Moderate and severe water stress effects on morphological and biochemical traits in a set of pepino (*Solanum muricatum*) cultivars

Juan Pacheco[a], Mariola Plazas[b], Ilaria Pettinari[a,1], Anbu Landa-Faz[a,2], Sara González-Orenga[c], Monica Boscaiu[c], Salvador Soler[a], Jaime Prohens[a,*], Oscar Vicente[a], Pietro Gramazio[d]

Affiliations

[a]*Institute for the Conservation and Improvement of Valencian Agrodiversity, Universitat Politècnica de València, Camino de Vera 14, 46022 Valencia, Spain*

[b]*Meridiem Seeds S.L., Paraje Lo Soler 2, 30700 Torre-Pacheco, Spain*

[c]*Mediterranean Agroforestry Institute, Universitat Politècnica de València, Camino de Vera 14, 46022 Valencia, Spain*

[d]*Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, 305-8572, Tsukuba, Japan*

[*] Corresponding author.

E-mail address: jprohens@btc.upv.es

[1]Present address: Department of Veterinary Medicine, Università degli Studi di Perugia, 06126 Perugia, Italy

[2]Present address: Centro de Investigación y de Estudios Avanzados, Instituto Politécnico Nacional, 07360 Ciudad de México, Mexico

Abstract

The pepino (*Solanum muricatum*) is a neglected crop from the Andean region with potential for expansion to many areas of the world. However, there is a lack of studies in pepino related to its response to water stress. In this study, we have subjected plantlets of seven pepino cultivars (Mur1-Mur7) to three treatments consisting of a fully irrigated control (C), a moderate water stress (WS-M), and a severe water stress (WS-S). Thirty-one traits related to growth, photosynthetic pigments, mono and divalent ions, osmolytes and antioxidants were measured. Significant differences were found among cultivars for most traits. The WS-M treatment did not affect most growth and biochemical parameters, while large differences with respect to the control were observed with the WS-S treatment. In general, the WS-S treatment induced an inhibition of the growth parameters, mainly the reduction of the fresh weight of leaves, stems and roots, as well as their water content. A principal component analysis (PCA) performed on the relative values of growth traits, together with the ANOVA for the traits for which significant interaction cultivar × treatment was detected, showed that cultivars Mur2 and Mur4 are the most tolerant to water stress. Although no clear-cut differences were observed among cultivars, the water-stressed plants of Mur2 and Mur4 displayed less variation with respect to the control than the other cultivars for the physiological and biochemical traits measured. Overall, photosynthetic pigments, malondialdehyde and total flavonoids decreased under severe water stress, while proline, Na^+ and K^+ contents increased significantly. The results obtained provide relevant information on the response to drought of pepino and have allowed identifying two cultivars better adapted to water stress that could be useful in breeding pepino for drought tolerance.

Keywords:

Solanum muricatum

Drought

Water stress

Photosynthetic pigments

Ions

Osmolytes

Antioxidant compounds

1. Introduction

The pepino (*Solanum muricatum* Aiton) is a neglected solanaceous crop from the Andean region with great potential, both for domestic markets and as an emerging crop in other regions of the world (Gurung et al., 2016). The pepino is a diploid (2n = 2x = 24), grown for its edible fruits and displays a great morphological variability amongst cultivars for fruit weight, shape and colour (Anderson et al., 1996; Herraiz et al., 2016). Pepino fruits have a high water content (92% of fresh weight) and are low in calories (250 kcal/kg) (Rodríguez-Burruezo et al., 2011). At maturity, it has a characteristic mild sweet flavour and intense fruity aroma (Prohens et al., 2005). The pepino fruit is usually eaten as fresh juicy fruit, although some cultivars are used in vegetable salads due to their higher acidity content and herbaceous flavour (Prohens et al., 2002). Different studies found that pepino displays antioxidant, antidiabetic, anti-inflammatory and antitumor properties (Hsu et al., 2011; 2018; Shathish and Guruvayoorappan, 2014; Sudha et al., 2011; Virani et al., 2020; Wang et al., 2019; Yue et al., 2019, 2020). One of the most interesting features of pepino

is its close phylogenetic relationship with the major crops potato and tomato (Särkinen et al., 2013; Spooner et al., 1993).

The pepino has traditionally been grown in the Andean zone in temperate climates and generally in the absence of drought stress (Prohens et al., 1996). However, its cultivation has been introduced in Mediterranean-type areas where the availability of water is a limiting factor, which is likely to be aggravated by climate change. Until now, not many studies have been performed on the response of pepino to drought (Duman and Sivaci, 2015). However, several studies exist on its performance under salinity conditions (Pluda et al., 1993; 2019; Prohens et al., 2003).

Determining the biochemical responses of pepino plants against drought stress is of great relevance for the development of cultivation techniques and for the selection and breeding programmes that allow a better crop management and the development of varieties with greater tolerance to drought (Fang and Xiong, 2015; Fita et al., 2015). However, to our knowledge, the biochemical responses of pepino to drought stress and the intraspecific variation in these responses have not yet been studied. Consequently, there is no information on biochemical tolerance markers that can be used as predictors of drought tolerance in pepino.

Metabolites and enzymes involved in the general responses of plants to water deficit are suitable candidates to be used as biochemical markers to assess the relative degree of drought tolerance of different cultivars. They include photosynthetic pigments, such as chlorophylls and carotenoids, which often decrease in drought-stressed plants, accompanying the inhibition of photosynthesis generally observed under stress (Batra et al., 2014; Kumar et al., 2017a; Reis et al., 2020; Szekely-Varga et al., 2020). Also, different inorganic and organic osmolytes accumulate in plant cells to maintain the cell turgor pressure under stress conditions, such as drought or salinity, that cause cell

dehydration (Seki et al., 2007; Singh et al., 2017; Al Hassan et al., 2016). Abiotic stress also induces, directly or as a secondary effect, oxidative stress in plants, which can be quantified by measuring the levels of specific markers (Del Rio et al., 2005; Kar, 2011). As a defence against oxidative stress, plants activate antioxidant systems; therefore, increases in the specific activities of antioxidant enzymes and/or the concentrations of antioxidant compounds are frequently observed in drought-stressed plants (Das and Roychoudhury, 2014; Kozminska et al., 2019; Plazas et al., 2019).

In this work, we have evaluated the response to water stress in seven pepino cultivars subjected to three different treatments under controlled greenhouse conditions: well-watered plants (control) and two degrees of water stress (reduction or complete withholding of irrigation). Once the treatments were finished, the plants were evaluated for growth parameters and photosynthetic pigments (chlorophyll a, chlorophyll b and total carotenoids) levels. Mono (Na^+, K^+, Cl^-) and divalent (Ca^{2+}) ion contents were measured in roots, stems and leaves, and leaf concentrations of proline (Pro) and total soluble sugar (TSS) (common plant osmolytes), malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) (oxidative stress biomarkers), and total phenolic compounds (TPC) and total flavonoids (TF) (representative antioxidant compounds) were also quantified. The final objective of this work was to determine the responses to water deficit in pepino and to evaluate the possible differences amongst varieties in these responses. These results will provide relevant information to better understand the drought-tolerance mechanisms in this species and may allow the identification of biochemical markers for the selection of cultivars more tolerant to this abiotic stress.

2. Materials and methods

2.1. Plant material and experimental design

The pepino cultivar '37-A' (Mur1), originating from Ecuador, and the improved varieties 'Sweet Round' (Mur2), 'Valencia' (Mur3), 'Turia' (Mur4), 'El Camino' (Mur5), 'Sweet Long' (Mur6), and 'Puzol' (Mur7), developed through different breeding programmes in Spain and New Zealand (Murray et al., 1992; Prohens et al., 2002; 2004; Rodríguez-Burruezo et al., 2004), were used for this study. These seven cultivars were selected based on their agronomic interest and genetic, phenotypic and composition diversity (Blanca et al., 2007; Herraiz et al., 2015; 2016) (Supplementary Data S1).

All the cultivars are maintained at the Solanaceae breeding laboratory at the COMAV, Universitat Politècnica de València (UPV; Spain). Pepino cultivars were vegetatively propagated *in vitro* and, after acclimatization, were transplanted to individual thermoformed pots (with a diameter in the upper part of 14.5 cm and 1.3 L capacity) containing commercial growing substrate N3 (Klasmann-Deilmann, Saterland, Germany). The plants were grown in a benched greenhouse with controlled environmental conditions. During the experiment, temperatures ranged between 17ºC and 30ºC, and humidity between 50% and 80%. After an initial period of three weeks in which the plants were watered to field capacity three times a week on Monday, Wednesday and Friday (starting the watering on a Wednesday) and when the plants reached the phenological stage 19 (nine or more leaves on the main shoot unfolded) of the specific pepino BBCH (Biologische Bundesanstalt, Bundessortenamt, CHemische Industrie) scale (Herraiz et al., 2015), three watering treatments were applied: control (C), moderate water stress (WS-M), and severe water stress (WS-S). Control and WS-M plants were irrigated with water (300 and 100 mL per pot, respectively) three times a week. Runoff water was freely allowed through the holes in the bottom of the pots, although for the

WS-M plants no runoff was observed. The WS-S water stress treatment consisted of the complete withholding of irrigation during the entire treatment period. Treatments were carried out for 19 days, with five replicates per cultivar and treatment arranged in a completely randomized design in the same greenhouse. The moisture of the substrate (% vol) was measured at the start of the experiment and at each irrigation date, just before the irrigation, with a WET-2 sensor (Delta-T Devices, Cambridge, United Kingdom). Traits measured in the plants at the end of the experiment are indicated in Table 1.

2.2. Growth parameters

The number of leaves (NL), stem length (SL), stem diameter (SD), and root length (RL) were measured at the end of the treatments (Table 1). Immediately after the experiment was finished, leaves, stems and roots were collected separately and weighed for obtaining fresh weight (LFW, SFW, and RFW, respectively). A fraction of the fresh material was stored at -80 ºC, and samples of the three organs were dried for 72 h in an oven at 65 ºC until a constant weight was achieved and then weighed again to calculate the dry weight (DW) of leaves, stems and roots (LDW, SDW and RDW, respectively). Water content percentage of each plant part (LWC, SWC and RWC), was calculated as follows (Gil et al., 2014): WC (%) = [(FW - DW/FW] × 100.

2.3. Photosynthetic pigments contents

Chlorophylls a and b (Chl a, Chl b) and total carotenoids (Caro) were determined following the protocols described by Lichtenthaler and Welburn (1983). To extract the pigments, 0.05 g of fresh leaf material was ground in 1 mL of ice-cold 80% (v/v) acetone

and mixed. After centrifuging for 15 min at 13,300 *g* and 4 ºC, the supernatant was collected and its absorbance was measured at 663, 646, and 470 nm. Chl a, Chl b, and Caro concentrations were calculated following Lichtenthaler and Welburn (1983) equations and expressed as mg g^{-1} DW. Determination of photosynthetic pigments, as well as all other UV/visible spectrophotometric assays described below, were carried out using a UV-1600PC spectrophotometer (VWR, Shanghai, China).

2.4. Ion content measurements

Contents of mono (Na^+, K^+, Cl^-) and divalent (Ca^{2+}) ions in leaves, stem and roots were determined according to Weimberg (1987), from 0.05 g of ground dry plant material mixed with 15 mL of deionised water. The samples were incubated at 95 ºC for 15 min in a water bath, cooled to room temperature and filtered through a 0.45 µm nylon filter (Gelman, NY, USA). Na^+, K^+ and Ca^{2+} concentrations were quantified with a PFP7 flame photometer (Burlington, VT, USA) and Cl^- with a chloride analyser (Sherwood, Cambridge, UK).

2.5. Osmolyte quantification

Proline (Pro) was extracted from 0.05 g dry leaf material with 2 mL of a 3% (w/v) aqueous sulphosalicylic acid solution and was quantified according to Bates et al. (1973). The extract was subsequently mixed with acid ninhydrin solution, incubated for 1 h at 95 ºC, cooled on ice and then extracted with two volumes of toluene. Absorbance of the organic phase was measured at 520 nm using toluene as a blank. Reaction mixtures containing

known amounts of Pro were run in parallel to obtain a standard curve. Pro concentration was expressed as µmol g^{-1} DW.

Total soluble sugars (TSS) contents were quantified following the method of Dubois, et al. (1956), mixing 0.05 g of fresh leaf material with 3 ml of 80% (v/v) methanol on a rocker shaker for 24 h. The extract was recovered by centrifugation, concentrated sulphuric acid and 5% phenol were added to the supernatant and the absorbance was measured at 490 nm. TSS contents were expressed as equivalents of glucose, used as the standard (mg eq. glucose g^{-1} DW).

2.6. Oxidative stress biomarkers and antioxidant compounds

Malondialdehyde (MDA) content was determined following the method of Hodges et al. (1999;), with some modifications (Taulavuori et al., 2001), using the same 80% methanol extracts prepared for TSS quantification. The samples were mixed with 0.5% thiobarbituric acid (TBA) prepared in 20% trichloroacetic acid (TCA), (or with 20% TCA without TBA for the controls), and then incubated at 95 ºC for 20 min. After stopping the reaction by cooling the samples on ice and centrifugation at 13,300 *g* for 10 min at 4 ºC, the supernatant absorbance was measured at 532 nm. MDA concentration was calculated using the equations described in Taulavuori et al. (2001) subtracting the non-specific absorbance at 600 and 440 nm.

The hydrogen peroxide content (H_2O_2) was determined according to a previously published method (Loreto and Velikova, 2001). H_2O_2 was extracted from 0.05 g fresh leaf material with a 0.1% (w/v) trichloroacetic acid (TCA) aqueous solution, followed by centrifuging the extract at 13,300 *g*. The supernatant was thoroughly mixed with one volume of 10 mM potassium phosphate buffer (pH 7) and two volumes of 1 M KI. The

absorbance of the sample was recorded at 390 nm. H_2O_2 concentrations were expressed as µmol g^{-1} DW.

Total phenolic compounds (TPC) were quantified in leaf methanol extracts by their reaction with sodium bicarbonate and the Folin–Ciocalteu reagent (Blainski et al., 2013). After 90 min of incubation at room temperature in the dark, the absorbance of the samples was measured at 765 nm. TPC concentrations were expressed as equivalents of gallic acid (GA), used as the standard (mg eq. GA g^{-1} DW).

Total flavonoids (TF) were measured by the method described by Zhishen et al. (1999), based on the nitration with $NaNO_2$ of aromatic rings carrying a catechol group, followed by reaction with $AlCl_3$ at alkaline pH. Absorbance was measured at 510 nm, and the concentration of flavonoids was expressed in equivalents of the standard catechin (C) (mg eq. C g^{-1} DW).

2.7. Data analyses

Statistical analysis was performed using a two factorial ANOVA, with cultivar and water stress treatments as main effects for all the parameters. Interactions between the effects (cultivar × treatment) were also analysed. The significance of differences ($p<0.05$) was assessed with Student-Newman-Keuls multiple range tests. For traits in which no interaction was observed, the main effects of the cultivar and treatment are presented in tables, whereas for those traits for which the interaction cultivar × treatment was significant, figures displaying the interaction are also included. To identify the most tolerant cultivars, for each cultivar the relative mean values of the WS-M and WS-S treatments in relation to the control were calculated. Subsequently, a principal component analysis (PCA) was performed on these data using two R packages: FactoMineR (Lê et

al., 2008) to compute PCA, and factoextra package (Kassambara, 2015) for extracting and visualising the results.

3. Results

3.1. Substrate moisture analysis

The moisture of the substrate in the pots showed the expected oscillations, according to the watering schedule. For the control plants, it was maintained at high levels during the 19 days of the treatment, with an average value of 61.6% at the end of the experiment (Figure 1). In contrast, for the WS treatments, the substrate moisture level suffered a sharp decrease during the first week, with a more pronounced reduction in the WS-S treatment, as compared to the WS-M treatment. After the water stress treatments, average moisture values of the substrate for WS-S and WS-M were of 7.8% and 18.8%, respectively. Within each treatment, all cultivars showed a similar pattern of temporal evolution of the pot substrate moisture (Figure 1).

3.2. Analysis of variance

The ANOVA revealed significant differences for both, cultivar and treatment main factors (Table 2). Out of the 31 traits analysed, 25 displayed significant differences for the cultivar effect and 17 for the treatment effect. For growth parameters, significant differences among cultivars were observed for all traits, except for the water content in leaves (LWC), stems (SWC), and roots (RWC). Similarly, differences between water stress treatments were highly significant for all growth parameters, except for the number

of leaves (NL) and the stem length (SL) (Table 2). For biochemical traits, significant differences between cultivars were observed for all parameters, except for the concentrations of Na^+ in roots, Cl^- in leaves, and H_2O_2. Contrarily, no significant differences were observed for most traits for the 'treatment' factor, except for Na^+ and K^+ in roots, K^+ in stems, proline (Pro), malondialdehyde (MDA), and total flavonoids (TF), which were found to be significant (Table 2). Significant differences were also found for the interactions between the 'cultivar' and 'treatment' factors, for three growth (NL, SD, and LFW) and five biochemical (K^+ in stems, K^+ and Cl^- in roots, Pro, and TF) traits.

3.3. Growth traits and identification of tolerant accessions

The results of the analysis of the mean effects on growth parameters of the factors 'cultivar' and 'treatment' are shown in Table 3. The number of leaves (NL) at the end of the experiment varied greatly in the seven selected cultivars, ranging from 16.8 leaves in Mur6 to 41.1 in Mur3, whereas no significant differences were observed between treatments (Table 3). Significant differences between cultivars were also observed for both stem parameters (SL and SD), being Mur2 and Mur6 the cultivars that registered the longest and the shortest stems, respectively; on the other hand, Mur1 to Mur5 had the broadest stem diameter and Mur7 the thinnest one (Table 3). Stem diameter (SD), but not stem length (SL), exhibited notable differences between water stress treatments. In this way, the average reductions of SD with respect to the control were 8.4% for WS-M and 33.4% for WS-S. Significant differences were found for root length between cultivars and also between treatments. Cultivars, Mur1, Mur2 and Mur3 had on average longer roots than those of the rest of the cultivars. The WS-M treatment resulted in significantly longer

roots than the control and WS-S plants, with no differences between these latter groups (Table 3).

Fresh weight of leaves, stem and roots displayed some significant differences among cultivars, as well as between treatments (Table 3). For most cultivars, only small, generally non-significant differences were observed in the fresh weights of the three organs, with some exceptions; for example, LFW was significantly higher in Mur3 than in all other cultivars, whereas Mur6 showed the lowest LFW, SFW and RFW values. On the other hand, considerable water stress-induced effects were observed for the WS-S treatment, leading to an average FW reduction of 64.5% in leaves, 56.9% in stems, and 73.5% in roots, compared to the corresponding controls; however, no significant differences were observed between the control and WS-M treatments.

The water content in leaves, stems and roots did not differ significantly between the seven cultivars (Table 3). The WS-S treatment had a strong impact on the water content of leaves and stems, with a 32.4% and 21.0% reduction, respectively, comparing to the corresponding controls. The WS-M treatment, on the other hand, only caused a significant decrease in the water content of roots, amounting to 9.4% of the control.

The effects of the WS treatments on those growth parameters for which a significant cultivar × treatment interaction was observed in the ANOVA, namely NL, SD and LFW (Table 2), are shown in Figure 2 for all cultivars. Regarding NL, no significant differences between the control and the water stress treatments (WS-M and WS-S) were observed in any of the cultivars except in Mur3, for which both, moderate and severe water deficit resulted in a substantial reduction of leaf number (up to 36.4%) (Figure 2A). For the stem diameter (SD), the WS-M treatment promoted a significant reduction only in Mur1 (13.8% of the control) and Mur7 (30.6%), whereas the WS-S treatment had a strong effect in all cultivars, particularly in Mur5 with a reduction of more than 50% of the control,

except in Mur3 and Mur4 (Figure 2B). Finally, leaf fresh weight (LFW) did not vary significantly in any of the seven cultivars, when comparing the well-watered controls and the plants subjected to the moderate water stress treatment. On the other hand, LFW decreased in most cultivars, in relation to the corresponding control, under WS-S conditions; the strongest reduction was observed in Mur5 (78.5%), followed by Mur1 (75.8%), Mur3 (71.9%) and Mur7 (70.2%). Mur2 and Mur4 were the only cultivars which did not display significant differences for LFW between the control and the WS-S treatments (Figure 2C).

The principal component analysis (PCA) for plant growth and water content traits, which allows the combined study of all traits in a single multivariate analysis, discriminated tolerant and sensitive cultivars. The first and second components (PC1 and PC2) performed on the relative values of growth and water content traits of the WS-M and WS-S treatments (expressed as percentages of the corresponding controls) accounted, respectively, for 69.1% and 14.9% of the total variation (Figure 3A). The variables that most contributed to the PC1 were those related to the water content and the fresh weight of the three tissues measured (leaf, stem and root), as well as the stem diameter (SD), which displayed high negative correlations ($r<-0.75$) with the PC1 (Figure 3A). Regarding PC2, the number of leaves (NL) and stem length (SL) were negatively correlated with this component and displayed the highest absolute values ($r<-0.60$) for the correlation with PC2 (Figure 3A). The PC1 clearly separated the two treatments, with the WS-S treatment being positively correlated with PC1, while the WS-M treatment was negatively correlated with PC1 (Figure 3B). The two cultivars of the WS-S treatment with the lowest values for the PC1 (i.e., associated with the smallest reduction of fresh weight and water content) were Mur2 and Mur4. Regarding PC2, these two latter cultivars were also associated with the lowest reduction of the number of leaves (NL) and stem length

(SL) under both, WS-M and WS-S treatments. The PCA data, together with the ANOVA analyses for the traits for which significant interaction cultivar × treatment was detected, indicates a greater tolerance to water stress of cultivars Mur2 and Mur4.

3.4. Photosynthetic Pigments

Regarding photosynthetic pigments (chlorophyll a, chlorophyll b and total carotenoids), significant differences were observed between the cultivars (Table 3). Mean Chl a and Chl b contents were lowest in Mur1 (7.35 and 1.74 mg g^{-1} DW, respectively) and highest in Mur3 (17.02 and 6.53 mg g^{-1} DW). Carotenoids concentrations in Mur5 were significantly higher than in the rest of cultivars. Regarding water stress treatments, no significant differences in the contents of the three pigments were observed between the control and the WS-M treatment, whereas the WS-S treatment resulted in significant reductions of their concentrations: 38.3%, 33.6% and 55.4% with respect to the corresponding controls, for Chl a, Chl b and Caro, respectively (Table 3).

3.5. Ion Accumulation

The mean concentrations of the monovalent (Na^+, K^+, Cl^-) and divalent (Ca^{2+}) ions in leaves, stems and roots generally varied between cultivars, with some exceptions. For example, the contents of Na^+ in roots or Cl^- in leaves did not differ significantly in the seven selected cultivars; on the other hand, Mur1 showed Ca^{2+} concentrations in roots significantly lower than in all other cultivars, whereas the highest levels of this cation in leaves were measured in Mur2 (Table 4). Despite differences among specific cultivars, some common trends were maintained; most important, the concentrations of all ions

were much higher in the aerial part of the plants than in the roots, and in all cases (except Ca^{2+} in Mur1) the ions accumulated predominantly in the stems, reaching levels ranging from 1.7 to 3.2-fold (for Na^{+}), 2.2 to 4.5-fold (for K^{+}), 4 to 7-fold (for Cl^{-}) or 3.3 to 15-fold (for Ca^{2+}) higher than in the roots, depending on the cultivar (Table 4).

When considering the main effects of the water stress treatments on the mean contents of the different ions in leaves, stems and roots, under moderate water stress conditions (WS-M treatment) no significant differences with the controls were found for any of the ions, in any organ, except for K^{+} in roots (1.3-fold higher than in the well-watered control). Regarding the WS-S treatment, significant differences were only observed in roots for Na^{+} (1.3-fold higher than in the control) and K^{+} (1.7-fold) (Table 4).

The effects of the WS treatments on the concentration of ions for which a significant cultivar × treatment interaction was observed in the ANOVA (K^{+} in stems and roots, and Cl^{-} in stems, see Table 2) are shown in Figure 4. In the WS-M treatment, K^{+} contents in stem increased significantly over the control only in Mur5 (1.6-fold) (Figure 4A); for the WS-S treatment, several cultivars showed a significant increase of K^{+} concentrations in the stem: Mur5 (2.1-fold over the control), followed by Mur7 (1.6-fold), and Mur6 and Mur3 (ca. 1.3-fold) (Figure 4A). Under moderate water stress conditions, the concentration of K^{+} in the root increased significantly in Mur2 (2.5-fold) and Mur3 (1.6-fold) (Figure 4B). Under WS-S treatment, in addition to Mur2 (ca. 3-fold increase) and Mur3 (1.9-fold), Mur6 also showed a significant increase in K^{+} stem contents, approximately 1.3-fold over the well-watered control (Figure 4B). For Cl^{-} contents in the stem, the only significant differences were observed in the WS-M treatment for cultivars Mur1 and Mur2, which accumulated 1.2 and 1.4-fold more Cl^{-} than the control, respectively (Figure 4C).

3.6. Osmolytes, oxidative stress markers and antioxidants

The main effects of the cultivar and treatment factors on the mean values of the analyzed osmolytes, oxidative stress markers and non-enzymatic antioxidants are shown in Table 5. Proline (Pro) was found highly variable in the seven cultivars, ranging from 14.8 µmol g^{-1} DW in Mur3 to 50.5 µmol g^{-1} DW in Mur1, which represents a 3.4-fold difference (Table 5). Average Pro levels varied significantly in the WS-S treatment, compared to the control plants, with an increase of 1.8-fold increase, approximately, whereas no differences were observed in the moderate water stress treatment (Table 5). Total soluble sugar (TSS) concentrations also displayed considerable differences between cultivars, from 54.86 (in Mur1) to 283.66 (in Mur2) mg eq. glucose g^{-1} DW, with intermediate values in the rest of cultivars; however, contrary to Pro, no significant differences were found between the control and water stress treatments (Table 5). MDA concentrations also varied between cultivars, but only about 2-fold, with the minimum value measured in Mur3 and the maximum one in Mur2; MDA contents decreased significantly, by more than 50% of the control, in the WS-S treatment (Table 5). Regarding hydrogen peroxide (H_2O_2) concentrations, no significant differences were detected, either between cultivars or when comparing the control and water stress treatments (Table 5). Significant differences were observed between cultivars for total phenolic compounds (TPC), with Mur6 displaying the highest concentrations and Mur2 and Mur3 the lowest; on the other hand, no significant differences were found for TPC between the different treatments (Table 5). Finally, total flavonoids (TF) contents did not vary in the pepino cultivars, except for Mur3, which showed a value significantly higher than all others, whereas a significant decrease of TF levels, of about 50% of the control, was detected only in the WS-S treatment (Table 5).

Two of the above traits, Pro and TF contents, showed significant cultivar × treatment interactions (Table 2). Average Pro concentrations increased significantly over the control only in the WS-S treatment, and only for Mur6 (ca. 3.7-fold) and Mur7 (ca. 2.6-fold) (Figure 5A). For TF, no significant differences were observed between the controls and the two WS treatments in most cultivars. However, in cultivar Mur3, which showed a mean control TF concentration about 10-fold higher than those of the remaining cultivars, severe WS conditions resulted in a reduction in TF contents of 65.7% of the control (Figure 5B).

4. Discussion

The pepino is a crop with a wide potential for expansion (Kumar et al., 2017b). However, many aspects related to the improvement of cultural practices and its tolerance to stresses, including drought, remain to be elucidated. The expansion of the crop to new areas outside the Andean region (Gurung et al., 2016), as well as the threat of climate change in its region of origin (Buytaert et al., 2010), makes it likely that stress due to drought will become more common in pepino cultivation in the near future. However, very little information is available on pepino responses to drought (Duman and Sivaci, 2015).

Evaluation of diversity for tolerance to drought within pepino genotypes may allow detecting sources of tolerance and could help to identify the most relevant mechanisms of response to water stress in this species. In other crops related to pepino, such as tomato and eggplant, diversity has been observed for tolerance to drought (Plazas et al., 2019; Raja et al., 2020). The work presented here represents the first systematic study of this type in pepino, assessing the effects of water stress on growth and biochemical responses in different pepino cultivars. There is a previously published report (Duman and Sivaci,

2015), which used a similar approach but was much more limited in scope, focused on a single cultivar ('Miski'), and characterised a narrower range of drought-induced responses. However, even though our study assessed more growth and biochemical parameters, it confirmed some trends observed in Duman and Sivaci (2015), like a decrease in water content, chlorophylls and carotenoids, and an increase in proline. In contrast, we did not observe a significative increase in the total phenolic compounds and MDA compared with the control; it should be mentioned, however, that the latter responses were observed only after persistent severe drought conditions (Duman and Sivaci, 2015).

In this work, we have found quantitative differences in growth and biochemical parameters, among pepino cultivars, both in control and drought-stressed plants, thus confirming at the physiological and molecular levels the already known high phenotypic and genetic diversity of pepino (Blanca et al., 2007; Herraiz et al., 2016). This opens the door to the exploitation of this diversity for selecting and breeding more drought-tolerant pepino varieties. In our study, in general, no inhibition of growth was observed under moderate water stress conditions (from 16.7 to 26.4% average percentage of substrate moisture from day 7), as no significant differences with the controls (from 46.9% to 71.7% average percentage of substrate moisture) were detected in most measured growth parameters. This led to hypothesise that pepino, in comparison with other crops, is moderately tolerant to drought and therefore lower amount of water can be applied without affecting severely the plant development. An interesting exception refers to root length, which increased significantly in the WS-M treatment; this response appears to mimic the behaviour of the plants in nature, where drought may induce root growth, as roots search for deeper and wetter layers of the soil (Kano et al., 2011). Similarly, with very few exceptions, the moderate water stress treatment did not affect the contents of the

determined biochemical variables, including photosynthetic pigments, ions, osmolytes, oxidative stress biomarkers and antioxidant compounds. On the contrary, the severe stress treatment (WS-S) induced significant differences in several of these biochemical parameters and caused a clear inhibition of growth, mostly reflected in the reduction of the fresh weight of leaves, stems and roots – a reduction partly due to dehydration of the three organs, as a decrease in their water content percentages was also observed. Therefore, as pepino seems to be relatively resistant to moderate water deficit conditions, it is likely that improvements in the water use efficiency of this crop can be achieved with proper irrigation management (Hatfield & Dold, 2019). Also, the analysis of the effects of drought on growth traits in the different cultivars led to the identification of two of them, Mur2 and Mur4, as more drought-tolerant than the rest of accessions, opening the way to the establishment of breeding programmes for tolerance to drought in pepino. The 'Sweet Round' cultivar (Mur2) was developed for being introduced in the Mediterranean climates, showing high productivity (around 30 and up to 67.5 t ha^{-1}), good tolerance to salinity, high levels of soluble solids (10.4%) and ascorbic acid (26 mg 100 g^{-1}) and an excellent flavour, texture and intensive scent. At commercial maturity, on average, fruit weights around 215 g and show yellow flesh and shiny golden-yellow purplish-striped skin, and is consumed mostly as a dessert (Ruiz et al., 1997, Supplementary Data S1). Contrarily to 'Sweet Round', which is more adapted to protected cultivation, the 'Turia' cultivar (Mur4) has shown good performance in a wide range of cultivations and environments. Also, it is mostly consumed in salads for its herbaceous-green aroma, firm flesh and medium soluble solids content (7-8º Brix) (Rodríguez-Burruezo et al., 2004). 'Turia' is highly productive (between 50 and 70 t ha^{-1}) and vigorous, and was the first pepino cultivar tolerant to tomato mosaic virus (ToMV), one of the main diseases affecting this crop. Phenotypically, 'Turia' has oval golden purple-striped fruits weighing

around 250-350 g and with yellow flesh (Supplementary Data S1). Ascorbic acid is also high, with values between 25 and 35 mg 100 g^{-1}. Both cultivars were developed at the Universitat Politècnica de Valencia.

The general responses of pepino to water deficit treatments, namely, inhibition of growth and degradation of photosynthetic pigments, are shared by other nightshade species, such as tomato and eggplant (Plazas et al., 2019; Raja et al., 2020), and by many other vegetable crops (Abid et al., 2018; Chmielewska et al., 2016; Zhou et al., 2018). What is not a general response to water stress in crop species, is the accumulation of monovalent ions, Na^+ and Cl^- (and, to a lesser extent, also K^+) to very high levels in the roots, mostly considering that the plants were grown under low salinity conditions; mean Na^+ and K^+ concentrations increased significantly in response to the water stress treatment. Furthermore, ion concentrations were even higher in the aerial parts of the plants, accumulating predominantly in the stems rather than in the leaves. This points to the presence in pepino of mechanisms for the active uptake by the roots and transport to the aboveground organs of these ions, which could contribute to cellular osmotic adjustment under water stress conditions and, therefore, to the (relative) drought tolerance of this species. The stem could act as a 'buffer' organ, limiting the transport of the toxic ions to leaf cells. The use of ions, such as Na^+ and Cl^-, as 'inorganic osmolytes' is a general mechanism largely contributing to salt tolerance in dicotyledonous halophytes (Flowers et al., 1977; Flowers and Colmer, 2008) but, in some cases, it has also been observed as a response to drought in drought-tolerant species (Xi et al., 2018). Typical glycophytes, on the contrary, tend to block their transport from the roots to the leaves in response to salt stress (Munns and Tester, 2008). Regarding the divalent cation Ca^{2+}, its participation in multiple stress signalling pathways is well established (Tuteja and Mahajan, 2007; Bose et al., 2011) and could also be involved in drought tolerance mechanisms in pepino,

as it accumulates to relatively high levels in the leaves, by active transport from the roots. However, if this is the case, those mechanisms should be constitutive since the water deficit treatments did not induce a significant increase in the average Ca^{2+} concentrations in the plants.

Proline is one of the most common plant osmolytes, accumulating in many species in response to different abiotic stress conditions, including drought; in addition to its role in osmotic adjustment, proline may participate in stress tolerance mechanisms as an osmoprotectant, by the direct stabilisation of proteins and macromolecular structures, as a ROS-scavenger and/or a signalling molecule (Szabados and Savouré, 2010; Akram et al., 2018). In the present study, proline contents increased in pepino cultivars under severe drought stress conditions. Similar findings have already been reported in many species of different families, such as tomato (Al Hassan et al., 2015; Raja et al., 2020), beans (Morosan et al., 2017), barley (Dbira et al., 2018), Norway spruce (Schiop et al., 2017), or different cultivars of ornamental species of the genus *Tagetes* (Cicevan et al., 2016), to give only a few examples. Since no positive correlation between the increment of proline levels and the relative drought tolerance of the pepino cultivars has been established, it is not clear whether proline is directly involved in the mechanisms of tolerance. In any case, proline could be a useful biochemical marker of water stress in this species, as it has been demonstrated in *Phaseolus vulgaris* cultivars (Arteaga et al., 2020). Soluble sugars are also functional osmolytes in many different plant species (e.g., Gil et al., 2013; Al Hassan et al., 2016; Plesa et al., 2019). It is, however, unlikely that these compounds play any relevant role in pepino responses to drought, as no significant changes in TSS levels were detected in the water-stressed plants, as compared with the controls. Similarly, the stress treatments did not induce an increase in the concentrations of the tested oxidative stress markers, MDA and H_2O_2; in fact, MDA levels even

decreased under severe WS. These data indicate that, under the specific conditions used in our experiments, there was no induction of oxidative stress as a secondary effect of the applied water deficit. Consequently, we also did not detect an increase in the levels of antioxidant compounds.

5. Conclusions

In our experimental conditions, pepino has shown to be relatively tolerant to moderate drought conditions even though it is affected by severe water stress, which was reflected in inhibition of growth, degradation of photosynthetic pigments and changes in several biochemical parameters. More intermediate water stress conditions between the two tested in this study will help to further adjust the water optimum requirements for pepino and study its physiological and biochemical response under drought stress. All tested pepino cultivars responded to water deficit in the same way, qualitatively, as should be expected for closely related genotypes, but with quantitative differences that allowed identifying two specific cultivars, Mur2 and Mur4, as relatively more tolerant to drought. Even though further studies will be required to elucidate the mechanisms of water stress tolerance in pepino, the active uptake of monovalent ions (Na^+, Cl^-, K^+) and their accumulation to very high concentrations in the aboveground organs of the plants, may be involved in those mechanisms, contributing to cellular osmotic adjustment under stress. The differences observed among cultivars in tolerance to water stress and the associated biochemical responses observed are relevant for the selection and breeding of more drought tolerant pepino cultivars.

Supplementary data

Supplementary Data S1: Pictures of leaves and fruits of the seven pepino cultivars assessed in this study, Mur1 (A and H), Mur2 (B and I), Mur3 (C and J), Mur4 (D and K), Mur6 (E and L), Mur6 (F and M), Mur7 (G and N).

Acknowledgements

The authors are grateful to Ana Maria Tofei, Weronika Spitalniak and Malwina Wegner for their assistance in the greenhouse work.

Funding

This work has been funded by Universitat Politècnica de València (research line 20200268). Pietro Gramazio is grateful to Japan Society for the Promotion of Science for a post-doctoral grant (P19105, FY2019 JSPS Postdoctoral Fellowship for Research in Japan [Standard]).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Akram, N. A., Iqbal, M., Muhammad, A., Ashraf, M., Al-Qurainy, F., and Shafiq, S. (2018). Aminolevulinic acid and nitric oxide regulate oxidative defense and

secondary metabolisms in canola (*Brassica napus* L.) under drought stress. *Protoplasma*, *255*, 163–174. https://doi.org/10.1007/s00709-017-1140-x

Al Hassan, M., Martínez Fuertes, M., Ramos Sánchez, F.J., Vicente, O., and Boscaiu, M. (2015). Effects of salt and water stress on plant growth and on accumulation of osmolytes and antioxidant compounds in cherry tomato. *Not. Bot. Hort. Agrobot. Cluj* 43, 1-11. https://doi.org/10.15835/nbha4319793

Al Hassan, M., Morosan, M., López-Gresa, M.P., Prohens, J., Vicente, O., and Boscaiu, M. (2016). Salinity-induced variation in biochemical markers provides insight into the mechanisms of salt tolerance in common (*Phaseolus vulgaris*) and runner (*P. coccineus*) beans. *Int. J. Mol. Sci.,* 17, *1582;* https://doi.org/10.3390/ijms17091582

Anderson, G. J., Jansen, R. K., and Kim, Y. (1996). The origin and relationships of the pepino, *Solanum muricatum* (solanaceae): DNA restriction fragment evidence. *Econ. Bot., 50*, 369–380. https://doi.org/10.1007/BF02866519

Arteaga, S., Yabor, L., Díez, M.J., Prohens, J., Boscaiu, M. and Vicente, O. (2020). The use of proline in screening for tolerance to drought and salinity in common bean (*Phaseolus vulgaris* L.) genotypes. *Agronomy*, 10**,** 817; *doi: 10.3390/agronomy10060817*

Bates, L. S., Waldren, R. P., and Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil*, *39*, 205–207. https://doi.org/10.1007/BF00018060

Batra, N. G., Sharma, V., and Kumari, N. (2014). Drought-induced changes in chlorophyll fluorescence, photosynthetic pigments, and thylakoid membrane proteins of *Vigna radiata*. *J. Plant Interact.*, *9*, 712–721. https://doi.org/10.1080/17429145.2014.905801

Blainski, A., Lopes, G. C., and De Mello, J. C. P. (2013). Application and analysis of the

Folin Ciocalteu method for the determination of the total phenolic content from *Limonium brasiliense* L. *Molecules*, *18*, 6852–6865. https://doi.org/10.3390/molecules18066852

Blanca, J. M., Prohens, J., Anderson, G. J., Zuriaga, E., Cañizares, J., and Nuez, F. (2007). AFLP and DNA sequence variation in an Andean domesticate, pepino (*Solanum muricatum*, Solanaceae): Implications for evolution and domestication. *Amer. J. Bot.*, *94*, 1219–1229. https://doi.org/10.3732/ajb.94.7.1219

Bose, J., Pottosin, I., Shabala, S., Palmgren, M.G., and Shabala, S. (2011). Calcium efflux systems in stress signaling and adaptation in plants. *Front. Plant Sci.*, *2*, 85, doi:10.3389/fpls.2011.00085

Buytaert, W., Vuille, M., Dewulf, A., Urrutia, R., Karmalkar, A., and Célleri, R. (2010). Uncertainties in climate change projections and regional downscaling in the tropical Andes: Implications for water resources management. *Hydrology and Earth System Sciences*, *14*, 1247–1258. https://doi.org/10.5194/hess-14-1247-2010

Cicevan, R., Al Hassan, M., Sestras, A.F., Prohens, J., Vicente, O., Sestras, R.E. and Boscaiu, M. (2016). Screening for drought tolerance in cultivars of the ornamental genus *Tagetes* (Asteraceae). *PeerJ*, 4, e2133; *doi: 10.7717/peerj.2133*.

Das, K., & Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front. Environ. Sci.*, 2, 1–13. https://doi.org/10.3389/fenvs.2014.00053

Dbira, S., Al Hassan, M., Gramazio, P., Ferchichi, A., Vicente, O., Prohens, J. and Boscaiu, M. (2018). Variable levels of tolerance to water stress (drought) and associated biochemical markers in Tunisian barley landraces. *Molecules,* 23, 613. *doi: 10.3390/molecules23030613.*

Del Rio, D.; Stewart, A.J.; Pellegrini, N. (2005). A review of recent studies on

malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr. Metab. Cardiovasc. Dis.* 15, 316–328.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, 28, 350–356. https://doi.org/10.1021/ac60111a017

Duman, S., and Sivaci, A. (2015). Investigation of drought stress in pepino (*Solanum muricatum* Ait. CV. Miski) leaves. *Pak. J. Bot.*, *47*, 1621–1627.

Fang, Y., and Xiong, L. (2015). General mechanisms of drought response and their application in drought resistance improvement in plants. *Cell. Mol. Life Sci.*, 72, 673–689. https://doi.org/10.1007/s00018-014-1767-0

Fita, A., Rodríguez-Burruezo, A., Boscaiu, M., Prohens, J., and Vicente, O. (2015). Breeding and domesticating crops adapted to drought and salinity: A new paradigm for increasing food production. *Front. Plant Sci.*, 6, 978. https://doi.org/10.3389/fpls.2015.00978

Flowers, T.J., and Colmer, T.D. (2008). Salinity tolerance in halophytes. *New Phytol.*, 179, 945-963. https://doi.org/10.1111/j.1469-8137.2008.02531.x

Flowers, T.J., Troke, P.F. and Yeo, A.R. (1977). The mechanism of salt tolerance in halophytes. *Annu. Rev. Plant Physiol.*, 28, 89–121. https://doi.org/10.1146/annurev.pp.28.060177.000513

Gil, R., Boscaiu, M., Lull, C., Bautista, I., Lidón, A., and Vicente, O. (2013). Are soluble carbohydrates ecologically relevant for salt tolerance in halophytes? *Funct. Plant Biol.*, 40, 805–818.

Gurung, S., Chakravarty, S., Chhetri, B., and Khawas, T. (2016). An introduction to pepino (*Solanum muricatum* Aiton): review. *Intl. J. Environ. Agric. Biotechnol.*, 1, 143–148. https://doi.org/10.22161/ijeab/1.2.8

Herraiz, F. J., Vilanova, S., Plazas, M., Gramazio, P., Andújar, I., Rodríguez-Burruezo, A., Fita, A., Anderson, G.J., and Prohens, J. (2015). Phenological growth stages of pepino (*Solanum muricatum*) according to the BBCH scale. *Sci. Hort.*, 183, 1–7. https://doi.org/10.1016/j.scienta.2014.12.008

Herraiz, F.J., Villaño, D., Plazas, M., Vilanova, S., Ferreres, F., Prohens, J., and Moreno, D.A. (2016). Phenolic profile and biological activities of the pepino (*Solanum muricatum*) fruit and its wild relative *S. caripense*. *Intl. J. Mol. Sci.*, 17. https://doi.org/10.3390/ijms17030394

Hodges, D.M., DeLong, J.M., Forney, C.F., & Prange, R.K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*, 207, 604–611. https://doi.org/10.1007/s004250050524

Hsu, C., Guo, Y., Wang, Z., and Yin, M. (2011). Protective effects of an aqueous extract from pepino (*Solanum muricatum* Ait.) in diabetic mice. *J. Sci. Food Agric.*, 91, 1517–1522. https://doi.org/10.1002/jsfa.4345

Hsu, J.Y., Lin, H.H., Hsu, C.C., Chen, B.C., and Chen, J.H. (2018). Aqueous extract of pepino (*Solanum muriactum* ait) leaves ameliorate lipid accumulation and oxidative stress in alcoholic fatty liver disease. *Nutrients*, 10, 931. https://doi.org/10.3390/nu10070931

Kar, R.K. (2011). Plant responses to water stress: Role of reactive oxygen species. *Plant Signal Behav.* 6, 1741–1745.

Kassambara, A. (2015). Multivariate Analysis 1: Practical Guide To Cluster Analysis in R. STHDA.

Kozminska, A., Al Hassan, M., Wiszniewska, A., Hanus-Fajerska, E., Boscaiu, M., and Vicente, O. (2019). Responses of succulents to drought: Comparative analysis of

four *Sedum* (Crassulaceae) species. *Sci. Hort.*, 243, 235-242; doi: 10.1016/j.scienta.2018.08.028

Kumar, D., Al Hassan, M., Naranjo, M.A., Agrawal, V., Boscaiu, M., and Vicente, O. (2017a). Effects of salinity and drought on growth, ionic relations, compatible solutes and activation of antioxidant systems in oleander (*Nerium oleander* L.). *PLOS ONE*, 12, e0185017. https://doi.org/10.1371/ journal.pone.0185017

Kumar, A., Adak, T., and Rajan, S. (2017b). Pepino (*Solanum muricatum* Ait.): A potential future crop for subtropics. *Tropical Plant Res.*, 4, 514–517. https://doi.org/10.22271/tpr.2017.v4.i3.067

Lê, S., Josse, J., and Husson, F. (2008). FactoMineR: An R package for multivariate analysis. *J. Stat. Soft.*, 25, 1–18. https://doi.org/10.18637/jss.v025.i01

Lichtenthaler, H.K., & Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Transact.*, 11, 591–592. https://doi.org/10.1042/bst0110591

Loreto, F., and Velikova, V. (2001). Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiol.*, 127, 1781–1787. https://doi.org/10.1104/pp.010497

Morosan, M., Al Hassan, M., Naranjo, M.A., López-Gresa, M.P., Boscaiu, M., and Vicente, O. (2017). Comparative analysis of drought responses in *Phaseolus vulgaris* (common bean) and *P. coccineus* (runner bean) cultivars. Eurobiotech J., 1, 247-252. doi: 10.24190/ISSN2564-615X/2017/03.09

Munns, R., and Tester M. (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59, 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Murray, B.G., Hammett, K.R.W., and Grigg, F.D.W. (1992). Seed set and breeding

system in the pepino *Solanum muricatum* Ait., Solanaceae. *Sci. Hort.*, 49, 83–92. https://doi.org/10.1016/0304-4238(92)90145-3

Plazas, M., Nguyen, H.T., González-Orenga, S., Fita, A., Vicente, O., Prohens, J., and Boscaiu, M. (2019). Comparative analysis of the responses to water stress in eggplant (*Solanum melongena*) cultivars. *Plant Physiol. Biochem.*, 143, 72–82. https://doi.org/10.1016/j.plaphy.2019.08.031

Plesa, I.M., Al Hassan, M., González-Orenga, S., Sestras, A.F., Vicente, O., Prohens, J., Boscaiu, M., and Sestras, R.E. (2019). Responses to drought in seedlings of European larch (*Larix decidua* Mill.) from several Carpathian provenances. *Forests*, 10, 511. https://doi.org/1*0.3390/f10060511*

Pluda, D., Rabinowitch, H.D., and Kafkafi, U. (2019). Pepino dulce (*Solanum muricatum* Ait.) quality characteristics respond to nitrogen nutrition and salinity. *J. Amer. Soc. Hort. Sci.*, 118, 86–91. https://doi.org/10.21273/jashs.118.1.86

Pluda, D., Rabinowitch, H.D., and Kafkafi, U. (1993). Fruit set and yield of pepino dulce response to nitrate-nitrogen and salinity levels and thinning of side branches and trusses1. *J. Plant Nutr.*, 16, 2121–2133. https://doi.org/10.1080/01904169309364678

Prohens, J., Sánchez, M.C., Rodríguez-Burruezo, A., Cámara, M., Torija, E., and Nuez, F. (2005). Morphological and physico-chemical characteristics of fruits of pepino (*Solanum muricatum*), wild relatives (*S. caripense* and *S. tabanoense*) and interspecific hybrids. Implications in pepino breeding. *Eur. J. Hort. Sci.*, 70, 224–230.

Prohens, J, Ruiz, J.J., and Nuez, F. (2003). Vegetable crop diversification in areas affected by salinity: the case of pepino (*Solanum muricatum*). *Acta Hort., 618*, 267–273. https://doi.org/10.17660/ActaHortic.2003.618.30

Prohens, J., Leiva-Brondo, M., Rodríguez-Burruezo, A., and Nuez, F. (2002). 'Puzol': A facultatively parthenocarpic hybrid of pepino (*Solanum muricatum*). *HortScience*, 37, 418–419.

Prohens, J., Ruiz, J.J., and Nuez, F. (1996). The pepino (*Solanum muricatum*, Solanaceae): A "new" crop with a history. *Econ. Bot.*, 50, 355–368. https://doi.org/10.1007/BF02866518

Raja, V., Qadir, S.U., Alyemeni, M.N., and Ahmad, P. (2020). Impact of drought and heat stress individually and in combination on physio-biochemical parameters, antioxidant responses, and gene expression in *Solanum lycopersicum*. *3 Biotech.*, 10, 208. https://doi.org/10.1007/s13205-020-02206-4

Rodríguez-Burruezo, A., Prohens, J., Leiva-Brondo, M., & Nuez, F. (2004). Turia pepino. *Canadian Journal of Plant Science*, *84*(2), 603–606. https://doi.org/10.4141/P03-108

Reis, L.C., Scalon, P.Q., Dresch, S., Foresti, A.C., Santos, C.C., and Pereira, Z.V. (2020). Chlorophyll a fluorescence as an indicator of water stress in *Calophyllum brasiliense*. *Not. Bot. Horti Agrobo.*, 48, 210–220. https://doi.org/10.15835/nbha48111757

Rodríguez-Burruezo, A., Kollmannsberger, H., Prohens, J., Nitz, S., and Nuez, F. (2004). Analysis of the volatile aroma constituents of parental and hybrid clones of pepino (*Solanum muricatum*). *J. Agric. Food Chem.*, 52, 5663–5669. https://doi.org/10.1021/jf040107w

Rodríguez-Burruezo, A., Prohens, J., and Fita, A.M. (2011). Breeding strategies for improving the performance and fruit quality of the pepino (*Solanum muricatum*): A model for the enhancement of underutilized exotic fruits. *Food Res. Intl.*, 44, 1927–1935. https://doi.org/10.1016/j.foodres.2010.12.028

Rodríguez-Burruezo, A., Prohens, J., and Nuez, F. (2004). 'Valencia': A new pepino (*Solanum muricatum*) cultivar with improved fruit quality. *HortScience*, 39, 1500–1502.

Ruiz, J. J., Prohens, J., & Nuez, F. (1997). “Sweet Round” and “Sweet Long”: Two pepino cultivars for Mediterranean climates. In *HortScience* (Vol. 32, Issue 4, pp. 751–752). https://doi.org/10.21273/hortsci.32.4.751

Särkinen, T., Bohs, L., Olmstead, R.G., and Knapp, S. (2013). A phylogenetic framework for evolutionary study of the nightshades (Solanaceae): A dated 1000-tip tree. *BMC Evol. Biol.*, 13, 214. https://doi.org/10.1186/1471-2148-13-214

Schiop, S.T., Al Hassan, M., Sestras, A.F., Boscaiu, M., Sestras, R.E., and Vicente, O. (2017). Biochemical responses to drought, at the seedling stage, of several Romanian Carpathian populations of Norway spruce (*Picea abies* L. Karst). *Trees Struct. Funct.*, 31, 1479–1490. *doi: 10.1007/s00468-017-1563-1.*

Seki, M., Umezawa, T., Urano, K., and Shinozaki, K. (2007). Regulatory metabolic networks in drought stress responses. *Curr. Opinion Plant Biol.*, 10, 296–302. https://doi.org/10.1016/j.pbi.2007.04.014

Shathish, K., and Guruvayoorappan, C. (2014). *Solanum muricatum* Ait. inhibits inflammation and cancer by modulating the immune system. *J. Cancer Res. Therapeut.*, 10, 623–630. https://doi.org/10.4103/0973-1482.138198

Singh, A., Sharma, M.K., and Sengar, R.S. (2017). Osmolytes: Proline metabolism in plants as sensors of abiotic stress. *J. Appl. Natural Sci.*, 9, 2079–2092. https://doi.org/10.31018/jans.v9i4.1492

Spooner, D.M., Anderson, G.J., and Jansen, R.K. (1993). Chloroplast DNA evidence for the interrelationships of tomatoes, potatoes, and pepinos (Solanaceae). *Amer. J. Bot.*, 80, 676–688. https://doi.org/10.1002/j.1537-2197.1993.tb15238.x

Sudha, G., Priya, M.S., Shree, R.I., & Vadivukkarasi, S. (2011). Antioxidant activity of ripe pepino fruit (*Solanum muricatum* Aiton). *Intl. J. Pharmacy Pharmaceut. Sci.*, 3, 257–261. https://doi.org/10.1111/j.1750-3841.2012.02944.x

Szabados, L. and Savouré, A. (2010) Proline: a multifunctional amino acid. *Trends Plant Sci.*, 15, 89–97. https://doi.org/10.1016/j.tplants.2009.11.009

Szekely-Varga, Z., González-Orenga, S., Cantor, M., Jucan, D., Boscaiu, M., and Vicente, O. (2020). Effects of drought and salinity on two commercial varieties of *Lavandula angustifolia* Mill. *Plants*, 9, 637; doi:10.3390/plants9050637

Taulavuori, E., Hellström, E. K., Taulavuori, K., and Laine, K. (2001). Comparison of two methods used to analyse lipid peroxidation from *Vaccinium myrtillus* (L.) during snow removal, reacclimation and cold acclimation. *J. Expt. Bot.*, 52, 2375–2380. https://doi.org/10.1093/jexbot/52.365.2375

Tuteja, N., and Mahajan, S. (2007). Calcium signaling network in plants. An overview. Plant Signal. Behav., 2, 79–85. https://doi.org/10.4161/psb.2.2.4176

Virani, D., Chaerunnisa, N. N., Suarsi, I., Dachlan, D. M., and Thahir, A. I. A. (2020). Pepino extract (*Solanum muricatum* Ait.) on HDL and LDL in type 2 diabetic rats. *Enfermería Clínica*, 30, 163–166. https://doi.org/https://doi.org/10.1016/j.enfcli.2019.10.061

Wang, N., Wang, L., Wang, Z., Cheng, L., & Wang, J. (2019). *Solanum muricatum* ameliorates the symptoms of osteogenesis imperfecta in vivo. *Journal of Food Science*, *84*, 1646–1650. https://doi.org/10.1111/1750-3841.14637

Weimberg, R. (1987). Solute adjustments in leaves of two species of wheat at two different stages of growth in response to salinity. *Physiol. Plantarum*, 70, 381–388. https://doi.org/10.1111/j.1399-3054.1987.tb02832.x

Xi J.J., Chen, H.Y., Bai, W.P., Yang, R.C., Yang, P.Z., Chen, R.J., Hu, T.M.. and Wang,

S.M. (2018). Sodium-related adaptations to drought: New insights from the xerophyte plant *Zygophyllum xanthoxylum*. *Front. Plant Sci*., 9, 1678. https://doi.org/10.3389/fpls.2018.01678

Yue, H., Xu, Q., Bian, G., Guo, Q., Fang, Z., and Wu, W. (2020). Structure characterization and immunomodulatory activity of a new neutral polysaccharide SMP-0b from *Solanum muricatum*. *Intl. J. Biol. Macromol.*, 155, 853–860. https://doi.org/10.1016/j.ijbiomac.2019.11.071

Yue, H., Xu, Q., Li, X., Elango, J., Wu, W., and Xu, J. (2019). Physicochemical characterization and immunomodulatory activity of a novel acid polysaccharide from *Solanum muricatum*. *Polymers*, 11, 1972. https://doi.org/10.3390/polym11121972

Zhishen, J., Mengcheng, T., and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.*, 64, 555–559. https://doi.org/10.1016/S0308-8146(98)00102-2

Table 1. List of the 31 traits with abbreviations and units used for the morphoagronomic and biochemical characterization measured in the seven pepino cultivars assessed in this study.

Code	Trait	Scale/Unit
Growth		
NL	Number of leaves	unit
SL	Stem length	cm
SD	Stem diameter	cm
RL	Root length	cm
LFW	Leaf fresh weight	g
SFW	Stem fresh weight	g
RFW	Root fresh weight	g
LWC	Leaf water content	%
SWC	Stem water content	%
RWC	Root water content	%
Photosynthtetic pigments		
Chl a	Chlorophyll a	mg g^{-1} DW
Chl b	Chlorophyll b	mg g^{-1} DW
Caro	Carotenoids	mg g^{-1} DW
Mono and divalent ions		
Na^+l	Sodium concentration in leaves	µmol g^{-1} DW
Na^+s	Sodium concentration in stems	µmol g^{-1} DW
Na^+r	Sodium concentration in roots	µmol g^{-1} DW
K^+l	Potassium concentration in leaves	µmol g^{-1} DW
K^+s	Potassium concentration in stems	µmol g^{-1} DW
K^+r	Potassium concentration in roots	µmol g^{-1} DW
Cl^-l	Chlorine concentration in leaves	µmol g^{-1} DW
Cl^-s	Chlorine concentration in stems	µmol g^{-1} DW
Cl^-r	Chlorine concentration in roots	µmol g^{-1} DW
$Ca^{2+}l$	Calcium concentration in leaves	µmol g^{-1} DW
$Ca^{2+}s$	Calcium concentration in stems	µmol g^{-1} DW
$Ca^{2+}r$	Calcium concentration in roots	µmol g^{-1} DW
Osmolytes		
Pro	Proline	µmol. g^{-1} DW
TSS	Total soluble sugars	mg eq. glucose g^{-1} DW
Antioxidants		
MDA	Malondialdehyde	nmol g^{-1} DW
H_2O_2	Hydrogen peroxide	µmol g^{-1} DW
TPC	Total phenolic compounds	mg eq. GA g^{-1} DW
TF	Total flavonoids	mg eq. C g^{-1} DW

Table 2. Two-way factorial ANOVA (F-values) for the traits measured in seven pepino cultivars under three drought stress treatments.

Trait	Cultivar	Treatment	Cultivar × treatment
Growth			
NL	16.65^{***}	3.09^{ns}	1.90^{*}
SL	9.73^{***}	2.16^{ns}	0.71^{ns}
SD	19.12^{***}	40.79^{***}	2.48^{**}
RL	9.52^{***}	10.95^{***}	1.30^{ns}
LFW	10.34^{***}	61.63^{***}	2.23^{*}
SFW	6.44^{***}	26.89^{***}	1.47^{ns}
RFW	11.19^{***}	49.69^{***}	1.50^{ns}
LWC	1.00^{ns}	36.05^{**}	1.01^{ns}
SWC	0.96^{ns}	29.04^{***}	0.65^{ns}
RWC	1.78^{ns}	72.26^{***}	0.99^{ns}
Photosynthtetic pigments			
Chl a	3.28^{**}	6.03^{**}	1.30^{ns}
Chl b	4.10^{***}	5.17^{***}	1.56^{ns}
Caro	2.41^{*}	22.87^{***}	1.00^{ns}
Mono and divalent ions			
$Na^{+}l$	7.75^{***}	0.43^{ns}	0.61^{ns}
$Na^{+}s$	16.88^{***}	2.04^{ns}	1.15^{ns}
$Na^{+}r$	1.59^{ns}	5.29^{**}	1.55^{ns}
$K^{+}l$	19.33^{***}	0.15^{ns}	0.72^{ns}
$K^{+}s$	11.80^{***}	13.78^{***}	4.08^{***}
$K^{+}r$	4.08^{**}	24.45^{***}	1.96^{*}
$Cl^{-}l$	1.51^{ns}	0.77^{ns}	1.21^{ns}
$Cl^{-}s$	9.16^{***}	1.67^{ns}	2.03*
$Cl^{-}r$	5.68^{***}	3.03^{ns}	1.01^{ns}
$Ca^{2+}l$	26.39^{***}	1.46^{ns}	0.56^{ns}
$Ca^{2+}s$	4.68^{***}	2.39^{ns}	1.69^{ns}
$Ca^{2+}r$	2.52*	1.15^{ns}	1.81^{ns}
Osmolytes			
Pro	4.78^{***}	8.27^{***}	1.95^{*}
TSS	12.92^{***}	0.20^{ns}	0.98^{ns}
Antioxidants			
MDA	2.30^{*}	10.50^{***}	0.48^{ns}
H_2O_2	2.05^{ns}	3.03^{ns}	1.46^{ns}
TPC	6.10^{***}	1.40^{ns}	1.80^{ns}
TF	76.82^{***}	11.53^{***}	8.37^{***}

^{ns}, *, **, *** indicate non-significant or significant at $p < 0.05$, 0.01, and 0.001, respectively.

Table 3. Mean effect of cultivar and treatment and the average standard error (SE) from the analysis of variance for growth and photosynthetic pigment traits in seven cultivars of pepino (Mur1 to Mur7) subjected to three drought stress treatments (Control; moderate water stress WS-M; severe water stress, WS-S). Different lowercase letters denote significant means differences within cultivar or treatments according to the Student-Newman-Keuls multiple range test ($p < 0.05$).

Factor	NL (n)	SL (cm)	SD (cm)	RL (cm)	LFW (g)	SFW (g)	RFW (g)	LWC (%)	SWC (%)	RWC (%)	Chl a (mg g-1 DW)	Chl b (mg g-1 DW)	Caro (mg g-1 DW)
Cultivar													
Mur1	24.5 bc	22.1 c	4.46 c	30.8 b	9.54 b	3.53 c	2.27 d	73.2 a	77.6 a	70.3 a	7.35 a	1.74 a	0.74 a
Mur2	23.7 bc	26.2 d	4.31 c	34.2 b	8.63 b	3.07 c	2.04 cd	84.1 a	84.3 a	76.3 a	16.07 b	6.19 b	0.66 a
Mur3	41.1 d	21.4 bc	4.49 c	32.9 b	13.49 c	2.98 c	2.35 d	80.3 a	79.3 a	70.6 a	17.02 b	6.53 b	0.66 a
Mur4	30.1 c	19.1 bc	3.40 c	23.0 a	9.05 b	2.79 c	1.59 bc	76.1 a	76.4 a	73.6 a	10.57 ab	4.45 ab	0.69 a
Mur5	25.8 bc	17.6 ab	4.09 c	24.2 a	7.40 b	1.73 ab	1.23 ab	76.4 a	76.7 a	65.4 a	11.88 ab	4.43 ab	1.03 b
Mur6	16.8 a	15.1 a	2.93 ab	20.0 a	4.70 a	1.39 a	0.77 a	72.3 a	76.2 a	65.1 a	11.14 ab	4.32 ab	0.62 a
Mur7	20.9 ab	19.7 bc	2.57 a	23.8 a	9.46 b	2.52 bc	0.97 a	80.2 a	80.7 a	68.7 a	16.45 b	6.38 b	0.68 a
SE	1.94	1.14	0.18	1.79	0.84	0.30	0.19	4.26	2.98	3.01	2.06	0.86	0.09
Treatment													
Control	27.6 a	21.1 a	4.36 c	24.3 a	11.37 b	3.25 b	2.23 b	86.6 b	84.7 b	83.2 c	14.81 b	5.20b	0.92 b
WS-M	27.2 a	20.4 a	3.99 b	31.5 b	11.29 b	3.06 b	1.99 b	87.4 b	84.7 b	75.4 b	14.85 b	5.94b	0.86 b
WS-S	23.6 a	19.0 a	2.90 a	25.2 a	4.03 a	1.40 a	0.59 a	58.5 a	66.8 a	51.4 a	9.13 a	3.45a	0.41 a
SE	1.27	0.75	0.12	1.18	0.55	0.20	0.13	2.79	1.95	1.97	1.35	0.57	0.06

Table 4. Mean effect of cultivar and treatment and the average standard error (SE) from the analysis of variance for mono- and divalent ion contents (μmol g^{-1} DW) in stem (s), leaves (l) and roots (r) in seven cultivars of pepino (Mur1 to Mur7) subjected to three drought stress treatments (Control; moderate water stress WS-M; severe water stress, WS-S). Different lowercase letters denote significant means differences within cultivars or treatments according to the Student-Newman-Keuls multiple range test ($p < 0.05$).

Factor	Na^+l	Na^+s	Na^+r	K^+l	K^+s	K^+r	Cl^-l	Cl^-s	Cl^-r	Ca^{2+}l	Ca^{2+}s	Ca^{2+}r
Cultivar												
Mur1	1,432 a	3,534 b	1,088 a	629 ab	2,007 c	544 ab	2,254 a	7,034 ab	1,007 ab	75.6 a	108.5 b	6.9 a
Mur2	2,069 ab	4,340 c	1,345 a	786 b	1,358 a	606 b	1,699 a	4,836 a	1,198 b	181.6 b	88.6 ab	17.7 b
Mur3	1,771 ab	3,609 b	1,224 a	777 b	1,300 a	464 ab	1,738 a	4,025 a	1,040 ab	48.9 a	68.0 a	15.6 b
Mur4	1,652 a	2,319 a	1,271 a	617 ab	1,742 bc	389 a	1,955 a	4,836 a	989 ab	49.0 a	77.5 a	22.5 b
Mur5	1,637 a	2,372 a	1,419 a	503 a	1,801 bc	482 ab	1,721 a	5,110 a	1,023 ab	67.9 a	107.9 b	19.3 b
Mur6	2,069 bc	2,355 a	1,106 a	1,065 c	1,571 ab	387 a	1,616 a	4,318 a	699 a	75.7 a	90.6 ab	20.0 b
Mur7	2,320 c	2,270 a	1,298 a	1,166 c	1,399 a	439 a	2,520 a	4,044 a	899 ab	83.7 a	77.1 a	23.1 b
SE	108	209	96	54.51	80	40	279	359	94	24.1	7.5	2.4
Treatment												
Control	1,832 a	2,915 a	1,094 a	805 a	1,430 a	350 a	1,758 a	4,663 a	937 a	79.5 a	88.3 a	20.2 a
WS-M	1,751 a	2,812 a	1,270 ab	777 a	1,549 a	459 b	1,949 a	4,874 a	946 a	62.8 a	81.1 a	19.3 a
WS-S	1,827 a	3,187 a	1,386 b	793 a	1,812 b	610 c	2,080 a	5,260 a	1,055 a	108.0 a	96.0 a	15.0 a
SE	71	137	63	35.73	52	26	183	235	62	15.8	4.9	1.6

Table 5. Mean effect of cultivar and treatment and the average standard error (SE) for osmolytes and antioxidants in seven cultivars of pepino (Mur1 to Mur7) subjected to three drought stress treatments (Control; moderate water stress WS-M; severe water stress, WS-S). Different lowercase letters denote significant means differences within cultivars and treatments according to the Student-Newman-Keuls multiple range test ($p < 0.05$). GA: gallic acid; C: catechin.

Factor	Pro (µmol. g-1 DW)	TSS. (mg eq. glucose g-1 DW)	MDA (nmol g^{-1} DW)	H_2O_2 (µmol g^{-1} DW)	TPC. (mg eq. GA g^{-1} DW)	TF (mg eq. C g^{-1} DW)
Cultivar						
Mur1	50.51 c	54.9 a	517.6 ab	68.06 a	15.76 bc	1.62 a
Mur2	37.15 abc	283.7 c	553.6 b	31.02 a	9.05 a	3.44 a
Mur3	14.80 a	220.7 bc	257.0 a	75.00 a	7.38 a	15.61 b
Mur4	24.19 ab	223.5 bc	519.0 ab	93.35 a	12.60 abc	1.37 a
Mur5	44.06 bc	233.9 bc	522.3 ab	49.93 a	10.98 ab	1.64 a
Mur6	25.79 ab	198.1 b	492.3 ab	75.03 a	17.47 c	1.40 a
Mur7	30.63 abc	192.2 b	445.5 ab	73.84 a	12.85 abc	1.35 a
SE	5.70	20.30	66.71	13.97	1.46	0.62
Treatment						
Control	25.34 a	203.8 a	583.3 b	81.10 a	10.97 a	4.22 b
WS-M	27.28 a	205.2 a	520.2 b	66.23 a	13.03 a	4.86 b
WS-S	44.72 b	194.3 a	313.9 a	50.71 a	12.90 a	2.25 a
SE	3.74	13.6	43.71	9.15	0.96	0.41

Figures

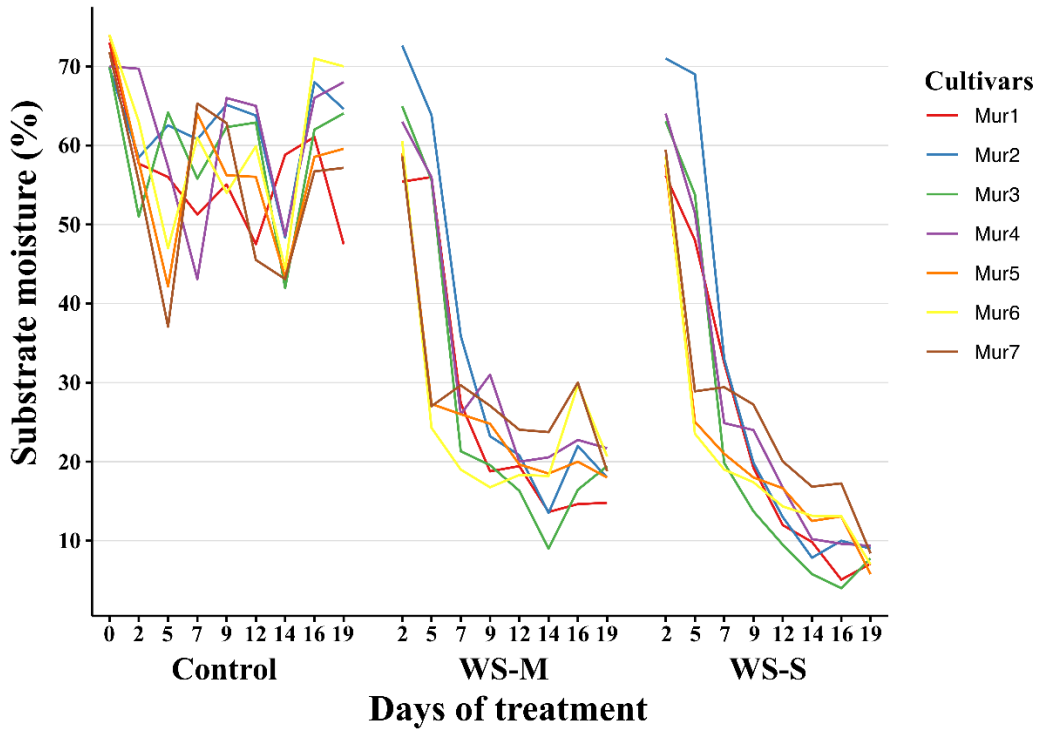

Figure 1. Average percentage of substrate moisture measured every two or three days for the control, moderate water stress (WS-M) and severe water stress (WS-S) treatments during the 19 days of the experiment.

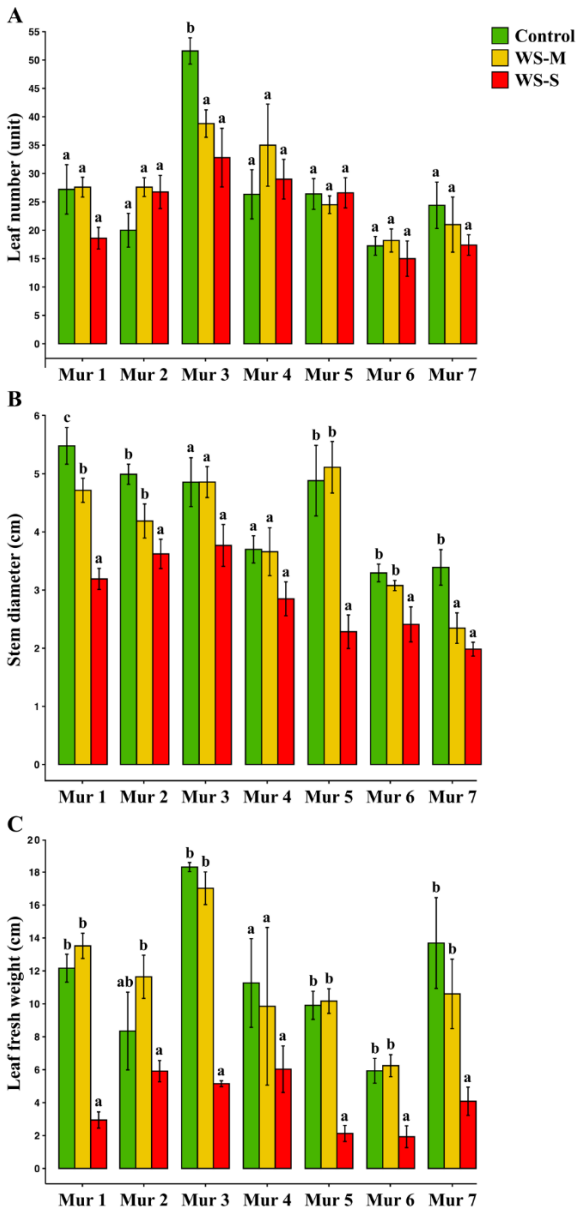

Figure 2. Growth parameters that exhibited significant cultivar × treatment interactions. (A) number of leaves, (B) stem diameter and (C) leaf fresh weight in seven pepino cultivars after 19 days of treatment as mean values with SE (n = 5) for the control (green bars), moderate water stress (WS-M) (yellow bars), and severe water stress (WS-S) (red bars) treatments. Different lowercase letters above the bars indicate significant differences between treatments for each cultivar, according to the Student-Newman-Keuls multiple range test ($p < 0.05$).

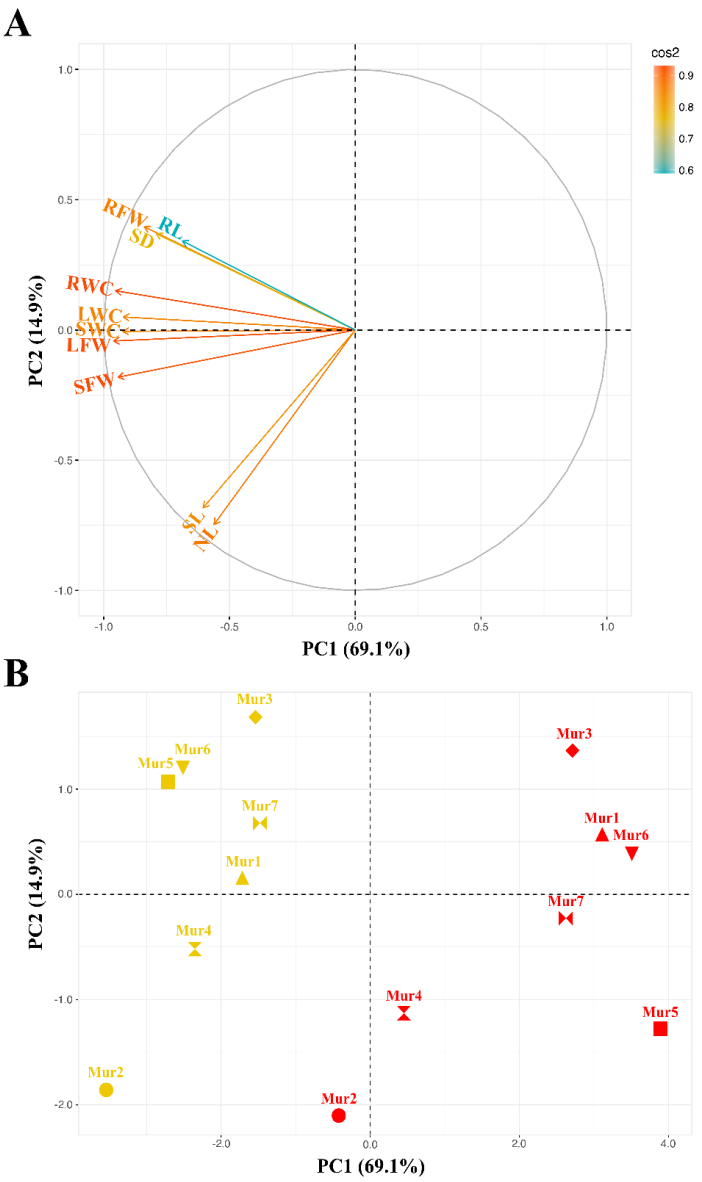

Figure 3. Principal component analysis (PCA) similarities based on the characterization of the values of 10 growth-related traits, expressed as percentages of the corresponding controls, of seven cultivars of pepino under moderate (WS-M) and severe (WS-S) water stress treatments. A) The variable correlation plot indicates the relationships between variables and the PC1 and PC2. Variables that are close to the circumference are more correlated to the first two PCs and those that are grouped together are positively correlated among them. B) Graph of cultivars under moderate water stress (WS-M) (yellow symbols), and severe water stress (WS-S) (red symbols) treatments. Each symbol represents one cultivar.

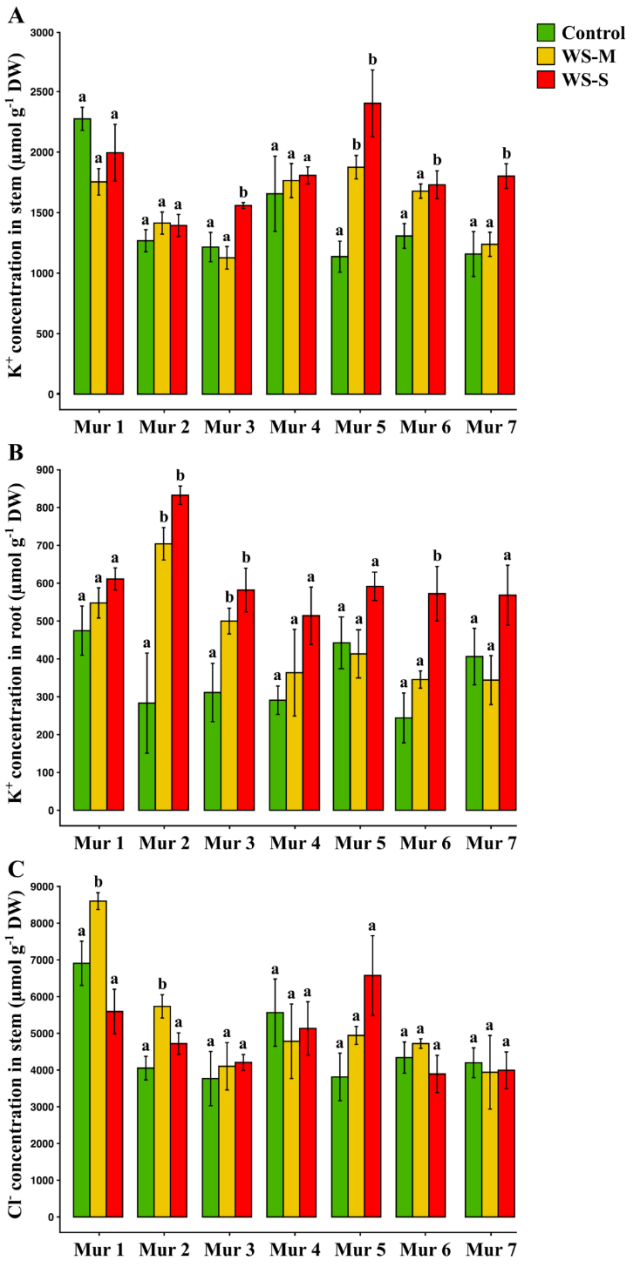

Figure 4. Ions that exhibited cultivar × treatment interactions. (A) Potassium concentration in stem, (B) potassium concentration in root and (C) chlorine concentration in stem in seven pepino cultivars after 19 days of treatment as mean values with SE (n = 5) for the control (green bars), moderate water stress (WS-M) (yellow bars), and severe water stress (WS-S) (red bars) treatments. Different lowercase letters above the bars indicate significant differences between treatments for each cultivar, according to the Student-Newman-Keuls multiple range test ($p < 0.05$).

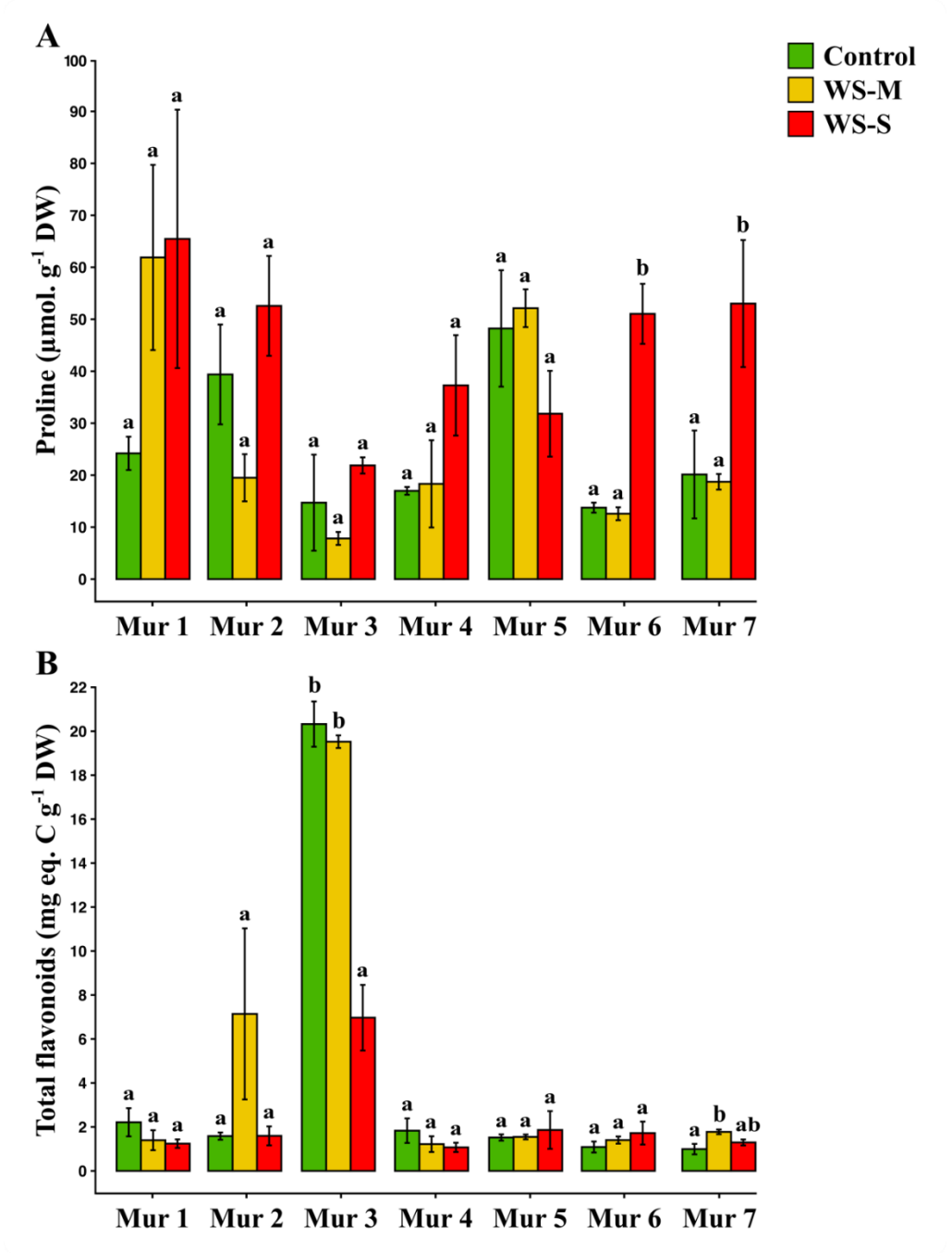

Figure 5. Osmolytes and antioxidants that exhibited cultivar × treatment interactions. (A) Proline and (B) total flavonoids in stem in seven pepino cultivar after 19 days of treatment as mean values with SE (n = 5) for the control (green bars), moderate water stress (WS-M) (yellow bars), and severe water stress (WS-S) (red bars) treatments. Different lowercase letters above the bars indicate significant differences between treatments for each cultivar, according to the Student-Newman-Keuls multiple range test ($p < 0.05$).