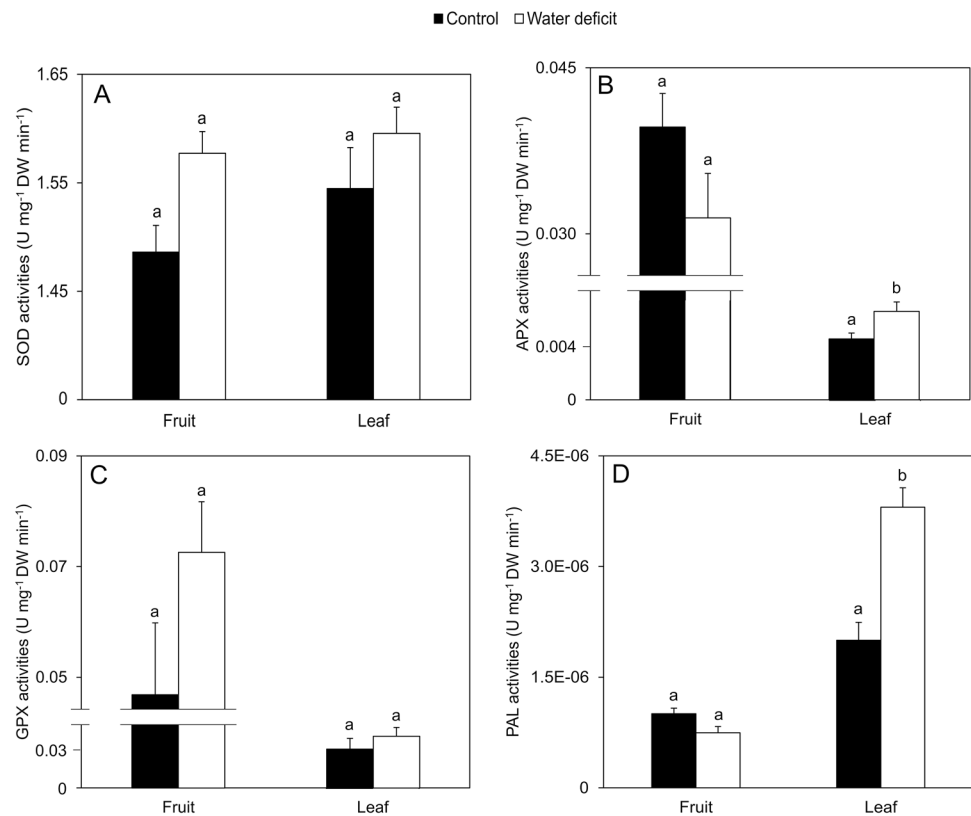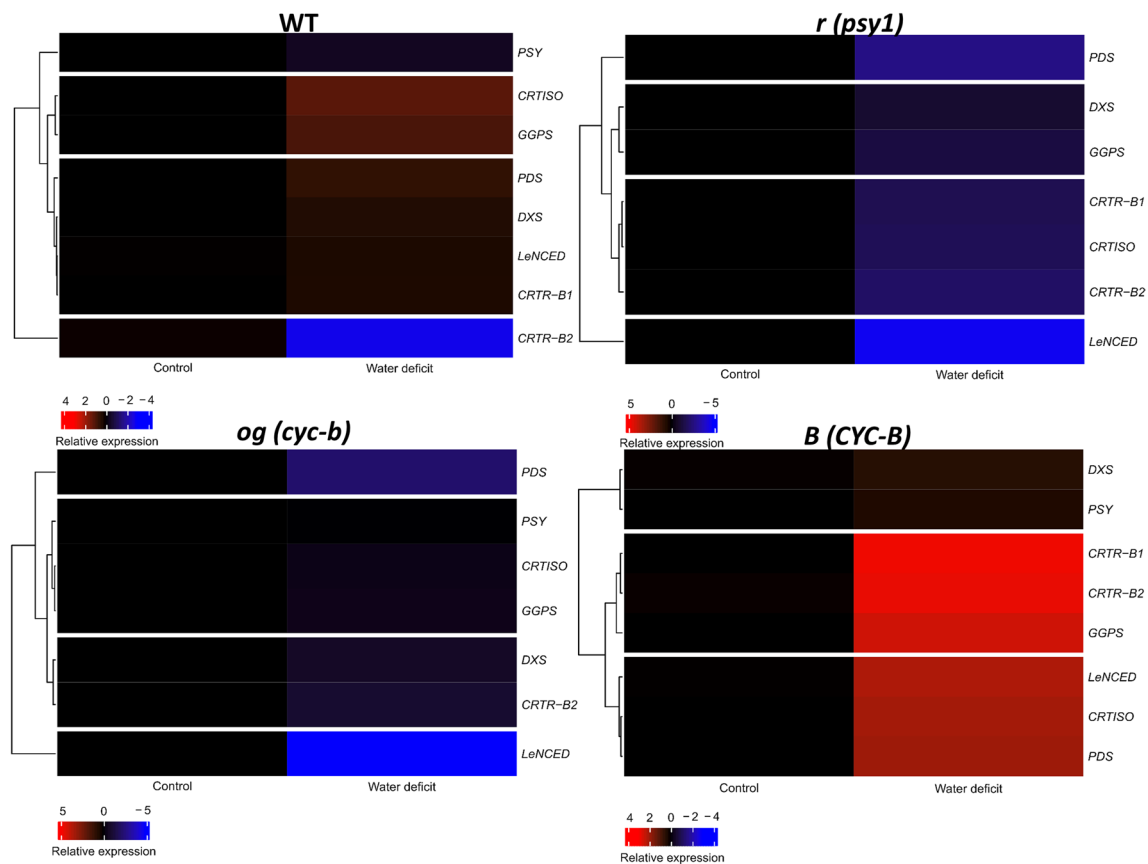

Fig. 7 Gene expression of the carotenoid biosynthesis genes in fruits of *Solanum lycopersicum* L. cv. Santa Clara is subjected to different water regimes. Expression levels were normalized to the corresponding expression value of the gene *ACT*

monitored. Ψw decreased according to water reduction (Fig. 1a), with minimum mean values of -0.38 MPa for the control and -0.53 MPa for the water deficit treatment. Reduction in the water potential values was observed for a commercial tomato line under mild water deficit conditions, even when grafted in a drought-tolerant rootstock (Nilsen

et al. 2014). According to Chaves et al. (2003), drought tolerance can be defined as the plant's capacity to cope with low leaf water potential under water deficit. Our results indicate that the plants were already acclimated to the water deficit at the B stage.

One of the most important physiological responses of plants to water deficit is the inhibition or reduction of photosynthesis, which consequently leads to loss of yield (Perez-Martin et al. 2014). In Santa Clara, the effect of moderate water deficit also reflected in the decrease of *A*, *gs*, *E* and *Ci/Ca* (Table 1), with a significant relationship with Ψw (Fig. 2). The *gs* of plants under water deficit conditions showed a smaller variation than that of the control plants, in which the stomata may be contributing to different degrees of opening, revealing the importance of stomatal limitation in water deficit conditions. Photosynthetic efficiency is most affected by water deficit due to the impairment of photosystem II (PSII), one of the most sensitive components (Gururani et al. 2015).

Plants have developed several strategies to avoid damage caused by dehydration (Hsieh et al. 2010). According to our results, the carboxylation efficiency was lower under water deficit conditions since the decrease in stomatal conductance contributes to the reduction of the internal concentration of CO_2. This relationship between photosynthetic rate and stomatal conductance confirms the important role of internal CO_2 diffusion in promoting

Fig. 8 Gene expression of the carotenoid biosynthesis genes in fruits of near-isogenic lines (NILs) in *Solanum lycopersicum* L. cv. Micro-Tom harboring the mutations *yellow flesh* (*r*), *Beta-carotene* (*B*) and *old-gold crimson* (*og*), subjected to control (irrigated) and water deficit treatments. Expression levels were normalized to the corresponding expression value of the gene *ACT*. *WT* wild-type

photosynthesis (Terashima et al. 2011; Pallozzi et al. 2013). Reduced CO_2 flux at the carboxylation sites is one of the main causes for the reduced photosynthetic rates since water controls the stomatal opening (Bosco et al. 2009). Thus, carboxylation efficiency is affected by the availability of CO_2 in the leaf mesophyll, as well as by temperature, light amount, and enzymatic activity (Taiz et al. 2017).

Minimizing water loss from stomata is a valuable approach for improving water use efficiency and drought tolerance (Hsieh et al. 2010; Kim et al. 2012). In Santa Clara, the *RWC* was not significantly different between the two water regimes (Fig. 1b), while the intrinsic and instantaneous water use efficiencies were significantly higher in the water deficit treatment. Yu et al. (2020) performed a meta-analysis to examine the impact of water deficit on water use efficiency and observed that water use efficiency and productivity in tomato plants can be improved under water deficit conditions. The wild tomato species *S. pennellii* shows greater water use efficiency than cultivated tomato and for this reason used as a source of drought resistance (Borba et al. 2017; Oliveira et al. 2021).

The leaf chloroplast pigments were not significantly different between the water treatments in Santa Clara (Fig. 3). However, together with *Ψw,* the effect of water deficit was pronounced. Abiotic stresses generally reduce chlorophyll contents in plants, and it may be related to the generation of reactive oxygen species (Silva and Asaeda 2017). A 45.4% decrease in total chlorophyll content was found in tomato seedlings under water deficit compared to the control conditions, a similar pattern also observed with chlorophylls *a* and *b* (Raja et al. 2020).

Another symptomatic parameter of stress-induced damage is plant growth, which is linked to energy resources generated by photosynthesis. Stress exposure leads to excessive excitation of the photosynthetic electron transport chain, altering the activity of the photosystem and causing decreased energy synthesis necessary for photosynthesis (Bechtold and Field 2018). Among the plant growth-related data, the variables with significant alterations induced by moderate water deficit were stem diameter, total plant height, number of leaves and fruits, and leaf, stem and total biomass (Table 2). These results are consistent with other studies that report a reduction in these variables, especially

in cultivated tomato, which is highly susceptible to drought. Wahb-Allah et al. (2011) showed that plant height was impaired by reduced irrigation, with significant variations observed among the different genotypes analyzed. Regarding stem diameter, Conti et al. (2019) noted a slight drop in different tomato varieties 16 days after withholding irrigation.

The biomass allocation to different plant organs was similar between treatments, but significant differences were observed in leaf and stem biomass allocation (Fig. 4). The root dry mass ratio did not differ between treatments. Liu and Stützel (2004) developed a study similar to ours and observed a gain in the allocation of root biomass in stressed plants, indicating that plants under water deficit invest more energy in root development for better use of available water in the soil.

The relative growth rate had a significant effect between the water regimes, leading to its decrease in plants under water deficit conditions, as also observed by Bian et al. (2019). This can be explained by the reduction of carboxylation efficiency in plants because of partial stomatal closure under water stress conditions. Net assimilation rate and leaf area ratio had no significant changes under water deficit conditions in Santa Clara (Table 2).

Fruit quality is associated with different characteristics, including SSC and pH. In our study, the effect of moderate water deficit was significant in the fruit weight, upper polar diameter and equatorial diameter (Table 3). These were the variables that most contributed as indicators to estimate fruit quality (Fig. 5). Despite the variations observed, pH and SSC did not show a significant difference between the two water regimes, corroborating the results found in previous studies (Di Gioia et al. 2013; Nicoletto et al. 2013; Djidonou et al. 2017; Lang et al. 2020). Although there is no significant variation in these parameters between irrigation treatments, Grieneisen et al. (2018) suggest that pH and SSC measurements continue to be included in evaluations, since these variables can show large differences depending on the stress conditions imposed.

The partial closure of the stomata in response to water deficit in plants causes a reduction in CO_2 and an increase in O_2, thus causing an imbalance of electrons in the photosystem and the formation of reactive oxygen species (Carvalho et al. 2011). In the present study, the enzymatic activities of *SOD*, *APX*, *GPX* and *PAL* in fruits did not differ between the two treatments (Fig. 6). In tomato leaf tissue from the work of Raja et al. (2020), *SOD* activity increased 2.5-fold in plants under water deficit conditions compared to control, while *APX* activities increased three-fold. These differences in relation to our results are probably due to the different duration of the water deficit period, which was only 10 days in the study by Raja et al. (2020) and 50 days in our study, thus allowing the plants to become acclimatized to the water deficit. Consequently, the fruits that matured in this situation

of more prolonged water deficit showed no differences in enzymatic activities between treatments.

In the final development stage of the tomato fruit, the color changes from green to red due to carotenoid accumulation and chlorophyll reduction (Rodrigo and Zacarias 2007). RNA-Seq data showed that the genes of the carotenoid biosynthesis pathway *DXS*, *IPI*, *GGPS*, *PSY*, *PDS*, *ZDS*, *CRTISO*, *ZISO*, *CRTR-B1*, *CRTR-B2*, *ZEP*, *NSY*, *NCED* and *LeNCED* were significantly responsive to the effects of ABA and NDGA (Fig. S1). In our study, the effects of moderate water deficit on the expression of these genes in fruits of 'Santa Clara' and WT 'Micro-Tom' were either negative or negligible, except for the carotene isomerases (Figs. 7 and 8 and Table S2). Two clusters of anticorrelated genes were observed (Fig. S2), indicating that their expression patterns were mutually exclusive. The carotenoid biosynthesis genes that most contributed to the variations in expression profiles under water deficit conditions were *LeNCED* and *PSY*, followed by the genes *CRTR-B1* and *CRTR-B2* (Fig. S2). These results are not quite surprising, as these enzymes catalyze limiting steps in the carotenoid biosynthesis pathway (Galpaz et al. 2006; Li et al. 2009; Zhang et al. 2009). Our study using the *r*, *og* and *B* mutants also confirmed the hypothesis that changes in carotenoid metabolites are able to shift the water deficit effects on the expression of the carotenoid biosynthesis genes in fruits (Fig. 8 and Table S2). These results suggest that there is an interaction between water deficit and carotenoids (*r* mutant) and that the increase in the concentrations of lycopene (*og* mutant) and beta-carotene (*B* mutant) in fruits produce signals that are transduced to either negatively or positively regulate, respectively, the transcription of the carotenoid biosynthesis genes in response to water deficit. The fact that carotenoid biosynthesis genes and enzymes are subjected to feedback and feedforward regulation by carotenoid metabolites supports our observations (Kachanovsky et al. 2012; Enfissi et al. 2017). Therefore, our results suggest the involvement of carotenoid metabolites in regulating feedback and feedforward the water deficit-mediated responses of pathway genes.

Conclusion

Solanum lycopersicum L. cv. Santa Clara showed adaptive responses to moderate water deficit since the photosynthetic efficiency was higher under these conditions, and with the reduction of transpiration rate, the RWC did not differ significantly from plants under control conditions. This adaptive response was also observed with an increase in the intrinsic and instantaneous water use efficiencies, as well as in the control of oxidative stress and changes in the expression profiles of carotenoid biosynthesis genes in fruits. However, these adaptive responses were not sufficient to maintain the

relative growth rate of the plant and the fruit weight, diameter and yield under conditions of moderate water deficit.

Author contribution statement DSRJ, LRC and MGCC conceived and designed the experiments. DSRJ, ACOB, IAB and NSL conducted the experiments. DSRJ, LRC and MGCC analyzed the data and drafted the manuscript. MGCC supported the project. All authors read and approved the final manuscript.

Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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