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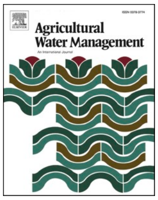

Comparison of physiological and biochemical responses of local and commercial tomato varieties under water stress and rehydration

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ABSTRACT

Water scarcity presents an increasingly urgent challenge with global implications for the production of irrigated vegetables. Among these crops, tomatoes stand out as one of the most widely cultivated. Given their vulnerability to water stress, it is crucial to ensure efficient and sustainable water management for tomato irrigation. This study aims to compare physiological and biochemical parameters among three local and three commercial resilient tomato varieties in response to water stress and rehydration. We subjected tomato plants to either two brief periods of water stress (WE1) or one extended period of water stress (WE2), followed by rehydration. Our results did not reveal significant differences in the response to water stress among the varieties, which could be attributed to their respective origins. Following rehydration, the plants quickly returned to their normal physiological values. An exploration of oxidative stress markers revealed that oxidative damage occurred solely during the second episode of water stress in WE1 plants, or towards the conclusion of the prolonged water stress period in WE2 plants. However, after rehydration, tomato plants returned to normal oxidative parameters values, indicating the absence of irreversible damage. Although the severe water stress did not compromise the viability of the plants, all treatments and varieties exhibited a predictable and substantial growth inhibition. In conclusion, the different tomato varieties studied exhibited similar responses to water stress, primarily characterized by the inhibition of gas exchange processes and heightened oxidative stress. Nonetheless, none of the plants suffered irreversible damage from this stress.

1. Introduction

Water scarcity is one of the greatest challenges of our times. This is particularly serious in the cultivation of irrigated vegetables, which are often grown in areas with limited water supplies (Gallardo et al., 2006). Tomato (*Solanum lycopersicum* L.) is one of the most widely-produced irrigated vegetable crops in the world. Spain, with 56,110 ha of tomato cultivation, ranks as the second-largest tomato producer in the European Union, just behind Italy (FAOSTAT, 2023). In southeastern Mediterranean regions of Spain, greenhouses are the leading providers of tomatoes to Western Europe (Castilla, 2007; Acebedo et al., 2022). Here, water supply is a limiting factor for crop production, so improved irrigation efficiency is essential to maintaining the sustainability of these systems.

Tomato plants are especially vulnerable to drought stress and require an abundance of water for vegetative and reproductive growth, especially during their flowering and fruit enlargement stages (Jangid and Dwivedi, 2016). While water stress may improve a crop's resilience and adaptability, it is often extremely damaging. This damage is often manifested through stomatal closure that reduces CO_2 intake, subsequently diminishing photosynthetic efficiency, increasing the formation of free radicals, and resulting in a drastic decrease in crop yield (Landi et al., 2017; Conti et al., 2019; Hao et al., 2019). Therefore, measurements of stomatal conductance, transpiration, and leaf surface temperature are useful for determining the plant's water status and its ability to avoid this stress, while oxidative stress and antioxidants help to identify oxidative damage and biochemical defenses (Sánchez-Rodríguez et al., 2010; Nankishore and Farrell, 2016). When plants are exposed to water stress, they respond with the abscisic acid (ABA) hormone, which stimulates the closure of stomatal guard cells to prevent water loss. This leads to the plant's inability to exchange gases, resulting in a drastic decrease in net photosynthesis (Saradadevi et al., 2017). The speed at which gas exchange declines as water stress intensifies can provide insights into drought tolerance. On the other hand, reactive oxygen species

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(ROS) accumulations are a consequence of gas exchange reduction, resulting in O_2 accumulation, CO_2 limitation, and the concomitant over-reduction of the photosynthetic electron transport chain during photorespiration (Laxa et al., 2019; Molina-Moya et al., 2019). ROS are responsible for inducing oxidative damage to lipids, proteins, carbohydrates, and DNA, leading to reduced growth, diminished harvest yields, and even plant death (Nemeskéri and Helyes, 2019). To counteract these damages, plants can activate a range of antioxidant mechanisms, both enzymatic and non-enzymatic. These mechanisms work to maintain ROS levels that are compatible with the normal functioning of the cells (Peco et al., 2021). Among them, phenolic acids and flavonoids act as a reducing agents and free radical scavengers, constituting relevant resistance mechanisms against both abiotic and biotic stressors (Sánchez-Rodríguez et al., 2011; Nakabayashi et al., 2014).

Understanding the recovery of gas exchange parameters after rehydration is crucial for comprehending the effects of water stress and possible acclimatization in plants (Bruce et al., 2007). As mentioned earlier, tomato plants exposed to water stress exhibited a rapid decline in gas exchange values. Under mild and moderate water stress, stomatal limitations prevailed over non-stomatal factors, whereas severe stress was characterized by non-stomatal limitations (Flexas et al., 2002). Consequently, during mild and moderate stress, these plants usually restore their normal values upon rehydration within a few days (Iovieno et al., 2016; Giorio et al., 2018; Hao et al., 2019). Regarding biochemical changes, Hao et al. (2019) observed several alterations in oxidative stress markers throughout the water stress period. However, these changes were reversed to their baseline values once the tomato plants were rehydrated. Additionally, the use of consecutive stress periods revealed a "memory effect" in tomato plants, as indicated by the increased accumulation of proline reported by Ioveno et al. (2016). It is important to emphasize that while studies involving rehydration periods offer valuable insights into the behavior of tomato plants under water stress, research on this specific subject remains limited at present.

Nowadays, the scientific community is making great efforts to find a solution to water scarcity. Significant progress has been made in two major areas: the search for and improvement of drought-resistant varieties, and the implementation of water-saving agriculture techniques (Chai et al., 2015; Nankishore and Farrell, 2016; Conti et al., 2019). The agronomic types of this species show vast genetic variations, which determine how they respond to environmental limitations such as water stress (Sánchez-Rodríguez et al., 2010, 2011; Conti et al., 2019; Villena et al., 2023a). As such, it is important to develop more resilient varieties that can handle water stress while still maintaining acceptable yield. In this sense, genetic biodiversity can be seen as a treasured resource for boosting crop yields and improving quality in challenging conditions (Salgotra and Chauhan, 2023). Local tomato crops, often cultivated in arid lands and frequently facing water scarcity conditions, have developed specific characteristics that enhance their adaptation to such environments (Villena et al., 2023b). These traits result from natural selection and the cultivation practices employed in these regions and could prove useful for improving new commercial varieties (Sousaraei et al., 2021). Additionally, the usage of water-saving agriculture techniques takes advantage of the understanding of the various stages of fruit growth and their sensitivity to water stress, enabling the efficient use of water while lessening the impact of decreased irrigation on yield and fruit quality (Galindo et al., 2018). Therefore, further research is necessary to comprehend the physiological response of tomatoes to water stress and rehydration. Such research could enhance the adaptability and resilience of tomato plants, thereby making water-saving techniques more effective.

Our objective is to compare the physiological responses of three local and three commercial tomato varieties during their vegetative period when subjected to water stress and subsequent rehydration. To achieve this, we will assess various growth and physiological parameters, including biomass content, stem water potential, leaf gas exchange, and oxidative stress markers. This research is motivated by the understanding that distinct farming practices and the emphasis on high production, without considering potential limitations in commercial plants, may lead to divergent physiological responses compared to local plants when subjected to water stress.

2. Material and methods

2.1. Plant material and growth conditions

In this study, six varieties of tomatoes (*Solanum lycopersicum* L.) were evaluated: three commercial varieties ('Sintonía' ('SN'), 'Marejada' ('MR') and 'Valenciano' ('VL')) and three local varieties ('SL-'82′' ('82′), 'SL-'264′' ('264′) and 'SL-'260′' ('260′)). Descriptions of the genotypes used in the study can be found in Table 1. Three different morphologies of local tomatoes were used, and their commercial counterparts were sought for comparison. The commercial varieties were secured from an agricultural company in Southern Spain (Marín et al., 2021), and the local varieties were obtained from the gene bank of the Higher Technical School of Agricultural Engineers at the University of Castilla-La Mancha located in Ciudad Real (Moreno et al., 2010; Villena et al., 2023a, 2023b).

Tomato seeds were germinated and grown in porex seedbeds under controlled conditions of lighting, temperature, and humidity until they reached the phenological stage 104 (fourth true leaf of the main stem unfolded) according to the BBCH scale (Feller et al., 1995). Subsequently, the seedlings were transplanted and grown in 40 L pots filled with 7.5 Kg of a sand and plant substrate mixture (1:2) in a greenhouse located at the Agriculture Research Area of the Polytechnic University of Madrid (40°26'21.8"N 3°44'15.7"W) for two months. The daily average, maximum (day) and minimum (night) temperatures in the greenhouse were 23.2 °C, 29.6 °C, and 14.6 °C, respectively, with an average relative humidity of 51.8%. For a month prior to the start of the water stress treatments, tomato plants were acclimated and watered daily to water-holding capacity (WHC) of the soil. The experimental design was focused on the seedling expansion phase of tomato plant growth, chosen due to its stable characteristics for studying behavior under water stress. Conducting the study during the flowering or fruiting stages would introduce additional variables that could affect water relations and vegetative growth (Guichard et al., 2005).

The varieties were arranged in a randomized complete block design with three treatments, four replicates per treatment, and six varieties, totaling 12 pots per variety and 72 pots in total. Each treatment was applied as follows:

- Control (C): Served as the control and were irrigated every day to their WHC.
- Water Stress 1 (WE1): Two periods of water stress were imposed. Five days after the initiation of the experiment, plants were not watered for 10 days. On the fifteenth day, the plants were irrigated to their WHC, and on the sixteenth day, they were deprived of water for 15 days. Finally, plants were rehydrated to their WHC.

Table 1
List and description of tomato genotypes grown in this experiment.

Name	Code	Type	Description	Growth
Sintonía	'SN'	Commercial cultivar	Cherry red variety	Indeterminate
Marejada	'MR'	Commercial cultivar	Medium black-red variety	Indeterminate
Valenciano	'VL'	Commercial cultivar	Medium Red variety	Indeterminate
SL-82	'82′	Local landrace	Cherry red variety	Indeterminate
SL-264	'264′	Local landrace	Medium black-red variety	Indeterminate
SL-260	'260′	Local landrace	Medium red variety	Indeterminate

- Water Stress 2 (WE2): One long period of water stress was applied. Five days after the experiment began, plants were not watered for 25 days. However, when the stem water potential was below − 1.4 MPa, plants were irrigated with 100 ml to maintain them in a state of severe water stress while still ensuring their survival. At the end of the experiment, plants were rehydrated to their WHC.

During both treatments (WE1 and WE2), the application of severe water stress was ensured following the criteria established by Zgallaï et al. (2006), and Silva et al. (2012), who defined it as a plant water potential ranging between − 1 and − 1.4 MPa.

2.2. Morphological parameters

At the end of the experiment, the leaf area of each plant was estimated. To accomplish this, a photograph of each tomato leaf was taken and the total area was analyzed and calculated using the ImageJ program (version 2.1.0). Additionally, aerial and root matter were oven-dried at 60 °C for 72 h, weighed, and the ratio between both was calculated (Peco et al., 2021).

On the other hand, the stomatal densities were measured by taking three impressions of the abaxial face of the leaf per plant with transparent nail polish, and then examining it under an Olympus BX51 light microscope. For this purpose, only random leaves located at the midpoint of the plant's height were selected from adequately hydrated plants at the halfway point of the experiment. For this purpose, only random leaves at half height of the plant were selected from well-watered plants. This process was only done in C plants, as water stress causes a stoppage in the vegetative growth of the plant and, given the duration of the experiment, no results would be found in WE1 and WE2 plants (data not shown).

2.3. Stem water potential

The stem water potential (Ψ_{stem}) was assessed at midday using a Scholander pressure chamber (PMS Instrument Company). The measurements were carried out on the fifth leaves from the shoot apex of each plant, with one leaf selected per plant. The leaves were covered with opaque bags two hours prior to the measurement. The results were expressed in megapascals (MPa). Measurements were conducted at nine specific points, twice weekly. This frequency of measurement allowed for a comprehensive exploration of the dynamics associated with water stress and subsequent rehydration processes.

2.4. Gas exchange parameters and leaf temperature

Transpiration (E), stomatal conductance (Gs), and leaf temperature (T) were measured at midday on the fifth leaves from the shoot apex of each plant. A CIRAS-3 DC CO2/H2O Gas Analyzer (PP-Systems, Amesbury, MA, USA), equipped with an automatic universal leaf cuvette (PLC6-U, PP-Systems), was used for these measurements. The gas exchange results were expressed as a percentage variation relative to the C treatment for each variety. These measurements were conducted simultaneously with the assessment of Ψ_{stem}.

2.5. Laboratory measurements

The laboratory measurements involved randomly selecting two leaves per plant, situated at a midpoint along each plant's height. These leaves were promptly frozen and stored at − 80 °C until analysis. Samples were collected at four specific time points during the experiment, corresponding to critical stages of stress and rehydration: days 14, 16, 28, and 33. To ensure accuracy, the results were corrected to account for variations in water content among samples taken from different sampling points. The following analyses were performed.

2.5.1. Lipid peroxidation

Lipid peroxidation was estimated via the extraction of malondialdehyde (MDA) content, following the method described by Buege and Aust (1978) with some modifications done by Peco et al. (2021) and Nascimento et al. (2020). Homogenization of leaves was carried out with 0.1% (w/v) trichloroacetic acid (TCA) in a rotary homogenizer. Afterwards, the homogenate was centrifuged at 10000 g for 15 min and aliquots of the supernatant were added to the reaction medium composed of 0.5% (w/v) thiobarbituric acid, 10% (w/v) trichloroacetic acid and 0.01% (w/v) butylated hydroxytoluene. The reaction was performed at 95ºC for 15 min and then quickly cooled on ice. Absorbance was measured at 535 nm and the results were obtained using a calibration curve for MDA. They were expressed as a percentage variation relative to the C treatment for each variety.

2.5.2. Free radical-scavenging activity

The total antioxidant capacity (AC) was analyzed according to the ABTS assay (Jiménez-Escrig et al., 2003). Methanolic extracts of the sample were evaluated using 80% MeOH (v/v) and 1% HCl (v/v). The ABTS (2,2′-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($\text{ABTS}^{\bullet+}$) was obtained by reacting a 7 mM ABTS solution with 2.45 mM potassium persulfate for 16 h in the dark at room temperature. The $\text{ABTS}^{\bullet+}$ solution was then diluted with water to an absorbance of 0.70 at 730 nm. The extract was mixed with the $\text{ABTS}^{\bullet+}$ solution, and after 20 min in the dark at room temperature, the absorbance was read at 730 nm. The results were then calculated using a calibration curve for Trolox (Sigma–Aldrich), and they were expressed as a percentage variation relative to the C treatment for each variety.

2.5.3. Total phenolics and flavonoids content

Methanolic extracts of leaves for both measurements were prepared in 70% MeOH (v/v). Total phenolics content was determined by the Folin–Ciocalteau method (Singleton and Rossi, 1965). 0.25 ml of the supernatant was mixed with 0.5 ml of water and 0.25 ml of Folin-Ciocalteau's reagent and incubated for 1 min in the dark at room temperature. Then, 1 ml of 20% Na_2CO_3 (w/v) was added and incubated for a further 2 h. After centrifugation, the absorbance was measured at 760 nm. The results were calculated using a calibration curve for gallic acid.

Total flavonoids content was determined by the aluminium chloride spectrophotometric method (Zhishen et al., 1999). 0.5 ml of the supernatant was mixed with 0.5 ml of aluminium chloride and incubated for 15 min in the dark at room temperature. The absorbance was then measured at 430 nm. The results were calculated using a calibration curve for quercetin.

2.6. Statistical analysis

All data reported were tested for homogeneity of variance and normality. Analysis of variance (ANOVA) was then performed using SPSS v.24, followed by a Duncan test between the means of the treatments to determine significant differences ($p \leq 0.05$). Regression analyses were conducted using Microsoft Excel, and correlation coefficients (R^2) and equations were calculated using Statistix 10.

3. Results

3.1. Water status of tomato varieties during the experiment

Ψ_{stem} had a downward trend in both WE1 and WE2 plants under water stress (Fig. 1 and Table S1). This decrease was expected and confirms that the WE plants had been successfully subjected to severe stress for the predetermined period. Fully irrigated plants (C) were kept with relatively constant Ψ_{stem} values; however, a marginal decline was noted.

On days 9 and 13, as a consequence of the suppression of irrigation in WE1 and WE2 plants, there was a gradual decrease in Ψ_{stem} in all

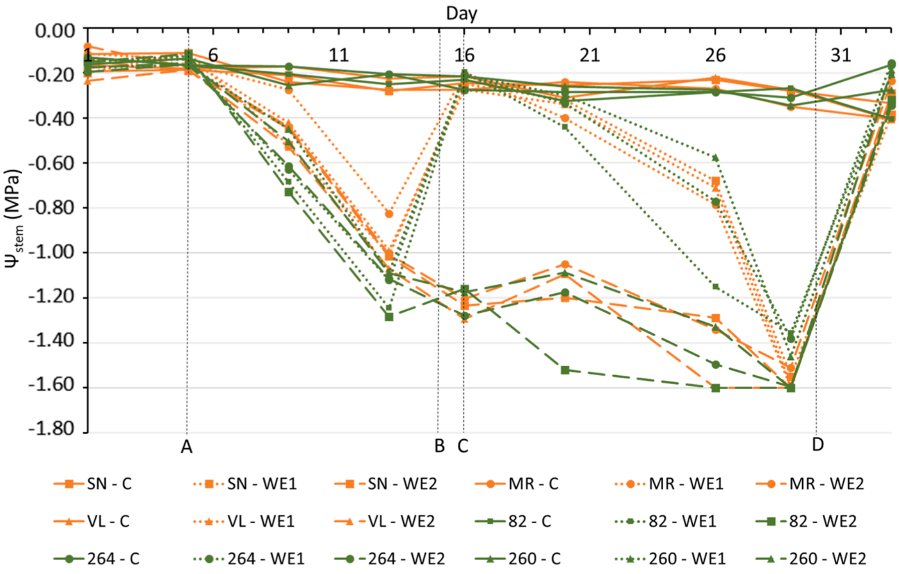


Fig. 1. Evolution of stem water potential (Ψ_{stem}) in six tomato varieties exposed to full irrigation (C), water stress treatment 1 (WE1) and water stress treatment 2 (WE2). Vertical line A (onset of water stress in WE1 and WE2 plants), B (end of water stress in WE1 plants), C (onset of second water stress in WE1 plants) and D (end of water stress in WE1 and WE2 plants). Values show the means of four replicate. Significant differences between treatments are shown in Table S1.

varieties studied. Nevertheless, 'MR' plants had a slower decrease, not appreciating significant differences with respect to the control until day 13. The lowest values were reached by '82′ variety (−1.27 MPa) and the highest by 'MR' plants (- 0.92 MPa). Due to the rehydration of WE1 plants, the sampling on day 16 indicated a complete recovery of all the varieties corresponding to this treatment (WE1). Irrigation was suppressed again for this group of plants (WE1), and consequently, there was a gradual drop in Ψ_{stem}, reaching its minimum value on day 29 (approximately −1.6 MPa). During the first and second water stress periods in WE1 plants, WE2 plants were maintained at minimum water potentials, ensuring their survival with minimal rehydration, maintaining values between – 1.10 and – 1.6 MPa. Finally, on day 30, WE1 and WE2 plants were rehydrated, and by day 33, their Ψ_{stem} values reached similar values to C plants.

3.2. *Morphological parameters*

The varieties exhibited distinct growth rates, as can be seen in their leaf areas (Table 2). Control plants of varieties 'VL' and '82' grew to the largest sizes, while the lowest growth rate was observed in varieties 'MR' and '264', with the remaining varieties exhibiting intermediate sizes.

After subjecting the plants to water stress treatments, notable discrepancies in leaf area were observed between the control group (C) and the plants exposed to water stress treatments (WE1–2) (Table 2). However, no statistically significant differences were found between the leaf areas of WE1 and WE2, although there was a trend indicating that WE2 plants were slightly smaller compared to WE1 plants (WE1 48–67% and WE2 63–72% smaller than C). The reduction in leaf area for both WE1 and WE2 plants was similar across different varieties, but more pronounced in the following order: 'VL' > 'SN' > '260' > 'MR' > '82' > '264' for WE1, and 'SN' > 'VL' > '260' > '264' > 'MR' > '82' for WE2.

The ratio between aerial parts and roots only showed significant differences between WE and C treatments in 'MR' and 'VL' varieties; greater growth in the root was observed compared to the aerial part for C plants (Table 2). Regarding stomatal density analysis in well-irrigated plants, the '264' variety had the highest number of stomata per surface area. 'MR' variety showed an intermediate density, and the lowest density was observed in 'SN' variety. The other genotypes ('VL', '82′ and '260′) presented intermediate densities between 'SN' and 'MR', being in all cases significantly lower than '264′ (Fig. 2).

Table 2
Leaves area and aerial/root dry matter ratio of six tomato varieties exposed to full irrigation (C), water stress treatment 1 (WE1) and water stress treatment 2 (WE2). Values show the means of four replicate. Values represent the mean ± standard error. Values with different lowercase letters are significant different between treatments of each variety and values with different capital letters were significantly different between varieties of the same treatment, at $P \leq 0.05$ (ANOVA, Duncan test).

Variety	Treatment	Leaves area (m^2)			Dry matter of aerial part/dry matter of root		
SN	C	2.0 ± 0.2	a	AB	14.3 ± 4.5	a	AB
	WE1	0.7 ± 0.1	b	A	10.8 ± 2.0	a	A
	WE2	0.5 ± 0.1	b	A	11.2 ± 1.6	a	AB
MR	C	1.5 ± 0.3	a	A	27.6 ± 4.8	a	B
	WE1	0.7 ± 0.1	b	A	15.4 ± 2.9	b	A
	WE2	0.5 ± 0.1	b	A	11.8 ± 1.0	b	AB
VL	C	2.4 ± 0.3	a	B	21.1 ± 2.6	a	AB
	WE1	0.8 ± 0.1	b	A	13.0 ± 2.5	b	A
	WE2	0.7 ± 0.1	b	AB	16.7 ± 0.6	b	B
82	C	2.3 ± 0.4	a	B	16.7 ± 7.4	a	AB
	WE1	1.2 ± 0.2	b	B	16.7 ± 2.5	a	A
	WE2	0.8 ± 0.1	b	B	14.8 ± 1.9	a	AB
264	C	1.8 ± 0.3	a	A	11.0 ± 3.4	a	A
	WE1	0.9 ± 0.1	b	AB	11.7 ± 1.6	a	A
	WE2	0.5 ± 0.1	b	A	9.6 ± 1.7	a	A
260	C	1.9 ± 0.1	a	AB	14.5 ± 4.3	a	AB
	WE1	0.8 ± 0.1	b	AB	13.5 ± 1.5	a	A
	WE2	0.6 ± 0.1	b	AB	14.2 ± 4.0	a	AB

3.3. *Transpiration rate (E), stomatal conductance (Gs) and leaf temperature under water stress and rehydration*

A similar behavior was observed in transpiration (E) and stomatal conductance (Gs) when a water restriction was applied (Fig. 3A and B, and Tables S2 and S3). E and Gs of WE1 and WE2 plants were negatively impacted as a response to the first water stress from day 9 in all varieties, with the exception of 'MR', in which no significant differences were observed until day 13. By day 16, after plant rehydration, it was observed that WE1 plants, except '264′, reached E and Gs values similar to those of C plants, while WE2 plants remained at minimum values.

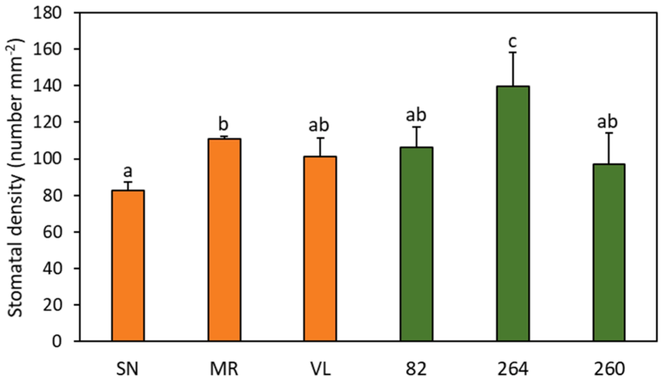


Fig. 2. Stomatal density of control plants in six tomato varieties. Different small letters show significant differences between varieties at $P \leq 0.05$ (ANOVA, Duncan test).

'264′ WE1 plants, despite being rehydrated (according to the Ψ_{stem}, Fig. 1), showed 44.1% and 58.4% less E and Gs than '264′C. On day 20, following the second water restriction, a strong drop of E and Gs was observed in WE1, until reaching values close to WE2. However, 'MR' WE1 and '260′ WE1 exhibited a slower decline, showing intermediate values on day 20 and reaching values close to WE2 on day 26. On day 33 after WE1–2 plants were rehydrated (on day 30), all plants recovered values close to C, except for '264′ WE2 which presented E and Gs values significantly lower than C (52.3% and 71.26%, respectively).

Leaf temperature showed an increase with respect to C plants during both regimes of water stress (Fig. 3C). This was significant on days 9 and 13 of the first stress in all varieties, except 'SN', and on day 29 in variety '82′ (Table S4).

3.4. Relationships between parameters

The correlation between Gs and Ψ_{stem} displayed similar trends across different varieties, exhibited reasonably accurate fits to exponential curves. The corresponding R^2 values were notably high, ranging from 0.6166 to 0.7247 (Fig. 4). There were no significant differences observed among the various varieties in either of the two coefficients of the exponential equations. However, the different equations obtained for each variety indicate a clear pattern of varying Gs values at the same Ψ_{stem}. In this regard, the order of the varieties from highest to lowest Gs values would be as follows: '264′ > 'MR' > '260′ > 'SN' > '82′ > 'VL'. In addition to the adjustments made to the exponential curves, the graph reveals two distinct regions indicating the response of Gs to Ψ_{stem}. The approximate threshold for these regions was identified at – 0.5 MPa. Values above this threshold did not exhibit a directly proportional response to the Ψ_{stem}. On the other hand, values below this threshold exhibited a clear dependency of Gs on Ψ_{stem}.

The relationship between E and Gs demonstrates a robust correlation, characterized by well-fitted quadratic curves and a high R^2 value of 0.97 (Fig. 5). We observe two distinct behaviors in this regard. When the conductance values are below 450 mmol H_2O m^{-2} s^{-1}, there is a proportional increase in E corresponding to Gs. However, beyond this threshold, although the diffusion of gases through the leaves increases (Gs), E remains relatively unchanged.

3.5. Effect of water stress and rehydration on oxidative stress parameters

A similar trend was observed in MDA content and AC index (Fig. 6A and B, and Tables S5 and S6), showing a decrease with respect to C treatment during the first part of the experiment and an increase during the second part in both water stress treatments.

On day 14, during the initial stress period, the MDA content displayed a decrease in both WE1 and WE2 plants in the 'VL' and '264′ varieties. However, the remaining varieties did not exhibit any significant changes (Fig. 6A). Despite the rehydration of WE1 plants on day 16, a decrease in MDA content was observed in the '82′ and '260′ plants of both WE1 and WE2 treatments. On day 29, which coincided with the second stress period in WE1 and the final phase of stress in WE2 plants, higher MDA content was detected in WE1 and WE2 plants of the 'MR', 'VL', '82′, and '260′ varieties. Finally, after rehydration of all the plants, no significant changes were observed in any of the measured varieties compared to C plants (Table S5).

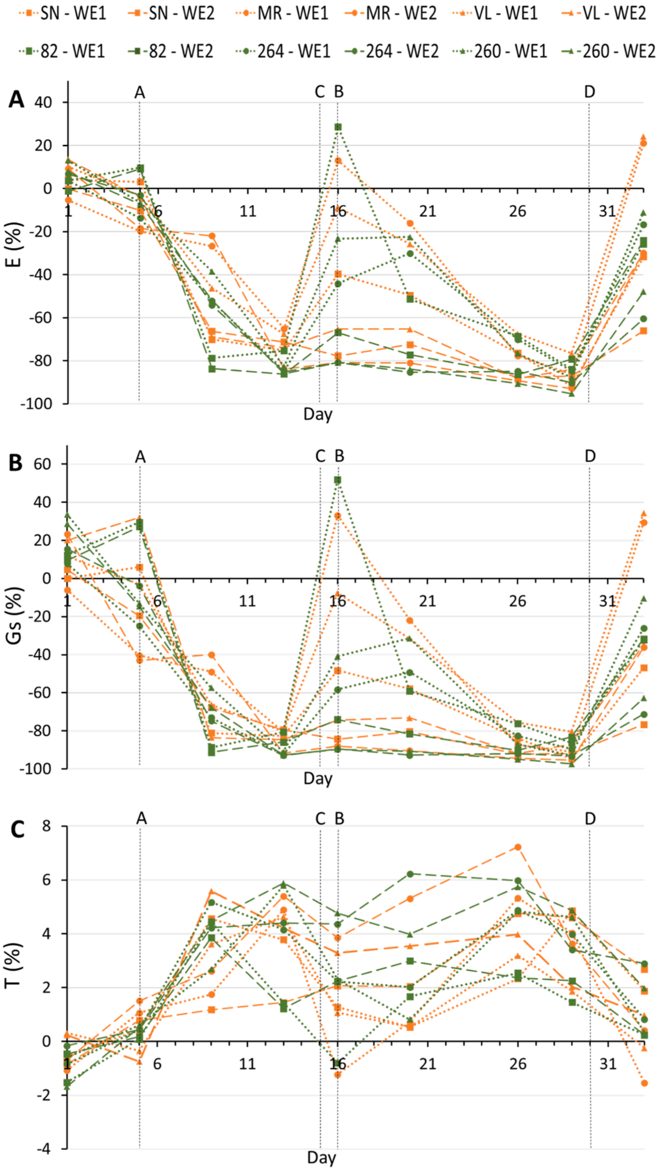
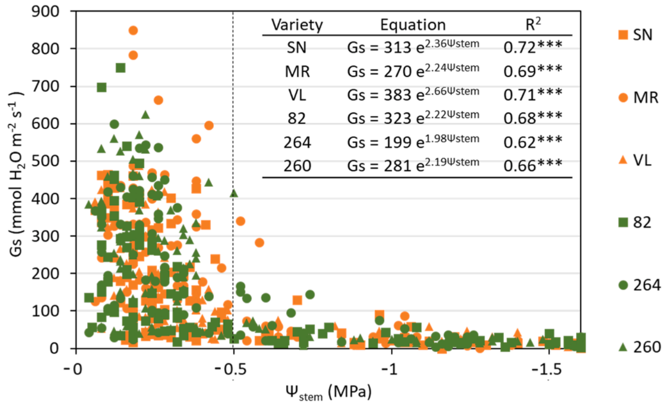


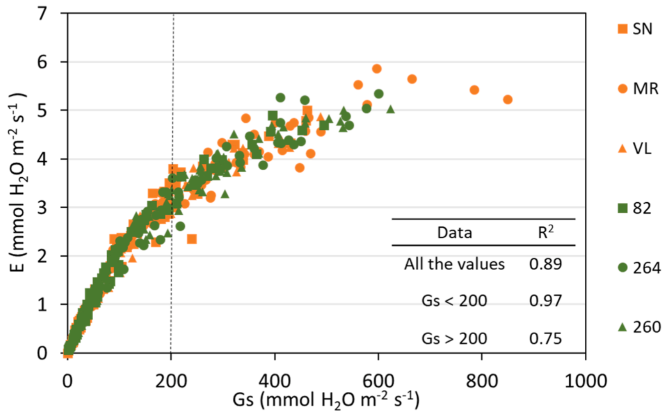
Fig. 3. Evolution of transpiration (E, A), stomatal conductance (Gs, B) and leaf temperature (T, D) in six tomato varieties exposed to full irrigation (C), water stress treatment 1 (WE1) and water stress treatment 2 (WE2). Vertical line A (onset of water stress in WE1 and WE2 plants), B (end of water stress in WE1 plants), C (onset of second water stress in WE1 plants) and D (end of water stress in WE1 and WE2 plants). Values show the means of four replicate. Results were expressed in % variation with respect to control treatment. Significant differences between treatments are shown in Tables S2–S4.

During the initial stress period, a downward trend in the AC index was observed in WE1 and WE2 plants of the 'MR' and '264′ varieties (Fig. 6B). Upon rehydration of the plants on day 16, a reduction was observed only in WE1–2 plants of 'SN', 'MR', and '82′. During the second stress period on day 29, an increase in the AC index was observed in all



Variety	Equation	R^2
SN	$Gs = 313\ e^{2.36\Psi stem}$	0.72***
MR	$Gs = 270\ e^{2.24\Psi stem}$	0.69***
VL	$Gs = 383\ e^{2.66\Psi stem}$	0.71***
82	$Gs = 323\ e^{2.22\Psi stem}$	0.68***
264	$Gs = 199\ e^{1.98\Psi stem}$	0.62***
260	$Gs = 281\ e^{2.19\Psi stem}$	0.66***

Fig. 4. Relationship between stomatal conductance (Gs) and stem water potential (Ψ_{stem}) in six tomato varieties. The upper right corner displays the corresponding equation and R^2 value for each exponential fit of each variety. The dotted vertical line represents the threshold at $\Psi_{stem} = -0.5$ MPa, which distinguishes between two distinct behaviors of the parameters. * **$P \leq 0.001$.



Data	R^2
All the values	0.89
Gs < 200	0.97
Gs > 200	0.75

Fig. 5. Relationship between transpiration (E) and stomatal conductance (Gs) in six tomato varieties. The R^2 values for each of the linear fits are displayed in the lower right corner. The dotted vertical line represents the threshold that distinguishes between two distinct behaviors of the parameters.

WE1 and WE2 plants, except in WE1 of 'SN' and 'MR'. Finally, following rehydration of all the plants, no significant changes were detected compared to C plants (Table S6).

3.6. Changes in phenols and flavonoids under water stress and rehydration

Phenol content exhibited diverse responses to water stress among the studied varieties (Fig. S1). On day 14, during the initial stress period, the phenol content decreased in WE1 and WE2 plants compared to the well-watered plants (C), although no significant differences were found in variety '82′. Upon rehydration of WE1 plants on day 16, a recovery of phenol content was observed, reaching similar values to the control in 'MR', 'VL', '264′, and '260′ varieties, while the 'SN' variety showed an increase compared to C plants. Generally, WE2 plants continued to exhibit lower levels of phenols compared to the control plants, but no differences were observed in the 'SN' and '82′ varieties. During the second water stress episode, no differences were observed in the WE1 plants compared to C, but a significant increase was detected in the WE2 plants of the 'MR', 'VL', '82′, and '260′ varieties. Post-rehydration, an increase in phenol content was only observed in 'SN' WE2 plants, while a decrease was observed in both plants of the '264′ variety.

Similar to the phenol content described earlier, variations in flavonoid content were observed among varieties exposed to water stress (WE1 and WE2) (Fig. S2). Plants subjected to two peaks of water stress (WE1) did not exhibit significant changes compared to C on any of the analyzed dates, except for the 'SN' variety, which showed a slight increase on the final day. However, plants exposed to longer periods of water stress (WE2), specifically from day 16 in the '260′ variety and day 28 in the 'SN'-'MR' varieties, displayed a significant increase in flavonoid content. Nevertheless, after rehydration, this increase was sustained only in the 'SN' and '82′ varieties.

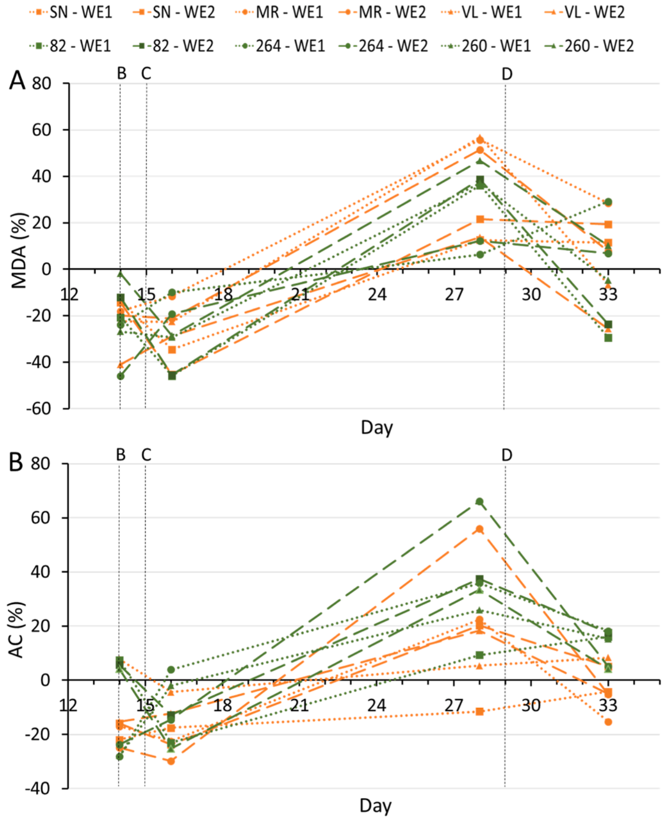


Fig. 6. Lipid peroxidation measured as content of MDA (MDA, A) and antioxidant capacity (AC, B) of Sintonía (SN), Marejada (MR), Valenciano (VL), SL82 (82), SL264 (264) and SL260 (260) tomato varieties exposed to three irrigation treatments: full irrigation (C), water stress 1 (WE1) and water stress 2 (WE2). Vertical line B (end of water stress in WE1 plants), C (onset of second water stress in WE1 plants) and D (end of water stress in WE1 and WE2 plants). Values show the means of four replicate. Results were expressed in % variation with respect to control treatment. Significant differences between treatments are shown in Tables S5 and S6.

4. Discussion

4.1. Effects of water deficit and rehydration on gas exchange parameters in genotypes of *Solanum lycopersicum*

The results obtained in this work provide an integrated view on physiological and biochemical behavior from water stress and rehydration experiments carried out on three commercial and three local landraces varieties. Despite the differences between the origin of the commercial and local varieties, there were no important differences in their resistance to water stress that could be attributed to either group. Indeed, this work provided valuable insights into the behavior of various tomato genotypes in response to water stress and rehydration. The findings shed light on how different tomato varieties cope with water scarcity and recover from stress conditions.

Stomatal conductance (Gs) indicates the rate of gas diffusion, such as CO_2, water and O_2, through the stomata of a plant. This parameter is closely linked to photosynthetic assimilation and transpiration rates, maintaining an appropriate balance between CO_2 uptake for photosynthesis and water loss (Nemeskéri and Helyes, 2019). When plants are subjected to water stress, their primary response is closing the stomata, leading to a greater water conservation but also drastically decreasing the rates of photosynthetic assimilation and transpiration (E) (Nankishore and Farrell, 2016; Patanè et al., 2016; Yuan et al., 2016; Jangid and Dwivedi, 2016; Giorio et al., 2018; Hao et al., 2019; Liang et al., 2020,). This is evidenced by the results in which water stress produced a strong reduction in Gs and E, reducing vegetative growth of stressed plants. The response observed is likely a result of the maturity of the plant, as seedling stages are the most critical owing to the absence of acclimatization mechanisms and the accelerated vegetative growth (Hao et al., 2019). The capacity of a plant to keep its stomata partially open, achieving a balance between water conservation and CO_2 exchange, indicates the ability to tolerate certain episodes of water stress as it allows for photosynthetic activity at lower Ψ_{stem} (Pirasteh-Anosheh et al., 2016). In the present work, '264′ genotype reduced their Gs values more slowly than the other varieties at decreasing values of Ψ_{stem}, sustaining its photosynthetic activity for longer. In contrast, the 'VL' variety reduced the values of Gs faster than the others. Both behaviors can be observed in the exponential equations shown in Fig. 4. However, it is essential to recognize that plants with a greater vegetative growth are affected sooner by periods of drought due to the larger leaf area, which increases water loss through transpiration. In this way, during stress caused by water scarcity, the '264′ variety had one of the smallest leaf sizes and therefore less water loss, while the 'VL' variety presented the largest size and the highest water loss. As such, its alleged resistance to water shortages mentioned earlier would be negated due to its lower vigor. Furthermore, Gs is primarily regulated by stomatal aperture but is also affected by the morphological features of the stomata, such as the stomatal size and density (Lawson, 2009; Pirasteh-Anosheh et al., 2016). The '264′ variety had a higher density of stomata than the other varieties, which could also contribute to the maintenance of a higher Gs rate throughout the water stress.

On the other hand, it is well-known that under conditions of water scarcity, when CO_2 fixation is limited, plants have several mechanisms for dissipating the excess excitation energy in a non-destructive way (Isoda, 2010). Many plants adapt to water stress through mechanisms of dissipation of excess excitation energy thermally by the downregulation of photosystem II activity to protect it from photodamaging effects (Lu et al., 2003). This, in combination with reduced transpiration, which typically carries away heat from the plant's surface, results in an increase of leaf temperature (Zhang et al., 2019). During the experiment, along with the decrease in E and Gs caused by the fall in Ψ_{stem}, an increase in leaf temperature was observed in all tomato varieties, which was returned to normal levels once the plants were recovered.

Correlations between the parameters studied yielded interesting insights. The correlation between Gs and Ψ_{stem} showed similar trends across different varieties, displaying reasonably accurate fits to exponential curves. All varieties behaved similarly, and no significant differences were observed among them. Two distinct zones were identified in which these parameters exhibited different relations between them, with a separation threshold located at − 0.5 MPa. Values above this threshold exhibited erratic responses, likely due to the influence of multiple factors, in addition to the Ψ_{stem}, on stomatal response. However, below this threshold, leaf stomatal conductance was clearly controlled by Ψ_{stem}. This threshold coincided with the mean values observed in tomato plants under optimal water conditions (Coyago-Cruz et al., 2019). Thus, the gas exchange in tomato leaves is dependent on the modulation of stomatal aperture when the water potential falls below − 0.5 MPa.

On the other hand, the correlation analysis revealed a strong relationship between Gs and E. However, based on Gs values, two distinct groups were identified: Gs greater than 450 and Gs less than 450 mmol $H_2O\ m^{-2}\ s^{-1}$. The influence of stomata on E differed between these groups. When Gs was less than 450 mmol $H_2O\ m^{-2}\ s^{-1}$, there was highly consistent stomatal control over E. In this range, the E response was directly proportional to stomatal opening. However, when Gs exceeded 450 mmol $H_2O\ m^{-2}\ s^{-1}$, the control of E by stomata became uncoupled and no longer occurred in a direct and proportional manner. Instead, the E response depended on the vapor pressure deficits between the leaf and the atmosphere. This implies that factors beyond stomatal opening, such as specific vapor pressure differentials, influenced the regulation of E in this range (Leonardi et al., 2000). Furthermore, variations in water vapor exchange could also contribute to the observed differences in E rates, which might be influenced by factors such as the degree of stomatal opening, stomatal size, and stomatal density (Endres et al., 2010).

Once the water stress in WE1 and WE2 plants had ended, all the plants recovered, except '264′WE2, rapidly, reaching similar E and Gs values to C plants. This indicates that short and medium-term water stress did not cause irreversible damage to the plants. Usually, the recovery of gas exchange parameters after moderate water stress is rapid and almost complete, attributed principally to the closure of stomata (Torrecillas et al., 1995; Ramanjulu et al., 1998; Dichio et al., 2006; Campos et al., 2014; Giorio et al., 2018; Hao et al., 2019). In contrast, after severe water stress, the recovery of gas exchange is progressive and slow (can take several days to weeks) and is usually incomplete, due to stomatal and non-stomatal alterations (Souza et al., 2004; Miyashita et al., 2005). In our case, plants exposed to water stress reached values of − 1.6 MPa, compared with other authors who estimated a severe stress value of around − 1 to − 1.4 MPa (Zgallaï et al., 2006; Silva et al., 2012). Therefore, we can conclude that our plants were exposed to one (WE2) or two (WE1) strong stresses. All the varieties used in this experiment, except '264′, were able to recover E and Gs values similar to C in a few days. This suggests that any minor alterations occurring in the photosynthetic machinery were easily reversed with the rehydration of the plants. Similar results were described by Torrecillas et al. (1995) and Hao et al. (2019) after 7–18 days of water stress, when tomato plants recovered similar gas exchange values to well-watered plants in 2–3 days. This quick recovery of Gs and E values would indicate the occurrence of only stomatal limitations of photosynthesis, as discussed above. However, Giorio et al. (2018), in an experiment with local tomato landraces exposed to severe water stress and easily recovering in 2–3 days, concluded that there were stomatal and non-stomatal limitations to photosynthesis, as evidenced by the relationship between CO_2 assimilation and Gs. Therefore, it is likely that if small alterations occurred in the photosynthetic machinery during this experiment, all of them were easily reversed with the rehydration of the plants.

The acclimatization of some genotypes to water scarcity is related to advantageous anatomical and biochemical modifications (Pirasteh-Anosheh et al., 2016). As has been proved, tomato water resistance genotypes show changes in regards to stomata density and morphology, and modifications in leaf mesophyll density and/or thickness (Lo Niinemets et al., 2009; Galmés et al., 2011, 2013). They also respond through osmotic adjustment, increasing solute concentration, which helps to retain water and maintain cell turgor pressure (Srinivasa Rao et al., 2000; Sánchez-Rodríguez et al., 2010; Landi et al., 2017; Giorio et al., 2018). It is well-documented that water stress may stimulate the excessive production of reactive oxygen species (ROS) due to excess excitation energy in the photosynthetic electron transport chain (Sánchez-Rodríguez et al., 2010; Yuan et al., 2016). Although these data will be discussed in the next section, it is pertinent to mention the overproduction of antioxidants to deal with ROS, as another biochemical mechanisms of acclimatization against water stress (Sánchez-Rodríguez et al., 2010, 2011; Yuan et al., 2016; Landi et al., 2017). Plants also have the ability to increase root system growth in order to become more efficient in the use of water present in the soil (Franco et al., 2011; Xu et al., 2015; Moles et al., 2018). The dimensions of the pots employed in our study were sufficient to prevent any adverse influence on the root system. Consequently, this phenomenon could be observed in our experiment with 'MR' and 'VL' varieties, as a reduction in the ratio of aerial and root parts. Taking into consideration all these anatomical and biochemical modifications explained, we could think that an exposure to a short severe water stress may improve the tolerance of plants to the same stress. However, the behavior of tomato plants was similar under water stress and subsequent rehydration in WE1 and WE2 plants. Therefore, the short and severe exposure tested in this experiment apparently did not involve mechanisms that improved the response to water stress in subsequent exposure. In some species such as cotton, after water stress a compensation effect was found when they recover after rehydration. In this case, an overcompensation response was observed for both photosynthesis and aboveground dry mass within one to three days after rehydration (Luo et al., 2016). Little is known about whether these adaptations disappear when plants recover or if they provide resistance in the medium to long term against new episodes of water stress. In leaves and roots of young olive (*Olea europaea* L.) trees, it was observed that total osmotic adjustment increased with increasing severity of drought stress, but decreased during the rehydration period (Dichio et al., 2006). Similarly, an activation of antioxidant mechanisms of enzymatic type was observed in tomato plants exposed to water stress and, once the plants were rehydrated, these activities returned to their original values (Hao et al., 2019). Contrary to the aforementioned authors, Giorio et al. (2018) and Iovieno et al. (2016) found a memory effect in tomato plants exposed to two consecutive water stresses. In this case, both authors concluded that osmotic adjustment through the proline accumulation was higher in the subsequent stress period than the first period. Therefore, while there might be an improvement in the plant's ability to respond a second time to the same stress, no differences were found in the behavior of the tomato plant when it was subjected to the first and second stress.

4.2. Effects of water deficit and rehydration on oxidative stress parameters

It is well established that water stress induces oxidative stress in plants due to greater leakage of electrons towards O_2 during photosynthetic and respiratory processes, resulting in a toxic increase in ROS (Hasanuzzaman et al., 2014; Çelik et al., 2017; Laxa et al., 2019; Molina-Moya et al., 2019). The accumulation of ROS, once surpassed a certain threshold, attacks membrane lipids, inactivates essential enzymes and damages nucleic acids, with the final consequence of cell death (Hasanuzzaman et al., 2014). Our results showed a similar trend in most of the varieties analyzed, showing a decrease in the oxidative degradation of lipids in WE plants compared to well-watered plants in the first days and an increase in degradation on the last days. This suggests that when tomato plants are exposed to short periods of water stress, although severe, they are able to control the overproduction of ROS and avoid oxidative damage to their membrane lipids. However, consecutive periods of water stress (WE1) or a longer period of water scarcity (WE2) causes strong oxidative damage to membrane lipids. Time-wise examination of MDA content by Yuan et al. (2010) revealed that on the first day of water stress, there was a decrease in MDA content, which progressively increased as the duration of stress increased. The Zarina tomato variety exposed to water stress also showed a decrease in MDA content, indicative of its resistance compared to other varieties analyzed (Sánchez-Rodríguez et al., 2012). Similarly, the analysis of lipid peroxidation in several tomato varieties revealed that water stress caused more oxidative damage in some varieties than in others, indicating their tolerance (Sánchez-Rodríguez et al., 2010, 2012; Çelik et al., 2017). Lipid peroxidation also seems to be affected by the intensity of the stress to which the tomato plants are subjected; thus, when plants were exposed to different levels of soil water content, they showed proportional increases to the intensity of the stress (Yuan et al., 2016). The same authors also examined its effect on different stages of tomato development from the seedling to maturity, showing it increased as the plant aged (Yuan et al., 2016). All these results, along with those obtained in our experiment; indicate that the trend in MDA content observed would be mainly determined by the variety, intensity, duration and age of the plant. Notably, like the rest of the measured parameters, lipid peroxidation caused by water stress was quickly reversed with the rehydration of the plants. This result is in line with the findings from cotton plants, which showed an increase in lipid peroxidation as a response to severe stress, yet, once rehydrated, they recovered values close to those of well-watered plants (Yi et al., 2016).

The antioxidant levels of a plant are an excellent indicator of the redox state, which is necessary for the development of stress tolerance (Laxa et al., 2019). Antioxidant capacity tests show the plant's ability to reduce pro-oxidant substances, which is indicative of its ability to combat oxidative damage caused by oxidative stress (Figueroa-Pe et al., 2014). Our results show a behavior that is similar to the one previously described for lipid peroxidation. This indicates that when a stressed tomato plant begins to experience the effects of oxidative stress caused by drought, the plant is able to react to this by increasing the amount of antioxidants present, in an effort to reduce and control this pro-oxidant situation. Antioxidant capacity has been proposed as a biochemical indicator for the selection of cultivars, which are less susceptible to water stress. By comparing the susceptibility to water stress of various tomato genotypes, it was found that the most resilient varieties had reduced antioxidant capacity, as well as weaker oxidative and lipid peroxidation stresses (Sánchez-Rodríguez et al., 2010). The observed boost in antioxidant activity has been described in various species in response to water stress; however, this response is dependent on stress factors such as tolerance, intensity, and duration tolerance, intensity and duration of stress (Sánchez-Rodríguez et al., 2010; Rahimi et al., 2018; Talbi et al., 2020; Lipan et al., 2021). Finally, similar to lipid peroxidation, when the plants were rehydrated, the antioxidant capacity values returned to values close to the control. Thus, it can be concluded that only extreme and prolonged stress would bring about an increase in antioxidant activity and that when the stress and the subsequent oxidative stress cease, normal antioxidant activity is swiftly reestablished.

Another metabolic process, which is widely discussed for its association with water stress tolerance, is the production of phenolics and flavonoids. Phenols and flavonoids and their metabolism are used by plants to hinder the formation of ROS and, consequently, oxidative stress (Król et al., 2015; Talbi et al., 2020). It is well-known that water stress causes a notable increase in the production of phenolic and flavonoid compounds in many species (Sánchez-Rodríguez et al., 2010, 2011; Król et al., 2015; Rahimi et al., 2018; Gao et al., 2020). Nevertheless, different findings have been gathered in past studies, and it is unclear whether their production indicates plants with greater or lesser tolerance to water stress. Sánchez-Rodríguez et al. (2010) reported a remarkable increase in the most sensitive tomato genotypes in response to drought stress. However, in this study, these genotypes were the ones that experienced the most significant oxidative stress, thereby indicating their role in ROS detoxification. However, Gao et al. (2020) noted that as flavonoid and phenol content increases under water stress, the plants become better equipped to adapt to drought. It appears likely that certain native species that are adapted to drought raise the contents of these compounds in response to adverse environmental conditions as a strategy to prevent oxidative damage caused by drought. However, in plants that are not adapted to drought, it seems to be related to damage more than tolerance (Sánchez-Rodríguez et al., 2010; Gao et al., 2020; Talbi et al., 2020). Considering that the species used has high water requirements, the increase in these compounds is probably linked to the oxidative damage produced by water stress rather than with a potential tolerance, resulting in an increase in the phenol and flavonoid content in some genotypes. However, this cannot be consistently associated with a greater or lesser tolerance.

5. Conclusion

The findings from this study indicate that there were no significant differences in the response to water stress and rehydration between local and commercial tomato varieties during the vegetative period. The survival ability of tomato plants under severe water stress ($\Psi_{stem} \approx -1.6$ MPa) was mainly attributed to the regulation of stomatal opening, leading to reduced transpiration and gas exchange. This growth-stunting effect had a detrimental impact on the final size of the drought-exposed plants. However, upon rehydration, all plants returned to their pre-stress values, indicating the absence of irreversible damage despite the vulnerability of tomato plants to water stress. Exposure to two consecutive short episodes of water stress did not reveal any specific adaptive mechanisms.

The occurrence of oxidative damage was only observed during the second water stress episode and at the end of the extended water stress period. Similar to other physiological parameters measured, the plants returned to their pre-stress values following rehydration.

Despite the commercial varieties being selected primarily for maximizing production without considering potential limitations like water scarcity, both groups of tomato plants exhibited similar responses to drought and subsequent rehydration in this study. It is crucial to emphasize that while all the tested varieties exhibited good recovery in terms of their physiological parameters, it is likely that they would be significantly and possibly differentially affected by the tested water regime in terms of productivity. Further studies are necessary to investigate this aspect.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ruben Moratiel reports financial support was provided by Polytechnic University of Madrid. Ruben Moratiel reports a relationship with Polytechnic University of Madrid that includes: employment.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agwat.2023.108529.

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