

## Growth and antioxidant responses triggered by water stress in wild relatives of eggplant

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### ABSTRACT

Wild relatives of eggplant (*Solanum melongena*) are of interest for breeding for tolerance to drought. To assess the potential of eggplant wild relatives from different gene pools, 18 accessions belonging to eggplant and eight wild relatives were evaluated for water stress tolerance. Plants grown in pots were normally irrigated or subjected to water stress by stopping irrigation. Growth and biochemical parameters related to oxidative stress, including proline, malondialdehyde (MDA), total phenolics and total flavonoids contents, and catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) activities, were determined after 11 days of treatment. The reduction of dry matter content in the aerial part of the water-stressed vs. control plants allowed classifying the accessions into three groups: tolerant (< 25% reduction), intermediate (25–35% reduction), or susceptible (> 35% reduction). Proline concentration increased in all accessions under water stress, in particular in the more tolerant ones, which showed an average increase of more than 30-fold over control values, compared to ca. 8-fold in the susceptible accessions. The group of tolerant accessions, which included *S. incanum*, *S. pyracanthos*, *S. dasyphyllum* and *S. torvum*, was also characterised by unchanged MDA contents and a more pronounced increase in the mean levels of flavonoids (20.6% over the non-stressed controls vs. 3.4% in the intermediate accessions and 5.0% in the least tolerant ones). The activity of antioxidant enzymes was extremely variable within groups and even within the same species. The results obtained reveal a high diversity for drought tolerance in the wild relatives of eggplant and provide insights into the biochemical mechanisms involved in the response to drought in eggplant wild relatives. The tolerant materials identified are of interest for breeding programmes for developing rootstocks and new eggplant cultivars with higher drought tolerance.

### 1. Introduction

The genus *Solanum* is one of the most biodiverse within angiosperms, comprising around 1400 species distributed throughout all continents, except Antarctica, and adapted to a large variety of ecological conditions in different habitat types (Knapp et al., 2013). Eggplant (*Solanum melongena* L.) is the most important *Solanum* crop native to the Old World, being at present the sixth most cultivated vegetable crop in the world (FAO, 2019). Eggplant can tolerate mild water stress (Diaz-Perez and Eaton, 2015; Sarker et al., 2004), and is considered a relatively

drought-tolerant crop (Behboudian, 1977). However, drought, already one of the most restrictive environmental factors for crops in many world areas, will shortly affect more regions; it is expected that severe drought episodes will be more frequent and last longer than at present (IPPC, 2014). Therefore, drought represents a major threat to eggplant cultivation, decreasing its yield and quality (Badr et al., 2020; Delfin et al., 2013; Karam et al., 2011; Kouassi et al., 2021; Lovelli et al., 2007).

Improving stress tolerance of cultivated eggplant by hybridisation with its wild relatives is gaining interest in breeding programmes (Afful et al., 2018; Gramazio et al., 2017; Kouassi et al., 2021; Plazas et al.,

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2016). *Solanum melongena* is included in subgenus *Leptostemonum* Bitter, the largest of the 13 major *Solanum* clades, with ca. 450 species distributed worldwide (Knapp et al., 2013). Cultivated eggplant has limited genetic diversity, and this can be expanded by hybridisation with wild relatives, which represent an important source of variation (Daunay and Hazra, 2012; Ghani et al., 2020; Gramazio et al., 2019; Plazas et al., 2016; Rakha et al., 2020). Tolerance to abiotic and biotic stresses has been reported among eggplant wild relatives, and some of them are used for eggplant grafting (Gisbert et al., 2011; Khah et al., 2011; Rakha et al., 2020; Sabatino et al., 2018). Also, interspecific hybrids such as, for example, *S. melongena* x *S. aethiopicum* represent alternative rootstocks that may enhance eggplant performance (Sabatino et al., 2019). These related species also represent a valuable tool for improving drought tolerance in *S. melongena*, as many of them thrive in environments with harsh climatic conditions, even in semi-desertic or desertic areas (Davidar et al., 2015; Kaushik et al., 2016; Knapp et al., 2013; Vorontsova and Knapp, 2016). According to their phylogenetic proximity and their ability to interbreed with eggplant, wild relatives of eggplant are classified into primary, secondary and tertiary gene pools (Harlan and de Wet, 1971). The only primary gene pool species is *S. insanum* L., the wild ancestor of eggplant (Syfert et al., 2016), which is fully inter-fertile with *S. melongena*, producing fertile hybrids (Davidar et al., 2015; Knapp et al., 2013). *Solanum insanum* is an annual or perennial weed distributed in Southeast Asia, Madagascar and Mauritius (Knapp et al., 2013; Mutegi et al., 2015; Ranil et al., 2017). This species is a good candidate for improving abiotic stress tolerance in eggplant as it has been reported to grow in dry areas (Ranil et al., 2017) and displays tolerance to salinity (Brenes et al., 2020a).

The secondary gene pool of eggplant is constituted by several African and Southeast Asian "prickly" species (Vorontsova et al., 2013; Vorontsova and Knapp, 2016; Weese and Bohs, 2010), with different degrees of crossability with the cultivated species (Daunay and Hazra, 2012; Plazas et al., 2016; Rotino et al., 2014). Species belonging to the secondary gene pool are genetically very diverse, and some of them are resistant to pests and pathogens and unfavorable climatic conditions, such as drought (Kouassi et al., 2016; Plazas et al., 2016). *Solanum campylacanthum* Hochst. ex A. Rich., *S. incanum* L. and *S. linnaeanum* Hepper & P.-M.L. Jaeger are closely related to cultivated eggplant (Acquadro et al., 2017) and are of interest for eggplant breeding for tolerance to abiotic stresses. *Solanum campylacanthum* is an evergreen herbaceous shrub, common in disturbed habitats between sea level and 2000 m altitude (Vorontsova and Knapp, 2016). *Solanum incanum* is tolerant to biotic and abiotic stresses, growing in desertic areas (Knapp et al., 2013; Gramazio et al., 2017), and its vigorous hybrids with *S. melongena* have been found to be suitable rootstocks for eggplant (Gisbert et al., 2011). Whole-genome resequencing using seven eggplant and one *S. incanum* accessions identified potential footprints of ancient introgressions from *S. incanum* into eggplant materials (Gramazio et al., 2019). *Solanum linnaeanum* is a shrub growing mainly on sandy soils, native to southern Africa and naturalised in North Africa and southern Europe and other continents. In the Iberian Peninsula, it is frequent in coastal and warm ruderal environments, ditches, wastelands, peri-urban areas, and beaches (Sobrinho Vesperinas and Sanz Elorza, 2012). Apart from these three species, *S. anguivi* Lam. and *S. dasyphyllum* Schumacher and Thonn., the wild ancestors of cultivated scarlet (*Solanum aethiopicum* L.) and gboma (*Solanum macrocarpon* L.) eggplants (Plazas et al., 2014), belong to the sister "anguivi clade" and can be crossed with eggplant (Rotino et al., 2014), being also of interest for eggplant breeding. The more phylogenetically distant *S. pyracanthos* Lam. (Knapp et al., 2019; Särkinen et al., 2013) is also part of the eggplant secondary gene pool, grouped in the "Madagascar clade" (Vorontsova et al., 2013), and hybrids with eggplant have been obtained (Plazas et al., 2016). The species is native to southern Madagascar, where it grows in dry and often disturbed habitats (Vorontsova and Knapp, 2016). It is also cultivated as an ornamental, known as porcupine tomato, and it has become invasive in some world areas.

The most phylogenetically distant species of the subgenus *Leptostemonum*, including Old and New World species, are grouped within the tertiary eggplant gene pool. Their crossability with cultivated species is challenging, producing only sterile or low-fertility hybrids after embryo rescue or somatic hybridisation (Daunay and Hazra, 2012; Kouassi et al., 2016; Plazas et al., 2016; Rakha et al., 2020; Rotino et al., 2014). However, resistance to biotic (Bletsos et al., 2003; Gousset et al., 2005; Öçal et al., 2018) or abiotic (Brenes et al., 2020b) stresses makes these species important candidates for eggplant breeding. Some of them, such as *S. torvum* Sw. and *S. sisymbriifolium* Lam., are of great interest. *Solanum torvum* is native to southern Mexico and northern South America but has a secondary distribution in Africa, Asia and Australia, where it behaves as an invasive weed (Witt and Luke, 2017). It grows in wet shrublands, dry shrub plains, forest clearings and rocky slopes (Gentry Standley, 1974). Apart from being used as an eggplant and tomato rootstock (Petran and Hoover, 2014; Sabatino et al., 2018, 2019), it has been reported as tolerant to salinity (Brenes et al., 2020b). *Solanum sisymbriifolium* is native to warm temperate South America, widely cultivated as an ornamental and present as a weed in many countries, occurring in agricultural areas, including irrigated crops and pastures, in urban and semi-urban areas (Lanza et al., 1995); it is tolerant to several eggplant pests and pathogens (Perpétuo et al., 2021; Taher et al., 2020).

Besides its deleterious effects on plants by disturbing the osmotic balance, and inhibiting photosynthesis and numerous metabolic reactions, drought, as other types of stress, is associated with increased production of reactive oxygen species (ROS) (Apel and Hirt, 2004; Das and Roychoudhury, 2014; Dumanović et al., 2021). ROS are generated in the normal cell metabolism during photosynthesis and respiration (Rodrigo-Moreno et al., 2013; Sharma et al., 2012). In small amounts, ROS play multiple functions as signalling molecules involved in many normal physiological processes (Apel and Hirt, 2004; Foyer and Noctor, 2005; Mhamdi and Van Breusegem, 2018; Mittler et al., 2004; Miller et al., 2008; Waszczak et al., 2018). Under stressful conditions, ROS production increases and, when not counterbalanced by defence mechanisms, they accumulate in excess activating proteases and endonucleases (Shabala, 2009), affecting cell membrane integrity by the oxidation of unsaturated fatty acids in lipids, inhibiting enzyme activities and the function of the photosynthetic apparatus, altering the structure of DNA, and finally inducing cell death (Das and Roychoudhury, 2014; Sharma et al., 2012). Plants possess different mechanisms for limiting ROS accumulation, which can be classified as non-enzymatic and enzymatic antioxidant systems. To the first category belong a large variety of antioxidant compounds, such as phenolics, especially the subgroup of flavonoids, ascorbic acid, or glutathione; to the second, different enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) (and other peroxidases), glutathione reductase (GR), among many others (Dumanović et al., 2021; Ozgur et al., 2013).

The health benefits related to the high content of antioxidants in eggplants are well-known (Gürbüz et al., 2018; Singh et al., 2009), but few works have analysed the activation of antioxidant mechanisms under drought conditions. In a previous study (Plazas et al., 2019), we reported high proline accumulation in four cultivars of *S. melongena* subjected to water stress, whereas MDA, total phenolics and flavonoids increased only in some genotypes. Of the antioxidant enzymes, only CAT increased in all cultivars. Kiran et al. (2019a) compared salt-tolerant and salt-sensitive eggplant genotypes exposed to drought and found lower levels of MDA and higher activity of SOD, CAT, APX and GR in the tolerant ones. A similar study comparing drought and salinity susceptible genotypes grafted on a salt-tolerant genotype and subjected to water and salt stress resulted in increased antioxidant enzyme activities and lower lipid peroxidation levels in the grafted plants (Kiran et al., 2019b). In another study, Zhou et al. (2019) analysed the expression of SmCSD1 (encoding a copper/zinc superoxide dismutase) and the activities of SOD and Cu/ZnSOD in eggplant under treatment with ABA and different stresses. They found that SmCSD1 was a stress-responsive

gene, especially to salinity and drought, in this species. Finally, Moglia et al. (2019) identified six cytosine-5 DNA methyltransferases (C5-MTases) and five DNA demethylases in eggplant, and differential transcript abundance of those genes under salinity and drought stress. These studies reveal an important impact of drought stress in the plants' gene expression patterns and biochemical responses. Very few studies have analysed the variation of antioxidant levels in eggplant relatives subjected to drought, and none of them targeted wild species. For example, Mibei et al. (2016) reported a general reduction of carotenoids in the cultivated scarlet eggplant (*S. aethiopicum*) and gboma eggplant (*S. macrocarpon*). In the same species, proline, glutamate, sucrose, fructose and metabolites of the tricarboxylic acid cycle were positively correlated with imposed drought stress (Mibei et al., 2018).

Given the interest and potential of eggplant wild relatives for improving tolerance to drought in eggplant, this work aimed to assess the tolerance to water stress in eight wild relatives of eggplant (*S. anguivi*, *S. campylacanthum*, *S. dasyphyllum*, *S. incanum*, *S. insanum*, *S. linnaeanum*, *S. pyracanthos*, *S. sisymbriifolium*, and *S. torvum*) in comparison with cultivated eggplant. A detailed analysis of growth responses, biochemical changes and activation of the antioxidant machinery under stress has been performed to select drought-tolerant materials and to provide insight into the mechanisms of drought tolerance in eggplant wild relatives. The results will be of interest for the utilisation of eggplant wild relatives in breeding eggplant rootstocks and cultivars with increased tolerance to drought.

## 2. Material and methods

### 2.1. Plant material and experimental design

The material evaluated includes 18 accessions, of which two are from cultivated eggplant *S. melongena* (ANS26 and MEL2) and 16 from eight wild species of eggplant relatives from the primary, secondary and tertiary gene pools. Data on the materials used are summarised in Table 1. All materials are stored at the germplasm bank of Universitat Politècnica de València.

Seeds of all accessions were germinated according to the protocol of Ranil et al. (2015). Once germinated in Petri dishes, seeds were transferred to trays containing Humin-substrat N3 (Klasmann-Deilmann, Germany) substrate in a climatic chamber with a 14 h light/10 h dark photoperiod and a 25 °C (light)/18 °C (dark) temperature regime, until plantlets reached a height of 6–8 cm; then, they were transferred to 1.3 L

individual (14.5 cm of external diameter and 12.5 cm of height) thermoformed pots (Projar, Quart de Poblet, Spain) filled with the same Humin-substrat N3 substrate. Individual pots were transferred to a greenhouse with controlled temperature (maximum of 30 °C and minimum of 15 °C) and irrigated every two days to maintain high moisture levels in the substrate, allowing free drainage to avoid the build-up of salts in the substrate. Stress treatments were started when young plants developed at least five true leaves (Fig. 1). Ten plants per accession with similar size, five for the control and five for the water stress treatment, were selected and randomly distributed in a greenhouse bench. Plants from control treatments were watered every two days with tap water (300 mL/pot), allowing free drainage of excess water, whereas, for the water stress treatment, irrigation was ceased entirely. Pots of both control and water stress treatments were elevated from the bench surface by placing an inverted Petri dish (9 cm in diameter) below each pot to allow drainage in control plants and avoid absorption of drainage water through the base of the pot in the stressed plants. Substrate humidity (% vol) was measured every other day throughout the treatments with a WET-2 Sensor (Delta-T Devices, Cambridge, UK). The treatments were extended for eleven days when plants were harvested before flowering. Roots and aerial parts (stems and leaves) were harvested separately from each plant.

### 2.2. Analysis of morphological traits

Non-destructive growth parameters such as stem length and the number of leaves were measured at the beginning and end of the treatments, whereas the length of roots and the fresh weight of roots, stems and leaves were measured only when plants were sampled after 11 days of treatment. Part of the fresh material was weighed (fresh weight; FW), dried for four days at 65 °C, until constant weight, and then weighed again, and the dry weight (DW) of roots (RDW) and aerial part (ADW) of the plants, including the dry weight of stems and leaves, was calculated. The water content (WC) percentage was calculated according to Gil et al. (2014), separately for roots, stems and leaves. All traits were measured in each of the five individual plants of each combination of variety × treatment.

### 2.3. Analysis of biochemical parameters

#### Proline, MDA, total phenolics and total flavonoids

All biochemical analyses were performed on fresh leaf material.

**Table 1**  
Origin and main characteristics of the eggplant and related wild accessions.

Accession	Species	Accession code in the germplasm collection	Origin	Fruit weight (g)	Prickles
Cultivated eggplant					
ANS26	<i>S. melongena</i>	ANS26	Spain	150–200	few
MEL2	<i>S. melongena</i>	BBS146	Ivory Coast	200–300	none
Primary gene pool					
INS1	<i>S. insanum</i>	SLKINS1	Sri Lanka	15–25	many
INS2	<i>S. insanum</i>	SLKINS2	Sri Lanka	20–30	none
Secondary gene pool					
ANG1	<i>S. anguivi</i>	BBS119	Ivory Coast	0.7–1.5	none
ANG2	<i>S. anguivi</i>	BBS125/B	Ivory Coast	1.5–2.0	none
CAM5	<i>S. campylacanthum</i>	MM680	Tanzania	5.0–7.5	none
CAM6	<i>S. campylacanthum</i>	MM700	Kenya	2.0–3.0	few
CAM8	<i>S. campylacanthum</i>	MM1426	Tanzania	4.0–6.0	several-many
DAS1	<i>S. dasyphyllum</i>	MM1153	Uganda	15–25	very many
INC1	<i>S. incanum</i>	MM664	Israel	10–13	few
MM577	<i>S. incanum</i>	MM577	Israel	8–12	few
LIN1	<i>S. linnaeanum</i>	JPT0028	Spain	10–15	many
LIN3	<i>S. linnaeanum</i>	MM195	Tunisia	15–25	many
PYR1	<i>S. pyracanthos</i>	SOLN66	USA	0.7–1.3	very many
Tertiary gene pool					
SIS1	<i>S. sisymbriifolium</i>	SOLN78	USA	2.5–4.0	very many
SIS2	<i>S. sisymbriifolium</i>	1180	India	2.0–3.5	very many
TOR1	<i>S. torvum</i>	B&T	France	1.0–1.5	few



**Fig. 1.** Plants at the beginning of the stress treatments. First line, left to right: *S. melongena* (MEL2), *S. insanum* (INS1), *S. insanum* (INS2), *S. anguivi* (ANG2); second line, left to right: *S. campylacanthum* (CAM5), *S. dasyphyllum* (DAS1), *S. incanum* (INC1); third line, left to right: *S. linnaeanum* (LIN1), *S. pyracanthos* (PYR1), *S. sisymbriifolium* (SIS2), *S. torvum* (TOR1).

Proline (Pro) was quantified according to the ninhydrin-acetic acid method (Bates et al., 1973). Extracts obtained by grinding 0.15 g of fresh material in 2 ml of sulphosalicylic acid (3%) solution were mixed with acid ninhydrin and incubated in a water bath at 95 °C for 1 h, subsequently cooled to room temperature and extracted with toluene. The absorbance was measured at 520 nm, using toluene as blank. The concentration of proline was expressed in  $\mu\text{mol g}^{-1}$  DW.

Malondialdehyde (MDA), total flavonoids (TF) and total phenolic compounds (TPC) were determined in 80% (v/v) methanol extracts of 0.1 g fresh leaves. For MDA quantification, extracts were mixed with 0.5% thiobarbituric acid (TBA), prepared in 20% TCA (or with 20% TCA without TBA for the controls). They were then incubated at 95 °C for 20 min following the protocol by Hodges et al. (1999). After stopping the reaction on ice, the supernatant's absorbance was measured at 532 nm. The non-specific absorbance at 600 and 440 nm was subtracted, and the MDA concentration was calculated with the equations described in Hodges et al. (1999) and expressed in  $\text{nmol g}^{-1}$  DW. TPC were measured by reaction with the Folin-Ciocalteu reagent as described in Blainski et al. (2013). The methanol extracts were mixed with the reagent and sodium carbonate and maintained in the dark. After 90 min, absorbance was read at 765 nm, and the results were calculated in equivalents of gallic acid, used as the standard ( $\text{mg eq GA g}^{-1}$  DW). TF concentrations were determined by nitration of catechol groups with  $\text{NaNO}_2$  and then reaction with  $\text{AlCl}_3$  at basic pH, following Zhishen et al. (1999). The absorbance of the samples was measured at 510 nm, and TF contents were expressed in 'equivalents of catechin', used as the standard ( $\text{mg eq C g}^{-1}$  DW).

#### 2.4. Activity of antioxidant enzymes

Crude protein extracts were prepared from 0.15 g fresh leaf tissue as described by Gil et al. (2014). Protein concentration in the extracts was determined by the method of Bradford (1976), using a commercial reagent (Bio-Rad Laboratories, Barcelona, Spain) and bovine serum albumin as the standard. Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) activities were measured in the protein extracts.

SOD activity in the protein extracts was determined according to Beyer and Fridovich (1987), by following spectrophotometrically (at 560 nm) the inhibition of nitro blue tetrazolium (NBT) photoreduction in reaction mixtures containing riboflavin as the source of superoxide radicals. One SOD unit was defined as the amount of enzyme causing 50% inhibition of NBT photoreduction. CAT activity was determined as described by Aebi (1984), following the decrease in absorbance at 240 nm, which accompanies the consumption of  $\text{H}_2\text{O}_2$  added to protein extracts. One CAT unit was defined as the amount of enzyme that will decompose 1  $\mu\text{mol}$  of  $\text{H}_2\text{O}_2$  per minute at 25 °C. APX activity was determined according to Nakano and Asada (1981) by measuring the decrease in absorbance at 290 nm as ascorbate becomes oxidised in the reaction. One APX unit was defined as the amount of enzyme required to consume one  $\mu\text{mol}$  of ascorbate per minute at 25 °C. GR activity was determined according to Connell and Mullet (1986), following the oxidation of NADPH [the cofactor in the GR-catalysed reduction of oxidised glutathione (GSSG)] by the decrease in absorbance at 340 nm. One GR unit was defined as the amount of enzyme that will oxidise 1  $\mu\text{mol}$  of NADPH per minute at 25 °C.

#### 2.5. Statistical analysis

Data were analysed using the program Statgraphics Centurion XVI (Statgraphics Technologies, The Plains, VA, USA). All mean values throughout the text are based on five biological replicates. A two-way ANOVA was performed considering as factors the treatment, the accession (genotype) and their interaction and post hoc comparisons were made using Tukey's HSD test at  $p < 0.05$ . A multivariate principal component analysis (PCA) was performed for standardised data of all traits using pairwise Euclidean distances among means for combinations of accession and treatment.

### 3. Results

#### 3.1. Substrate moisture

The initial moisture was similar in all pots, with values above 70%.

After the first two days of treatments, a drop to less than 50% moisture was registered in all pots; nevertheless, control pots recovered initial moisture values after watering. This pattern was repeated till the end of the experiment. On the contrary, moisture in the pots from the water stress treatment gradually decreased, reaching similar values, below 5%, in all accessions studied.

### 3.2. Morphological analysis

The two-way ANOVA (Table 2) revealed that treatment and accession effects and their interaction were significant for all morphological traits. The greatest contributor to the sums of squares for RWC, ADW, SFW, LFW, SWC and LWC was the treatment effect, whereas for RL, RDW, RFW, Lno, and SLI, it was the accession effect. The interaction effect was not the main contributor to the sums of squares for any of the traits, whereas the residual effect was the most significant contributor for SD.

The drought treatment, in general, induced a decrease in root parameters (Table 3). However, the reductions of RL and RDW with respect to the corresponding controls (average of 18.9% and 19.8%, respectively) were lower than those of RFW (69.8%) or RWC (41.4%). The response varied largely depending on the accession. Maximum reductions were observed in CAM8 for RWC (59.9%), SIS2 for RFW (89.3%) and RDW (66.5%), and INS1 for RL (47.7%), whereas INS2 did not show significant reductions for the root parameters evaluated (Table 3).

Plants under water stress had only 7.61 leaves (Lno) on average, whereas the control had 10.35. Again, there were differences between the genotypes: PYR1, DAS1, TOR3, ANG1, and SIS1 did not show significant differences as a consequence of the drought treatment, whereas MEL2 had 41.2% and INC1 41.6% fewer leaves under drought

**Table 2**

Two-way analysis of variance (ANOVA) of treatment, accession, and their interactions for the traits considered. Numbers represent percentages of the sum of squares.

Trait	Treatment (A)	Accession (B)	A x B	Residuals
<b>Morphological traits</b>				
Root length (RL)	14.87***	57.03***	10.64***	17.45
Root dry weight (RDW)	6.18***	62.61***	5.59*	25.59
Root fresh weight (RFW)	26.64***	49.67***	7.27***	16.39
Root water content (RWC)	74.09***	13.37***	6.99***	5.54
Leaf number (Lno)	22.80***	55.19***	5.42***	16.58
Stem length increase (SLI)	16.40***	63.34***	7.70***	12.54
Stem diameter (SD)	5.90**	22.94***	21.49***	49.87
Aerial dry weight (ADW)	40.87***	34.98***	5.38**	18.75
Stem fresh weight (SFW)	41.99***	30.35***	19.99***	7.48
Leaf fresh weight (LFW)	86.17***	3.94***	3.76***	6.12
Stem water content (SWC)	64.15***	26.26***	4.57***	5.00
Leaf water content (LWC)	73.68***	11.64***	8.29***	6.37
<b>Biochemical traits</b>				
Proline (Pro)	78.60***	10.00***	9.59***	1.81
Malondialdehyde (MDA)	4.97***	55.42***	3.66	35.93
Total phenolic content (TPC)	8.11***	71.57***	10.51***	9.79
Total flavonoids /TF)	0.97**	76.52***	8.53***	13.97
Superoxide dismutase (SOD)	0.91 <sup>ns</sup>	54.10***	15.00***	30.00
Catalase (CAT)	0.48 <sup>ns</sup>	57.25***	17.53***	24.72
Gluthation reductase (GR)	0.43 <sup>ns</sup>	60.60***	16.70***	22.20
Ascorbate peroxidase (APX)	0.93 <sup>ns</sup>	28.40***	17.20**	53.50

<sup>ns</sup>, \*, \*\*, \*\*\*, indicate non-significant, or significant at  $p < 0.05$ ,  $< 0.01$ , or  $< 0.001$ , respectively.

conditions. The stem growth arrested under drought conditions and plants elongated, except for INC1, which did not display significant differences with the control.

Plants subjected to drought also had lower stem and leaf fresh weights than under control conditions. This reduction in the fresh weight was highest for SFW in SIS2 (77.6%) and for LFW in LIN3 (83.3%), and lowest for INC1 with no significant SFW changes, and MM577, with a reduction of 55.1% for LFW. These weight changes were caused partly by reductions with respect to the corresponding controls in the water content of the stems and leaves, respectively. Interestingly, the genotypes with a lower reduction in SWC and LWC were MEL2 (15.5% and 19.6%, respectively) and SIS2 (16.7% and 18.2%, respectively), followed by TOR3 and DAS1. As expected, control plants could accumulate higher dry biomass than the water-stressed plants (8.3 vs. 5.6 g, on average). Nevertheless, several genotypes did not show significant differences in dry weight between treatments. This trait was used for ranking the cultivars regarding their susceptibility to water stress. According to the reduction of the aerial part dry weight (ADW) in the stressed plants in relation to their respective controls, three categories were established (Table 3 and Fig. 2): accessions that showed a reduction of less than 25% of their ADWs were considered as tolerant (MM577, PYR1, DAS1, INC1, TOR3), from 25 to 35% as intermediate (CAM8, ANG1, LIN3, LIN1, INS2, CAM5), and more than 35% as sensitive (CAM6, ANG2, SIS1, ANS26, INS1, MEL2, SIS2).

### 3.3. Biochemical analyses: proline, MDA, total phenolics and total flavonoids

The water stress treatment had a strong effect on proline (Pro) accumulation (Table 4). However, the effect of accession and its interaction with treatment were also significant (Table 2). Pro concentrations remarkably increased in all accessions, from 251.7% in ANG2 to over 9000% in PYR1 (Table 4). Values in control, non-stressed plants were low, below  $30 \mu\text{mol g}^{-1} \text{DW}$ , except for a few accessions, such as ANG2, ANS26 and SIS1, with values around  $100 \mu\text{mol g}^{-1} \text{DW}$ . The concentration of Pro in water-stressed plants ranged from  $170 \mu\text{mol g}^{-1} \text{DW}$  in DAS1 to approximately  $600 \mu\text{mol g}^{-1} \text{DW}$  in MM577 and ANG1. Pro-concentrations increased mostly in the more tolerant accessions, on average over 30-fold in water-stressed plants with respect to those in the control, whereas in the intermediate group, the increase was about 20-fold, and in the susceptible ones, around 8-fold. However, a clear relationship between Pro-concentrations and the degree of stress tolerance of the different genotypes could not be established, as marked differences were noticed not only within groups but also between accessions within species.

Although the two-way ANOVA showed a significant effect of both treatment and genotype (Table 2), leaf MDA contents displayed a significant change under water stress only in one *S. anguivi* accession (ANG1) and in the two accessions of *S. linnaeanum* (LIN1 and LIN3), all three belonging to the group with intermediate stress tolerance (Table 4). MDA values ranged from  $49.9 \text{ nmol g}^{-1} \text{DW}$  in DAS1 to  $143.6 \text{ nmol g}^{-1} \text{DW}$  in ANG2 in control plants and from  $58.9 \text{ nmol g}^{-1} \text{DW}$  in MM577 to  $169.8 \text{ nmol g}^{-1} \text{DW}$  in ANG1 in water-stressed plants (Table 4).

A significant effect of treatment, accession, and their interaction was detected for both total phenolic compounds and total flavonoids, with the accession effect contributing more to overall variation (Table 2). Total phenolic compounds (TPC) content increased significantly due to the drought treatment in all accessions grouped in the tolerant category, except in PYR1 (Table 4). In the intermediate group, TPC variation was significant only in CAM8, whereas in the group of sensitive accessions, high heterogeneity was observed, with significant increases in CAM6, INS1, INS2 and MEL2. Values in control ranged from the lowest, around  $11 \text{ mg eq GA g}^{-1} \text{DW}$  in the two accessions of *S. linnaeanum* (LIN1 and LIN3) to  $32.67 \text{ mg eq GA g}^{-1} \text{DW}$  in TOR 3, whereas in water-stressed plants, they varied from  $13.39 \text{ mg eq GA g}^{-1} \text{DW}$  in PYR1 to  $46.01$

**Table 3**

Effect of 11 days of water stress on growth parameters of cultivated eggplant *S. melongena* (ANS26 and MEL2), *S. anguivi* (ANG1 and ANG2), *S. campylacanthum* (CAM5; CAM6 and CAM8), *S. dasyphyllum* S (DAS1), 2 of *S. incanum* (INC1 and MM577), *S. insanum* (INS1 and INS2), *S. linnaeanum* (LIN1 and LIN3), *S. pyracanthos* (PYR1), *S. sisymbriifolium* (SIS1 and SIS2) and of *S. torvum* (TOR1). Values shown are the mean and percentage of change in the stress treatments (WS) in comparison to the control (C). The average standard error (SE; obtained from the ANOVA analyses) and least significant difference (LSD;  $p = 0.05$ ) for pairwise comparisons between averages of combinations of treatment  $\times$  accession, are provided. Asterisks and <sup>ns</sup> indicate, respectively, significant and non-significant differences at  $p < 0.05$  between values in C and WS ( $n = 5$ ). Abbreviations: root length (RL), root fresh weight (RFW), root dry weight (RDW), root water content (RWC), leaf number (L no), stem length increase (SLI), stem fresh weight (SFW), leaf fresh weight (LFW), stem water content (SWC), leaf water content (LWC), aerial dry weight (ADW, stem + leaves).

Accession	Treatment	RL (cm)	RDW (g)	RFW (g)	RWC (%)	Lno	SLI (cm)	SFW (g)	LFW (g)	SWC (%)	LWC (%)	ADW (g)
<b>Tolerant accessions</b>												
MM577	C	32.2	2.2	11.8	80.6	12.0	3.6	9.0	26.9	73.2	75.6	9.0
	WS	21.8	2.3	4.1	43.0	7.8	0.8	5.8	12.1	55.3	54.6	8.1
	Change (%)	-32.3*	4.5 <sup>ns</sup>	-65.2*	-46.7*	-35.0*	-77.8*	-35.6*	-55.0*	-24.5*	-27.8*	-10.0 <sup>ns</sup>
PYR1	C	33.0	2.8	19.5	85.9	7.8	14.4	12.1	20.1	78.3	79.6	6.7
	WS	26.6	2.1	5.1	56.5	7.2	2.5	4.91	7.5	51.8	51.9	5.9
	Change (%)	-19.4*	-25.0*	-73.8*	-34.2*	-7.7 <sup>ns</sup>	-82.6*	-59.4*	-62.7*	-33.8*	-34.8*	-11.9*
DAS1	C	40.4	4.5	23.4	80.7	7.2	1.4	6.4	26.9	77.9	82.5	6.1
	WS	36.2	4.6	8.8	64.4	6.0	0.2	3.64	10.7	53.4	64.4	5.1
	Change (%)	-10.4 <sup>ns</sup>	2.2 <sup>ns</sup>	-62.4*	-20.2*	-16.7 <sup>ns</sup>	-85.7*	-43.1*	-60.2*	-31.5*	-21.9*	-16.4 <sup>ns</sup>
INC1	C	20.4	1.4	9.9	85.9	7.2	1.0	4.7	20.3	73.5	80.5	5.3
	WS	21.2	1.9	3.9	51.1	4.2	1.0	3.4	6.3	54.4	56.6	4.3
	Change (%)	3.9 <sup>ns</sup>	35.7 <sup>ns</sup>	-60.6*	-40.5*	-41.7*	0.0 <sup>ns</sup>	-27.7 <sup>ns</sup>	-69.0*	-26.0*	-29.7*	-18.9 <sup>ns</sup>
TOR3	C	34.6	3.8	23.8	84.15	7.4	4.20	12.8	32.9	82.5	77.8	9.5
	WS	30.4	3.0	4.7	34.5	6.0	0.6	7.4	10.8	65.7	55.6	7.31
	Change (%)	-12.1 <sup>ns</sup>	-21.1 <sup>ns</sup>	-80.3*	-59.0*	-18.9 <sup>ns</sup>	-85.7*	-42.2*	-67.2*	-20.4*	-28.5*	-23.1 <sup>ns</sup>
<b>Mean</b>	C	<b>32.1</b>	<b>2.9</b>	<b>17.7</b>	<b>83.5</b>	<b>8.3</b>	<b>4.9</b>	<b>9.0</b>	<b>25.4</b>	<b>77.1</b>	<b>79.2</b>	<b>7.3</b>
	WS	<b>27.2</b>	<b>2.8</b>	<b>5.3</b>	<b>49.9</b>	<b>6.2</b>	<b>1.0</b>	<b>5.0</b>	<b>9.5</b>	<b>56.1</b>	<b>56.6</b>	<b>6.1</b>
	Change (%)	<b>-15.2</b>	<b>-5.4</b>	<b>-69.9</b>	<b>-40.2</b>	<b>-25.0</b>	<b>-79.2</b>	<b>-44.1</b>	<b>-62.7</b>	<b>-27.2</b>	<b>-28.5</b>	<b>-16.1</b>
<b>Intermediate accessions</b>												
CAM8	C	19.4	2.3	13.3	82.3	11.0	11.8	7.9	30.8	78.9	80.0	8.4
	WS	17.6	1.7	2.6	33.0	7.8	2.4	3.4	5.6	39.0	28.7	5.9
	Change (%)	-9.3 <sup>ns</sup>	-26.1 <sup>ns</sup>	-80.5*	-59.9*	-29.1*	-79.7*	-57.0*	-81.8*	-50.6*	-64.1*	-29.8*
ANG1	C	19.0	1.6	8.4	80.9	11.0	11.7	13.9	30.3	82.2	82.3	7.7
	WS	13.6	1.0	1.7	39.5	10.0	2.8	4.0	5.9	55.4	35.2	5.5
	Change (%)	-28.4*	-37.5 <sup>ns</sup>	-79.8*	-51.2*	-9.1 <sup>ns</sup>	-76.1*	-71.2*	-80.5*	-32.6*	-57.2*	-28.6*
LIN3	C	29.0	2.6	10.3	80.9	12.0	10.0	11.6	28.2	78.6	82.7	7.3
	WS	19.6	1.7	2.8	37.8	8.0	3.8	5.6	4.7	48.9	49.9	5.2
	Change (%)	-32.4*	-34.6 <sup>ns</sup>	-72.8*	-53.3*	-33.3*	-62.0*	-51.7*	-83.3*	-37.8*	-39.7*	-28.8*
LIN1	C	23.3	3.1	12.5	75.3	11.6	8.4	13.0	30.2	79.8	80.8	8.37
	WS	16.6	2.0	3.1	34.5	9.4	3.6	4.1	7.2	50.5	50.0	5.6
	Change (%)	-28.8*	-35.5*	-75.2*	-54.2*	-19.0*	-57.1*	-68.5*	-76.2*	-36.7*	-38.1*	-33.1*
CAM5	C	31.8	2.6	9.5	71.8	15.6	16.0	8.7	27.9	70.3	74.5	9.7
	WS	26	2.2	3.8	41.0	10.0	6.0	3.6	7.1	48.9	39.2	6.4
	Change (%)	-18.2*	-15.4 <sup>ns</sup>	-60.0*	-42.9*	-35.9*	-62.5*	-58.6*	-74.6*	-30.4*	-47.4*	-34.0*
<b>Mean</b>	C	<b>24.5</b>	<b>2.4</b>	<b>10.8</b>	<b>78.2</b>	<b>12.2</b>	<b>11.6</b>	<b>11.0</b>	<b>29.5</b>	<b>78.0</b>	<b>90.1</b>	<b>8.3</b>
	WS	<b>18.7</b>	<b>1.7</b>	<b>2.8</b>	<b>37.2</b>	<b>9.0</b>	<b>3.7</b>	<b>4.1</b>	<b>6.1</b>	<b>48.5</b>	<b>40.6</b>	<b>5.7</b>
	Change (%)	<b>-23.6</b>	<b>-29.5</b>	<b>-74.1</b>	<b>-52.5</b>	<b>-26.1</b>	<b>-67.9</b>	<b>-62.4</b>	<b>-79.3</b>	<b>-37.7</b>	<b>-49.3</b>	<b>-31.0</b>
<b>Susceptible accessions</b>												
CAM6	C	27.2	2.41	10.3	76.3	13.6	9.4	8.8	28.3	68.9	77.0	9.8
	WS	18.6	2.0	3.2	37.5	10.6	1.8	4.6	8.9	49.5	52.9	6.3
	Change (%)	-31.6*	-17.0 <sup>ns</sup>	-68.9*	-50.9*	-22.1*	-80.9*	-47.7*	-68.6*	-28.2*	-31.3*	-35.7*
ANG2	C	37.0	1.8	11.1	83.2	13.4	10.2	13.7	29.4	85.1	82.5	7.1
	WS	26.6	1.1	1.86	39.0	10.2	2.4	3.4	5.8	56.6	47.2	4.5
	Change (%)	-28.1*	-38.9*	-83.2*	-53.1*	-23.9*	-76.5*	-75.2*	-80.3*	-33.5*	-42.8*	36.6*
SIS1	C	37.0	1.8	8.9	80.0	11.0	18.6	27.0	31.9	89.0	83.9	8.4
	WS	28.8	1.0	1.7	42.4	8.6	6.1	6.6	7.2	65.8	55.4	5.3
	Change (%)	-22.2*	-44.4*	-80.9*	-47.0*	-21.8 <sup>ns</sup>	-67.2*	-75.6*	-77.4*	-26.1*	-34.0*	-36.9*
INS2	C	27.4	5.0	34.4	85.4	8.6	8.1	11.9	30.9	79.1	73.3	11.2
	WS	26.8	4.6	30.4	84.7	7.0	2.6	4.9	8.5	50.3	47.9	6.9
	Change (%)	-2.2 <sup>ns</sup>	-8 <sup>ns</sup>	-11.6 <sup>ns</sup>	-0.8 <sup>ns</sup>	-18.6*	-67.9*	-58.3*	-72.5*	-36.4*	-34.7*	-38.4*
ANS26	C	33.9	1.7	11.0	87.5	7.4	6.6	16.9	34.13	88.1	85.0	7.1
	WS	23.80	1.6	1.8	43.0	5.2	1.10	3.78	6.33	61.67	54.5	4.33
	Change (%)	-29.8*	-5.9 <sup>ns</sup>	-83.6*	-50.9*	-29.7*	-83.3*	-77.6*	-81.4*	-30.0*	-35.9*	-39.0*
INS1	C	31.4	2.8	11.9	75.8	11.8	11.00	13.7	28.8	78.9	78.9	9.0
	WS	16.4	1.6	2.6	35.5	7.6	3.0	3.1	5.8	55.3	38.5	5.1
	Change (%)	-47.8*	-42.9*	-78.2*	-53.2*	-35.6*	-72.7*	-77.4*	-79.9*	-29.9*	-51.2*	-43.3*
MEL2	C	21.5	5.6	34.4	84.3	8.5	1.75	8.0	31.1	79.0	83.9	8.6
	WS	30.2	3.1	8.7	61.3	5.0	0.7	4.5	11.1	66.7	67.4	4.6
	Change (%)	40.5*	-44.6*	-74.7*	-27.3*	-41.2*	-60.0*	-43.8*	-64.4*	-15.6*	-19.7*	-46.5*
SIS2	C	43.2	2.4	16.7	85.3	9.2	25.2	29.3	37.3	88.59	82.2	10.0
	WS	30.6	0.8	1.8	55.6	6.0	6.4	6.6	7.9	73.7	67.2	4.3
	Change (%)	-29.2*	-66.7*	-89.2*	-34.8*	-34.8*	-74.6*	-77.5*	-78.8*	-16.8*	-18.3*	-57.0*
<b>Mean</b>	C	<b>32.3</b>	<b>2.9</b>	<b>17.3</b>	<b>82.2</b>	<b>10.4</b>	<b>11.3</b>	<b>16.2</b>	<b>31.5</b>	<b>82.1</b>	<b>80.8</b>	<b>8.9</b>
	WS	<b>25.2</b>	<b>2.0</b>	<b>6.5</b>	<b>50.0</b>	<b>7.5</b>	<b>3.0</b>	<b>4.7</b>	<b>7.7</b>	<b>59.9</b>	<b>53.9</b>	<b>5.2</b>
	Change (%)	<b>-22.0</b>	<b>-32.8</b>	<b>-62.5</b>	<b>-39.3</b>	<b>-27.9</b>	<b>-73.5</b>	<b>-71.0</b>	<b>-75.6</b>	<b>-27.0</b>	<b>-33.3</b>	<b>-42.0</b>
SE		1.65	0.3	2.0	2.45	0.6	1.6	0.9	1.4	1.6	2.1	0.4
LSD ( $p = 0.05$ )		4.63	0.9	5.6	6.8	1.6	4.4	2.4	4.0	4.5	5.9	1.2

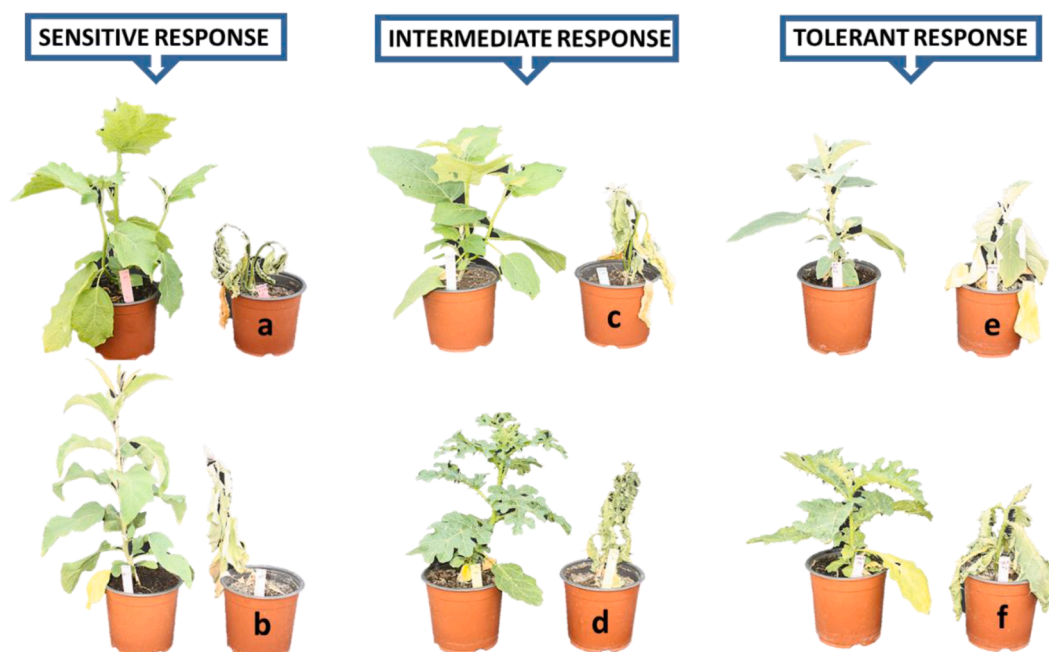


Fig. 2. Accessions susceptible (a: *S. anguivi* ANG2, b: *S. campylacanthum* CAM6), intermediate (c: *S. anguivi* ANG1, d: *S. linnaeanum* LIN1) and tolerant (e: *S. incanum* MM577, f: *S. dasyphyllum* DAS1) to drought after 11 days of ceasing irrigation.

mg eq GA g<sup>-1</sup> DW in TOR3 (Table 3). Total flavonoids contents were characteristic of each accession, ranging from the lowest values in *S. pyracanthos* (PYR 1), both in control (4.47 mg eq C g<sup>-1</sup> DW) and stressed plants (4.42 mg eq C g<sup>-1</sup> DW), to the highest values in *S. torvum* (TOR 3) in control (14.72 mg eq C g<sup>-1</sup> DW) and stressed plants (17.49 mg eq C g<sup>-1</sup> DW). Total flavonoids contents changed from control to water stress condition differently, depending on the accessions; some of them increased flavonoids levels significantly under stress, such as MM577, INC1, TOR3, ANG1, CAM6, INS2 and MEL2, whereas they decreased in others, such as CAM5 and SIS1. The tolerant genotypes under stress had a higher mean increase over the control in TPC (35%) and TF (20.6%) than the intermediate (14.4% and 3.3% respectively) and susceptible genotypes (19.2%, and 5%).

The ANOVA for the activity of antioxidant enzymes only showed significant effects for accession and interaction but not for the treatment (Table 2). Significant differences between the control and the water stress treatments were detected in only a few accessions (Table 4). None of the four enzymes analysed increased its activity in all accessions due to water stress. The variation was very high even between cultivars of the same species (Table 4).

### 3.4. Multivariate analysis

The principal component analysis (PCA), performed using the means of all traits measured in control and water stress treatments of the 18 accessions, resulted in six components with an eigenvalue greater than 1, which explained 85.1% of the total variation. The first component, which accounted for 37.6% of the variation, was positively correlated with growth parameters of stem and leaves and the length of the roots and negatively with Pro-concentrations (Fig. 3). The second component, accounting for an additional 15.7% of the variation, was positively correlated with total phenolics and flavonoids and the activity of GR and SOD, and negatively with the number of leaves and stem elongation. The PCA scatterplot revealed a clear separation of the treatments along the first axis, with control on the left and water stress on the right. In contrast, the accessions were primarily separated along with the second component (Fig. 3). In this way, in both the control and water-stress treatments, accessions TOR3, MEL2, INS2, ANS26, DAS1, and INC1

displayed positive values for the second PC, whereas SIS1, SIS2, PYR1, LIN1, LIN3, INS1, ANG1, ANG2 displayed negative values. No clear pattern of clustering of the three classes of tolerance could be observed in the PCA plot, with accessions of each group being intermingled with those of the other treatments (Fig. 3).

### 4. Discussion

Improving crop adaptation to drought through interspecific hybridisation and breeding with wild relatives can result in new cultivars with better resilience and adapted to the most important stresses associated with climate change (Gramazio et al., 2018; Prohens et al., 2017). In eggplant, being a vegetable crop primarily grown in tropical and subtropical regions, which are expected to suffer from increased drought stress (Sheldon, 2019), the development of drought-tolerant accessions is a major breeding objective (Kouassi et al., 2021). The analysis of the growth parameters indicated a large variability of responses to water stress in the eggplant wild relatives evaluated, matching its high genetic diversity (Acquadro et al., 2017; Vorontsova et al., 2013). Three groups could be established based on the reduction of the aerial (stems and leaves) dry weight of water-stressed plants in relation to their respective controls. The complete ceasing of irrigation even for only 11 days had a significant deleterious effect on the growth of eggplant wild relatives. Although eggplant is considered more tolerant to water stress than other vegetable crops (Sarker et al., 2004; Diaz-Perez and Eaton, 2015), this study proves that compared to many of its wild relatives, the accessions of *S. melongena* (ANS26 and MEL2) were sensitive to drought. At the end of the treatments, water-stressed plants of the two accessions, as of other accessions included in the most sensitive group, showed intense wilting and dehydration. Most of the morphological traits measured in plants ranked in this category showed a significant reduction compared to their respective controls. In the same group of sensitive accessions, the two accessions of *S. insanum*, considered as the eggplant wild progenitor (Rani et al., 2017), and the two accessions of *S. sylimbrifolium* (SIS1 and SIS2), were included. One of *S. anguivi* (ANG2) accessions was categorised as sensitive to drought, whereas the other accession (ANG1) was ranked in the intermediate group. Another wild species that displayed only moderate tolerance to water stress was *S. campylacanthum*, a

**Table 4**

Effect of 11 days of water stress on growth parameters of cultivated eggplant *S. melongena* (ANS26 and MEL2), *S. anguivi* (ANG1 and ANG2), *S. campylacanthum* (CAM5; CAM6 and CAM8), *S. dasyphyllum* S (DAS1), 2 of *S. incanum* (INC1 and MM577), *S. insanum* (INS1 and INS2), *S. linnaeanum* (LIN1 and LIN3), *S. pyracanthos* (PYR1), *S. sisymbriifolium* (SIS1 and SIS2) and of *S. torvum* (TOR1). Values shown are the mean and percentage of variation in the stress treatments (WS) in comparison to control (C). The average standard error (SE; obtained from the ANOVA analyses) and least significant difference (LSD;  $p = 0.05$ ) for pairwise comparisons between averages of combinations of treatment  $\times$  accession, are provided. Asterisks and <sup>ns</sup> indicate, respectively, significant and non-significant differences at  $p < 0.05$  between values in C and WS ( $n = 5$ ). Abbreviations: proline (Pro), malondialdehyde (MDA), total phenolic compounds (TFC), total flavonoids (TF), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR).

Accession	Treatment	Pro ( $\mu\text{mol g}^{-1}\text{DW}$ )	MDA (nmol $\text{g}^{-1}\text{DW}$ )	TPC (mg eq. GA $\text{g}^{-1}\text{DW}$ )	TF (mg eq. C $\text{g}^{-1}\text{DW}$ )	SOD activity (U. $\text{g}^{-1}$ protein)	CAT activity (U. $\text{g}^{-1}$ protein)	APX activity (U. $\text{g}^{-1}$ protein)	GR activity (U. $\text{g}^{-1}$ protein)
<i>Tolerant accessions</i>									
MM577	C	7.3	51.9	16.9	7.1	140.6	35.4	166.0	206.9
	WS	583.2	58.9	22.1	9.1	147.0	43.1	166.6	458.5
	Change (%)	7889.0*	13.5 <sup>ns</sup>	30.8*	28.2*	4.6 <sup>ns</sup>	21.8 <sup>ns</sup>	0.4 <sup>ns</sup>	121.6*
PYR1	C	4.4	69.7	13.3	4.5	51.1	18.3	469	308.4
	WS	421.0	74.7	13.4	4.4	40.5	19.3	82.9	744.6
	Change (%)	9468.2*	7.2 <sup>ns</sup>	0.8 <sup>ns</sup>	-2.2 <sup>ns</sup>	-20.8 <sup>ns</sup>	5.1 <sup>ns</sup>	-82.3 <sup>ns</sup>	141.4*
DAS1	C	13.5	49.9	15.2	6.1	121.5	49.2	227.5	711.3
	WS	170.8	69.2	19.6	7.6	64.5	15.3	137.3	346.1
	Change (%)	1165.2*	38.7 <sup>ns</sup>	28.9*	24.6 <sup>ns</sup>	-46.9 <sup>ns</sup>	-68.9 <sup>ns</sup>	-39.6 <sup>ns</sup>	-51.3 <sup>ns</sup>
INC1	C	15.1	63.6	19.9	8.8	29.8	38.3	351.7	2443.5
	WS	231.5	63.3	31.2	11.1	69.5	171.6	279.8	2275.9
	Change (%)	1433.1*	-0.5 <sup>ns</sup>	56.8*	26.1*	133.2 <sup>ns</sup>	348.0*	-20.4 <sup>ns</sup>	-6.9 <sup>ns</sup>
TOR3	C	11.5	72.8	32.7	14.7	157.9	38.0	442.7	1218.6
	WS	236.5	94.4	46.0	17.5	16.7	21.2	595.2	458.7
	Change (%)	1956.5*	29.7 <sup>ns</sup>	40.7*	19.0*	-89.4 <sup>ns</sup>	-44.2*	34.4 <sup>ns</sup>	-62.4*
<b>Mean</b>	C	<b>10.4</b>	<b>61.6</b>	<b>19.62</b>	<b>8.24</b>	<b>100.2</b>	<b>35.8</b>	<b>331.4</b>	<b>977.7</b>
	WS	<b>328.6</b>	<b>72.1</b>	<b>26.46</b>	<b>9.94</b>	<b>67.6</b>	<b>54.1</b>	<b>252.4</b>	<b>856.8</b>
	Change (%)	<b>3071.8</b>	<b>17.1</b>	<b>35</b>	<b>20.6</b>	<b>-32.5</b>	<b>50.9</b>	<b>-23.8</b>	<b>-12.4</b>
<i>Intermediate accessions</i>									
CAM8	C	19.9	104.3	16.3	7.8	117.9	147.5	175.2	882.2
	WS	404.5	128.1	20.1	10.1	296.8	162.3	381.8	1586.5
	Change (%)	1932.7*	22.8 <sup>ns</sup>	23.3*	29.5*	151.7*	10.0 <sup>ns</sup>	117.9 <sup>ns</sup>	79.8*
ANG1	C	25.6	118.1	16.9	7.6	92.2	31.8	241.8	1004.8
	WS	603.7	169.8	17.4	7.8	23.4	30.8	709.4	202.9
	Change (%)	2258.2*	43.8*	3.0 <sup>ns</sup>	2.7 <sup>ns</sup>	-74.6 <sup>ns</sup>	-3.1 <sup>ns</sup>	193.4*	-79.8*
LIN3	C	21.1	70.4	11.5	5.6	44.9	8.0	389.3	1370.8
	WS	440.6	104.8	13.6	5.9	72.3	5.1	134.8	463.3
	Change (%)	1988.2*	48.9*	18.3 <sup>ns</sup>	5.4 <sup>ns</sup>	61.0 <sup>ns</sup>	-36.3 <sup>ns</sup>	-65.4*	-66.2*
LIN1	C	19.2	59.0	11.3	4.8	59.2	46.2	401.9	499.3
	WS	470.1	91.1	13.6	5.3	78.5	6.29	245.6	463.3
	Change (%)	2348.5*	54.5*	20.4 <sup>ns</sup>	10.4 <sup>ns</sup>	32.6 <sup>ns</sup>	-86.4*	-38.9 <sup>ns</sup>	-7.2 <sup>ns</sup>
CAM5	C	17.1	131.9	19.7	12.9	144.8	40.6	342.5	794.9
	WS	277.0	142.0	21.9	10.9	153.2	56.6	420.0	836.9
	Change (%)	1519.9*	7.7 <sup>ns</sup>	11.2 <sup>ns</sup>	-15.5*	5.8*	39.4 <sup>ns</sup>	22.6 <sup>ns</sup>	5.3 <sup>ns</sup>
<b>Mean</b>	C	<b>20.6</b>	<b>96.7</b>	<b>15.1</b>	<b>7.7</b>	<b>91.8</b>	<b>54.8</b>	<b>310.1</b>	<b>910.4</b>
	WS	<b>439.2</b>	<b>127.2</b>	<b>17.3</b>	<b>8</b>	<b>124.8</b>	<b>52.2</b>	<b>378.3</b>	<b>710.6</b>
	Change (%)	<b>2034.0</b>	<b>31.4</b>	<b>14.4</b>	<b>3.4</b>	<b>36.0</b>	<b>-4.7</b>	<b>22.0</b>	<b>-22.0</b>
<i>Susceptible accessions</i>									
CAM6	C	10.6	75.2	15.9	7.8	267.3	33.6	392.9	581.1
	WS	277.0	95.6	23.1	10.1	282.8	131.1	280.1	923.2
	Change (%)	2513.2*	27.1 <sup>ns</sup>	45.3*	29.5*	5.8 <sup>ns</sup>	290.2*	-28.7 <sup>ns</sup>	58.9*
ANG2	C	109.7	139.6	20.0	10.1	69.6	19.2	225.0	1185.1
	WS	385.8	143.5	21.6	8.7	39.5	25.1	594.7	314.7
	Change (%)	251.7*	2.8 <sup>ns</sup>	8.0 <sup>ns</sup>	-13.9 <sup>ns</sup>	-43.2 <sup>ns</sup>	30.7 <sup>ns</sup>	164.3*	-73.4*
SIS1	C	98.5	66.5	18.7	7.6	74.1	9.3	49.5	860.9
	WS	428.7	66.2	15.6	5.2	96.8	12.1	83.2	285.7
	Change (%)	335.2*	-0.5 <sup>ns</sup>	-16.6 <sup>ns</sup>	-31.6*	30.6 <sup>ns</sup>	30.1 <sup>ns</sup>	68.1 <sup>ns</sup>	-66.8*
INS2	C	14.7	67.4	17.8	7.6	220.2	18.6	160.8	1387.3
	WS	580.5	87.9	27.5	12.0	190.5	7.6	199.0	1690.4
	Change (%)	3849.0*	30.4 <sup>ns</sup>	54.4*	57.9*	-13.5 <sup>ns</sup>	-59.2 <sup>ns</sup>	23.6 <sup>ns</sup>	21.8 <sup>ns</sup>

(continued on next page)



Table 4 (continued)

Accession	Treatment	Pro ( $\mu\text{mol g}^{-1}\text{DW}$ )	MDA (nmol $\text{g}^{-1}\text{DW}$ )	TPC (mg eq. $\text{GA g}^{-1}\text{DW}$ )	TF (mg eq. $\text{C g}^{-1}\text{DW}$ )	SOD activity (U. $\text{g}^{-1}\text{ protein}$ )	CAT activity (U. $\text{g}^{-1}\text{ protein}$ )	APX activity (U. $\text{g}^{-1}\text{ protein}$ )	GR activity (U. $\text{g}^{-1}\text{ protein}$ )
ANS26	C	94.8	92.5	24.6	15.4	208.2	103.3	413.0	412.7
	WS	347.0	95.3	24.6	13.7	100.6	80.8	125.7	510.4
	Change (%)	266.0*	-3.0 <sup>ns</sup>	0 <sup>ns</sup>	-11.0 <sup>ns</sup>	-51.7*	-21.8 <sup>ns</sup>	-69.6*	23.7 <sup>ns</sup>
INS1	C	37.9	77.8	14.7	6.6	27.6	39.6	313.6	506.2
	WS	504.6	93.8	17.9	5.1	42.5	40.2	393.5	377.3
	Change (%)	1231.3*	20.6 <sup>ns</sup>	21.8*	-22.7 <sup>ns</sup>	54.0 <sup>ns</sup>	-1.5 <sup>ns</sup>	25.5 <sup>ns</sup>	-25.5 <sup>ns</sup>
MEL2	C	12.7	65.6	17.4	9.5	233.3	18.6	137.4	1158.4
	WS	297.39	90.1	29.2	14.8	171.2	31.9	283.7	1619.6
	Change (%)	2241.7*	37.4 <sup>ns</sup>	67.8*	55.8*	-26.6 <sup>ns</sup>	71.6 <sup>ns</sup>	106.5 <sup>ns</sup>	39.8*
SIS2	C	14.8	87.3	17.3	6.1	163.7	51.5	140.9	161.0
	WS	304.0	87.9	15.0	4.6	53.9	12.7	312.5	273.7
	Change (%)	1954.0*	0.7 <sup>ns</sup>	-13.3 <sup>ns</sup>	-24.6 <sup>ns</sup>	-67.1*	-75.3 <sup>ns</sup>	121.8 <sup>ns</sup>	70 <sup>ns</sup>
Mean	C	49.2	84.0	18.3	8.8	158	36.7	229.1	781.6
	WS	390.6	95.0	21.8	9.3	122.2	42.7	284.1	749.4
	Change (%)	693.7	13.2	19.2	5.0	-22.6	16.3	24.0	-4.1
SEs		13.7	10.8	1.1	0.7	25.2	12.5	84.1	143.3
LSD		38.3	30.2	3.1	1.9	70.5	35.0	235.2	400.6

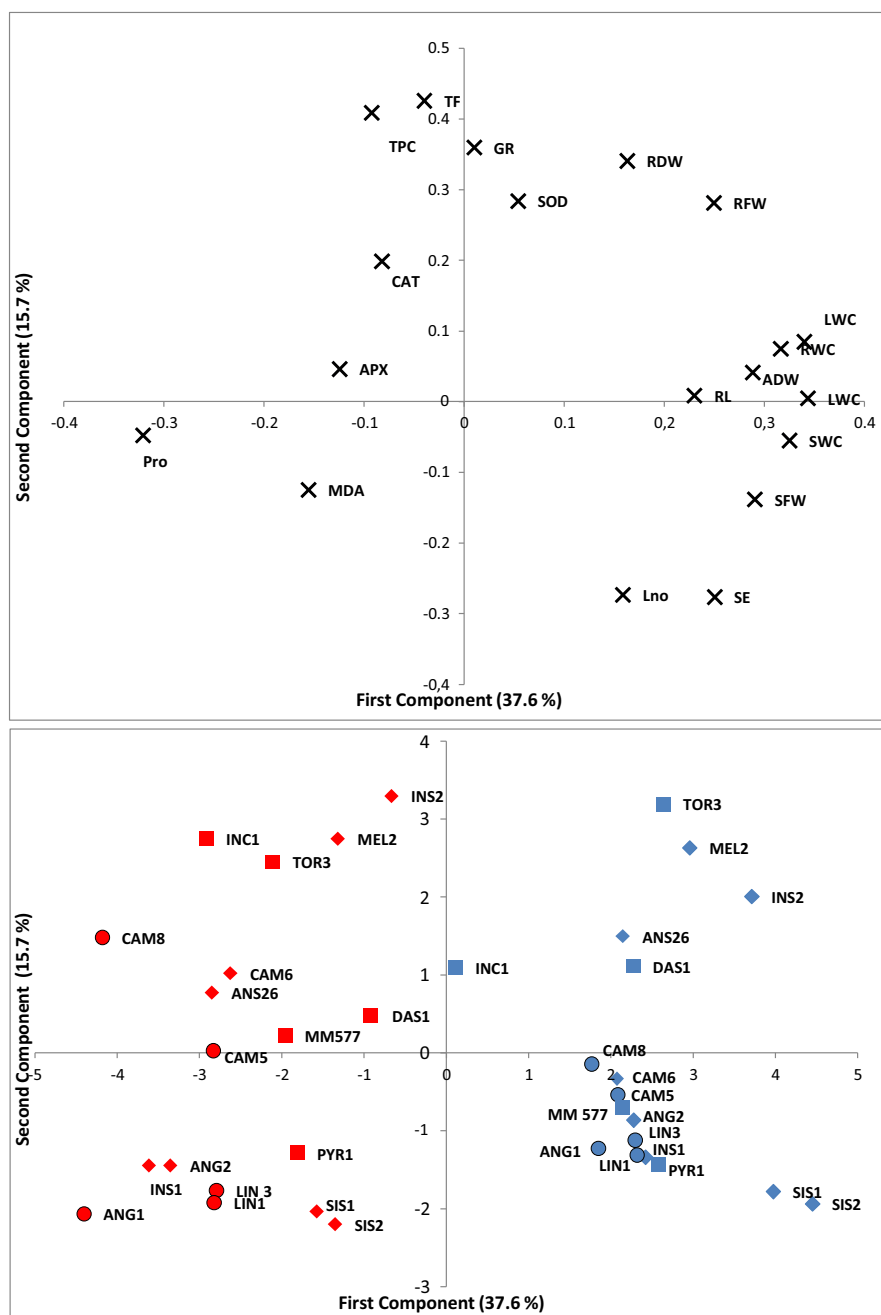
species widespread in Africa, characterised by a considerable morphological and genetic variation, including several ploidy levels (Anaso and Uzo, 1990; Knapp et al., 2013;). Out of the three accessions of *S. campylacanthum* analysed, CAM6 falls within the group of the most sensitive, whereas CAM8 and CAM5 are included in the intermediate genotypes. The two accessions of *S. limnaeanum* were also grouped in this latter category.

Based on a smaller variation of the dry weight of aerial parts and morphological traits of the stressed plants compared to those from the control, four species proved to be drought tolerant. The two accessions of *S. incanum* analysed (MM577 and INC1) ranked as tolerant, confirming previous information on its good drought tolerance (Gramazio et al., 2017). In fact, *S. incanum* grows in desertic and semidesertic areas of the Middle East and East Africa (Vorontsova and Knapp, 2016). The species belongs to the secondary gene pool of cultivated eggplant (Syfert et al., 2016), and crosses are relatively easy to obtain (Plazas et al., 2016), making it a valuable material for improving drought tolerance in eggplant. Accessions of two other species that responded with only a small reduction of growth under water stress belong to the secondary gene pool of eggplant. One of them was *S. pyracanthos*, which belongs to the more remote "Madagascar" clade (Vorontsova et al., 2013) and grows in dry areas (Vorontsova and Knapp, 2016). The second one, *S. dasyphyllum*, is primarily a forest species, but it can also be found in savannah and grassland environments (Vorontsova and Knapp, 2016), where water stress is common. Finally, *S. torvum*, also classified as tolerant, belongs to the tertiary gene pool (GP3), including phylogenetically distant species. In this case, pre- and post-zygotic barriers require specific breeding techniques – like embryo rescue – for hybridisation, and the progeny is often sterile (Plazas et al., 2016). Interestingly, *S. torvum* can grow in dry environments and is used as a rootstock for eggplant (Gisbert et al., 2011), and has also demonstrated tolerance to salinity (Brenes et al., 2020b).

Different abiotic stresses, such as water deficit and salinity, cause oxidative stress (Das and Roychoudhury, 2014.). Malondialdehyde (MDA) is one of the final products of membrane lipid peroxidation, and highly unsaturated fatty acids of cellular membranes are the macromolecules most susceptible to oxidative stress (Pamplona, 2008). For this reason, MDA is considered an excellent marker of the level of oxidative stress in plant and animal systems (Del Rio et al., 2005), widely used when ranking cultivars according to their stress tolerance (Mihaljević et al., 2021; Szekely-Varga et al., 2020). In the present study, a significant stress-induced increase of MDA was detected only in

some of the accessions, and only a slight difference was found between the mean values of the water-stressed and control plants. MDA did not show a significant increase in any of the accessions classified as tolerant to water stress, indicating that they do not suffer from oxidative stress and suggesting that their ROS scavenging mechanism may be more efficient. However, MDA cannot be used in screening for drought resistance in wild relatives of eggplant due to the large variability in MDA contents between accessions of the same group of tolerance. The reliability of this widely used biochemical marker has recently been questioned due to methodological pitfalls and possible misinterpretations of its variation (Morales and Munné-Bosch, 2019). The small increase in MDA levels in all studied accessions suggests the presence of strong antioxidant mechanisms that ensure rapid detoxification of free radicals.

Of all compounds analysed here, Pro is likely playing a major role in drought tolerance as it accumulated at very high concentrations under stress. An average increase of 13-fold in Pro was observed, considering all accessions examined, especially in the two most tolerant, namely *S. incanum* MM577 (79-fold) and *S. pyracanthos* PYR1 (95-fold). There are reports of Pro increase under different types of abiotic stress in cultivated eggplant, such as drought (Plazas et al., 2019; Sarker et al., 2005; Tani et al., 2018), salinity (Brenes et al., 2020a, b; Hassen and Al-Zubaidi, 2018; Shishira et al., 2016), or K stress (Marques et al., 2011). Proline is one of the most common osmolytes in plants, with an essential role in osmoregulation (Ashraf and Foolad, 2007; Kavi Kishor and Sreenivasulu, 2014; Parida et al., 2008; Szabados and Savouré, 2010). Besides its direct osmotic effects, Pro acts as an important redox buffer (Verslues and Sharma, 2010), and as an antioxidant, with a function in ROS scavenging; in some species, its activity as a free radical scavenger is more effective than its osmotic function (Hong et al., 2000). Smirnoff and Cumbes (1989) were the first to show that free and polypeptide-bound proline can act as scavengers by binding with  $\text{O}_2^-$  and OH to form a variety of hydroxyproline derivatives. Under water stress, Pro accumulates at very high levels in some species, generating an important redox buffer (Verslues and Sharma, 2010). Increased Pro-concentrations in chloroplasts of plants subjected to stress are related to a low NADPH/NADP ratio, stabilisation of redox balance, and reduced photoinhibition and damage of the photosynthetic apparatus (Hare and Cress, 1997; Hossain et al., 2014). In this way, Pro over-accumulating transgenic plants have been reported to cope with water deficit stress by contributing to osmotic adjustment and mitigating oxidative stress associated with drought (Ben Rejeb et al., 2014;



**Fig. 3.** Principal Component Analysis (PCA) conducted with 24 analysed parameters in 18 accessions of eggplant and wild relatives subjected to control and water deficit treatments. The first (PC1) and second (PC2) principal components account, respectively, for 37.6 and 15.7% of the total variation. Loading plot of the principal component analysis (A), where leaf number (Lno), stem elongation (SE), fresh weight of roots (RFW), stems (SFW) and leaves (LFW), water content of roots (RWC), stems (SWC) and leaves (LWC), dry weight of roots (RDW), aerial dry weight (ADW), proline (Pro), malondialdehyde (MDA), total phenolic compounds (TPC), total flavonoids (TF), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and ascorbate peroxidase (APX), are represented. Scatter plot of the PCA scores (B), where the control is in blue, and the water stress treatment in red, and tolerant accessions are represented by squares, intermediate accessions by circles, and susceptible accessions by diamonds (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Nguyen et al., 2019). Proline contributes to stabilising sub-cellular structures, scavenging free radicals, and buffering cellular redox potential (Gupta and Huang, 2014). It also chelates heavy metals, modulates different cellular functions, and regulates the expression of stress-responsive genes, acting as a component of the signalling pathways of a range of different stresses (Kaur and Asthir, 2015). Numerous comparative studies have reported a higher accumulation of proline in the more tolerant genotypes (related species or cultivars of a given species) than in the more stress-sensitive ones (Ashraf and Foolad, 2007; Ashraf and Harris, 2004). For example, in a comparative study of the responses to salt stress between eggplant and *S. insanum*, we have reported higher Pro accumulation in the more tolerant wild species (Brenes et al., 2020a).

The development of drought-tolerant cultivars or rootstocks in eggplant relies on the identification of germplasm of interest, which may allow the introgression of genes from this germplasm into eggplant lines

(Cattivelli et al., 2008; Fita et al., 2015). For this, a fast and effective assessment of drought tolerance in young plants is required. Proline was reported as an effective biochemical marker of stress in crops (Abbas et al., 2014; Cicevan et al., 2016; Mwadingeni et al., 2016), including eggplant (Plazas et al., 2019; Zayova et al., 2017), but also in many other plants, such as forest species (Schiop et al., 2015; Plesa et al., 2018), or even in wild halophytes (Al Hassan et al., 2016a). However, not always a higher Pro concentration is found in the more tolerant genotypes. In many cases, there is no correlation, or even a negative correlation, with the less tolerant genotypes having higher levels of proline, its accumulation being a mere stress response, not related to increased tolerance (Al Hassan et al. 2016b; Arteaga et al., 2020; Chen et al., 2007; Kozminska et al., 2018). In this study, higher absolute levels of proline were not correlated with the degree of tolerance. Therefore, the use of Pro for ranking eggplant-related wild species in screening for drought tolerance may be limited.

Under stress conditions, plants activate the synthesis of other low molecular compounds such as ascorbic acid (AA), phenolics, and flavonoids, among others (Gill and Tuteja, 2010; Miller et al., 2010). Eggplant is considered a functional food and belongs to the top 10 vegetables with antioxidant activity (Okmen et al., 2009; Peng et al., 2016). The species is reported to have a high phenolic compounds content, including flavonoids, highly diverse in different genotypes (Niño Medina et al., 2017; Prohens et al., 2013). In the 18 accessions analysed, foliar phenolics generally increased under stress, whereas the mean values were similar in the two treatments for flavonoids. Although there was large variability among genotypes, as in the case of Pro, the stress-tolerant genotypes showed a higher drought-induced mean increase in TPC and TF contents than the intermediate and susceptible genotypes. Likewise, the increase was significant in four out of the five accessions within the group of tolerant genotypes. However, TFC concentrations were variable even within the same species, and, for example, *S. melongena* cultivar MEL2 showed a significant increase, whereas ANS26 did not. In contrast, in the two *S. insanum* accessions analysed, the variation in TFC was significant, and in *S. linnaeanum*, the values were similar in the two accessions. A similar pattern was detected for total flavonoid (TF), with generally higher concentrations in the more tolerant cultivars. Due to the better ability to activate the synthesis of these strong antioxidant compounds, membrane lipid degradation was not detected by the MDA assay in this group of accessions.

Phenolics and flavonoids are potent antioxidants that act as secondary ROS scavenging systems activated only after the first line of defense, represented by antioxidant enzymes, is overcome. The antioxidant enzymatic machinery in plants is generally very efficient, including several key antioxidant enzymes, with different cellular localisation and working in concert to detoxify ROS (Das and Roychoudhury, 2014; Gill and Tuteja, 2010; You and Chan, 2015). Super-oxide dismutase (SOD) acts as the first line of defence against oxidative stress in plants by catalysing the removal of  $O_2^-$  through its rapid dismutation into  $H_2O_2$  and  $O_2$  (Alscher et al., 2002). The activity of this enzyme increases significantly in halophytes in response to stress, but studies in glycophytes have reported both increases and decreases under stress conditions (Bose et al., 2014). Catalase (CAT) catalyses the conversion of  $H_2O_2$  into water and oxygen (Willekens et al., 1997). Ascorbate peroxidase (APX) also removes hydrogen peroxide but uses ascorbate as a reductant (Shigeoka et al., 2002). The activity of these two enzymes, CAT and APX, is mainly up-regulated in salt- and drought-tolerant species (Bose et al., 2014; Laxa et al., 2019). Glutathione reductase (GR) is an oxidoreductase with a role in the maintenance of intracellular reduced glutathione (GSH), which is involved in a wide range of essential functions (Couto et al., 2016). The activity of this enzyme has been reported to increase, decrease or remain unchanged under stress conditions (Bose et al., 2014; Laxa et al., 2019). Susceptible plants are considered to activate the glutathione-dependent scavenging system predominantly, but tolerant species showed higher activation of the ascorbate-dependent one (Laxa et al., 2019). The four antioxidant enzymes showed substantial variation in our study, with no clear pattern distinguishable for the three cultivar groups. Moreover, high variability was detected between accessions of the same species, as previously reported by Plazas et al. (2019), who found a significant increase only in CAT in four eggplant cultivars analysed, and a high variability with respect to the other antioxidant enzymes. Also, different variations of antioxidant enzymes under water and salt stress were found in eggplant cultivars (Kiran et al., 2019a).

## 5. Conclusions

The analysis of growth parameters after a short period of 11 days of total irrigation retention allowed the selection of the most tolerant genotypes of eggplant wild relatives, which is consistent with the ecological conditions in their natural habitats since they are all taxa typical of arid zones or able to grow in dry environments. The biochemical

analysis reflected a large variability of responses. No specific traits could be established for the most tolerant or the most susceptible accessions, so neither Pro nor MDA concentrations are accurate markers in screening for drought tolerance in the wild eggplant-related species complex. However, increased proline levels in stressed plants, especially in tolerant genotypes, clearly indicate that this amino acid plays a crucial role in the response to water stress, conferring better adaptation when accumulated at higher levels. The intensity of oxidative stress induced by water deficit in eggplant and its relative wild species is relatively weak, probably due to the efficiency of the antioxidant mechanism. MDA did not increase in tolerant genotypes, which had a more significant increment in Pro and total phenolics and flavonoids contents. The pattern of enzyme activity was highly variable among accessions. Overall, the results obtained indicate a large diversity of tolerant accessions among the wild related species that can be exploited for eggplant breeding.

## CRedit authorship contribution statement

**Mariola Plazas:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Project administration, Funding acquisition. **Sara González-Orenga:** Methodology, Investigation, Data curation, Writing – original draft. **Huu Trong Nguyen:** Methodology, Investigation. **Irina M. Morar:** Methodology, Investigation. **Ana Fita:** Conceptualization, Writing – original draft, Visualization. **Monica Boscaiu:** Conceptualization, Formal analysis, Data curation, Writing – original draft, Visualization, Supervision, Project administration. **Jaime Prohens:** Conceptualization, Formal analysis, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Oscar Vicente:** Conceptualization, Writing – review & editing, Supervision, Project administration.

## 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.

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