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Research article

Morphological, physiological, and biochemical changes of herbaceous ornamental plants subjected to drought stress and recovery

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ABSTRACT

Drought episodes, which are very frequent in the Mediterranean region, have negative effects on plant growth. To reduce irrigation, which is no longer sustainable today, it is necessary to identify plants capable of resisting water shortage. The aim of the experiment was to investigate the eco-physiological and biochemical changes in three herbaceous plants, *Dimorphotheca hybrida*, *Pelargonium peltatum*, and *P. hybridum*, which are widely adopted for ornamental purposes in Mediterranean gardening. Their ability and the mechanisms they adopt to recover after water stress, following periodic suspension and restoration of the water supply, were studied. Three water regimes were evaluated: control plants (100% WC) and drought-stressed plants (60% and 40%WC). After one week of submission to drought, stressed plants were re-watered to 100% WC and then recovered for seven days. Subsequently, the plants were subjected to drought exposure for 15 days. The plants, after the second cycle of suspension of water, were kept in the same conditions of control plants for seven days. Plant growth, leaf gas exchanges, chlorophyll *a* fluorescence, relative water content, chlorophylls and carotenoids, proline, and malondialdehyde content were determined. The total dry biomass was influenced by species and drought treatment, but among the experimental factors there is not interaction. Under severe water stress (40% WC), all species showed significant reductions in total leaf area and leaf number. The greatest decrease in total leaf area was observed in *D. hybrida* (−41%), followed by *P. peltatum* (−27%) and *P. hybridum* (−23%). Leaf number decreased by 11% in *D. hybrida*, 14% in *P. peltatum*, and 32% in *P. hybridum*. The effect of treatments on leaf RWC at different times during the experiment determined values similar to those of the control plants in *P. hybridum*, and reduced during the second drought event in *P. peltatum* and *D. hybrida*. At the end of the trial, net photosynthesis under the most severe stress (40%WC) was significantly reduced in all species by −64% *D. hybrida*, −76% *P. peltatum*, −70% *P. hybridum*. The Fv/Fm ratio in *P. peltatum* and *D. hybrida* showed significant differences only during the second drought stress period. By the end of the

Abbreviations: A_N, net CO_2 assimilation rate; gs, stomatal conductance; E, transpiration rate; 100%, control 100% of Water Container Capacity (WC); RWC, Relative Water Content; F0, minimum fluorescence; Fm, maximum fluorescence; Fv/Fm, maximum quantum efficiency of PSII; Pro, proline; MDA, malondialdehyde; S-R, suspension/rewatering.

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second re-watering period, the stressed plants exhibited values similar to those of the control plants.

Results demonstrated that photosynthetic parameters were the most affected by antioxidants and osmolytes, such as proline. Considering all parameters, the species were ranked according to their drought tolerance as follows: *P. hybridum* > *D. hybrida* = *P. peltatum*, highlighting that each species exhibited distinct adaptations. These findings indicate that the combined evaluation of physiological and biochemical parameters under repeated drought and recovery conditions can enhance the selection of ornamental species better suited to low-water urban landscapes.

1. Introduction

The constant increase in the level of greenhouse gases, particularly CO_2, has led to an increment in the Earth's temperature, with an enhance in the frequency and intensity of drought episodes [1,2]. The last few years have been characterised by increasingly hotter summers with frequent heat waves, and an increasingly harsh climate is expected in the near future, intensifying the frequency and severity of water stress [3,4].

These phenomena will especially affect coastal communities with high population density, due to the increase in urban population, considering that 4.2 billion people live in urban areas globally, which could spread 6.7 billion by 2050 [5]. Promoting the reduction of water use in residential green areas for the irrigation of ornamental landscapes and gardens is essential to meet the growing demand for freshwater, as climate change and population growth are accentuating the reduction of resources [6].

The effects of climate change may be more intense in regions such as the Mediterranean, which is considered a hotspot for drought risk [7,8]. In the 21st century, the average increase in temperature in this environment was estimated to be 20% higher than the global average. A reduction in precipitation is expected to be approximately 20 mm/°K [9]; therefore, drought episodes are expected to be more frequent, long-lasting, and intense [10].

Drought episodes are very frequent in the Mediterranean region, where the climate is characterised by higher temperatures, a high vapour pressure deficit, high levels of radiation, and low rainfall during the growing seasons. These conditions negatively influence the growth of plants under stressful conditions [11]. To avoid harmful effects and loss of the ornamental value of plants used in landscaping, irrigation is used in arid and semi-arid areas, often with drinking water. In some areas of the US, it has been estimated that 30-70% of all drinking water is used for the irrigation of urban landscapes [12]. The shortage of water in some regions limits the use of drinkable water for ornamental greens.

During drought stress conditions different morphological, physiological, biochemical and molecular changes were observed in plants that can damage both plant growth and productivity [13,14]. Many morphological modifications to water stress affect the above-ground part of the plants. Leaf growth is, in fact, the growth process of plants is most sensitive to water deficits [15]. Unfortunately, many adaptation strategies affect the visual appearance of plants, and physiological alterations can lead to loss of the ornamental value of plants or green areas. Therefore, the selection of plants for drought tolerance is extremely important for landscape and urban green design. Selection of ornamental plants for drought tolerance involves identifying species and cultivars that exhibit adaptive mechanisms to withstand water scarcity. These mechanisms include morphological, physiological, and biochemical modifications, such as an increased root/shoot ratio, reduced growth, and changes in leaf anatomy, such as waxy and thick cuticula [16]. Interestingly, while some ornamental plants show traits indicative of drought tolerance, such as low leaf water potential and osmotic adjustment [17]. The selection of key physiological traits can aid in the identification of drought-tolerant species, contributing to sustainable landscaping practices in the face of climate change [16].

Among the most common physiological adaptations to water stress, the decrement of relative water content (RWC), leaf water potential, transpiration rate, stomatal conductance, and, hence, the decrease in the assimilation of CO_2, with inhibition of photosynthetic activity [18,19] are mostly reported in the scientific literature. In species sensitive to drought stress, reductions in chlorophyll [20] and protein concentrations have been observed [21]. Piveta et al. [22] in an experiment observed that water stress, in addition to inhibiting chlorophyll biosynthesis, reduces chlorophyll concentration because it increases chlorophyllase activity and the rate of its decomposition. Lower water availability induces photosynthesis reduction, and the excitation energy of light is higher than that used during the photosynthesis process. Due to drought stress, there is an overexcitation of photosynthetic pigments in the antennal pigments, which can cause the increasing of reactive oxygen species (ROS) in the chloroplasts [23].

After the cessation of drought stress, the occurrence of small amounts of rainfall can have a positive effect on plant function, both at the whole-plant level and on biochemical responses. Therefore, especially in herbaceous species, a group of plants is not always extensively investigated from this point of view, and the mechanisms adopted to tolerate water stress should be understood.

The capability of plants to adapt to prolonged periods of water shortage, at the end of which it recovers its functions, could be considered a mechanism of adaptation to the typical conditions of the Mediterranean environment [24]. Nevertheless, the response of plants certainly differs significantly according to the period of the stress, the plant species used, and the phase of development [25].

Private gardens and public green areas are attractive because of the presence of colourful bedding plants. As reported by Heidari et al. [26], when plants have a small root system and are grown in pots, they can suffer more from the effects of water stress.

To reduce water consumption, which is no longer sustainable today, it is necessary to identify plants capable of resisting water shortage. However, the water needs and responses of ornamental plants to drought are not yet well specified, principally for herbaceous plants, which represent a large part of ornamental vegetation in urban green areas [27].

The current study concerns African Daisy (*Dimorphotheca hybrida* DC.) of *Asteraceae* family and two species of geraniums - ivy geranium (*Pelargonium peltatum* (L.) L'Hér.) and pot geraniums (*Pelargonium hybridum* (L.) L'Hér) of the *Geraniaceae* family, which are widely adopted for ornamental purposes in Mediterranean gardening.

This experimental work started with the assumption that the final capacity of these species is linked to their ability to maintain high values of RWC under stress conditions and to recover rapidly after recovery periods through activation at the cellular level and the biosynthesis of osmotic compounds. The response of different ornamental plants to drought stress have been well reported in different studies; however, the morpho-physiological responses of herbaceous ornamental species to progressive drought and recovery after irrigation are relatively limited.

The aim of this experiment was to fit this framework, which had the objective of evaluating, on three ornamental herbaceous species, the ability to recover from water stress, following periodic suspension and restoration of the water supply, and study the mechanisms that support this ability. The effects of stress memory on subsequent drought stress applications were also evaluated. Results report in this work are original in terms of species considered and level of drought stress intensity. Physiological and biochemical approaches provide insight into ornamental species behaviour by increasing water stress conditions.

2. Materials and methods

The experimental trial was carried out in an unheated experimental greenhouse of the University of Catania (Italy) during the 2023 growing season. The experimental site was located at Catania (longitude 37°31′E, latitude 15°04′N, altitude 92 m). The cold greenhouse had a steel frame and was covered with polycarbonate sheets. *Dimorphotheca hybrida* DC. 'Dalina® Special Sunshine Beauty' = *D. hybrida*, *Pelargonium peltatum* (L.) L'Hér. 'Moonflair® Red' = *P. peltatum,* and *Pelargonium hybridum* (L.) L'Hér. 'Galaxy™ Red' = *P. hybridum* rooted cuttings were provided by the Florensis Italia company, Lazzate (MB, Italy). Rooted cuttings of the species were transplanted into 14 Ø pots (one plant per pot) filled with a substrate based on peat and perlite (2/1, v/v). The irrigation of the plants was regular for one month at a well-watered (100% WC) level. At the end of March, the plants were separated into three factors: control plants (100% WC) and drought-stressed plants (60%WC and 40%WC).

2.1. Irrigation management

WC was defined operationally as the percentage of container water-holding capacity (pot capacity), determined gravimetrically. Pot capacity (100% WC) was determined by fully saturating the substrate and allowing free drainage to cease, after which pots were weighed. This pot weight was used as the reference value. The 60% and 40% WC treatments were defined as fixed proportions of the water content a pot capacity. WC was monitored throughout the experimental period using a gravimetric method, estimating water loss as the difference between pot weight measured after the irrigation (once drainage had stopped), and the weight recorded before the subsequent irrigation event, following the method described by Toscano et al. [28]. Each pot (six plants per treatment, two per repetition) was positioned on an electronic balance (capacity 3.4 kg and, resolution of 0.01 g, Orma, model BCE4200). The cumulative amount of water supplied from the first drought stress period to the end of the experiment was 6.6, 4.7, 3.8 L for 100% CC, 60% CC, and 40% CC respectively for *D. hybrida*; 7.5, 5.4, 4.3 L for 100% CC, 60% CC, and 40% CC respectively for *P. peltatum*; 7.2, 5.2, 4.2 L for 100% CC, 60% CC, and 40% CC respectively for *P. hybridum*. The plants subjected to drought stress, after one week were watered at 100% WC and left in these conditions for 7 days. After, the plants were subjected to exposure to drought for 15 days. After the second cycle of drought stress, the plants were irrigated at 100% for another 7 days. The total duration of the experimental trial was 66 days (Fig. 1).

2.2. Plant materials

Leaf materials were taken weekly for physiological and biochemical analyses. Fresh plant material was frozen in liquid N_2 and stored at − 80 °C to determined proline, chlorophyll, carotenoids, and malondialdehyde.

At the end of the trial, roots, after being cleaned, were separated from the epigeal portion. The leaves and stems were also separated into nine plants per treatment (three per repetition). Root, leaves, and steam were dried (65 °C) and dry biomass were recorded. Total leaf area was measured with a leaf area meter (Delta-T Devices Ltd., Cambridge, UK).

Temperature and relative humidity data were recorded using a data logger CR1000 (Campbell Scientific Ltd., Loughborough, UK) during the experimental period. Mean temperatures ranged between 18 and 22 °C, and relative humidity (RH) ranged between 50 and 66% (Fig. 2).

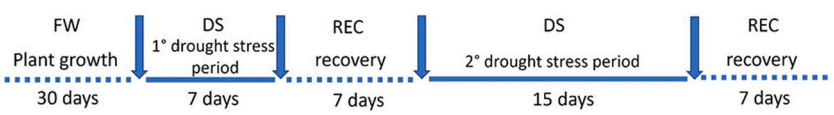

Fig. 1. Timeline for suspension/rewatering (S-R) treatments used in the experiment. FW = full watering; DS = drought stress event; REC = recovery.

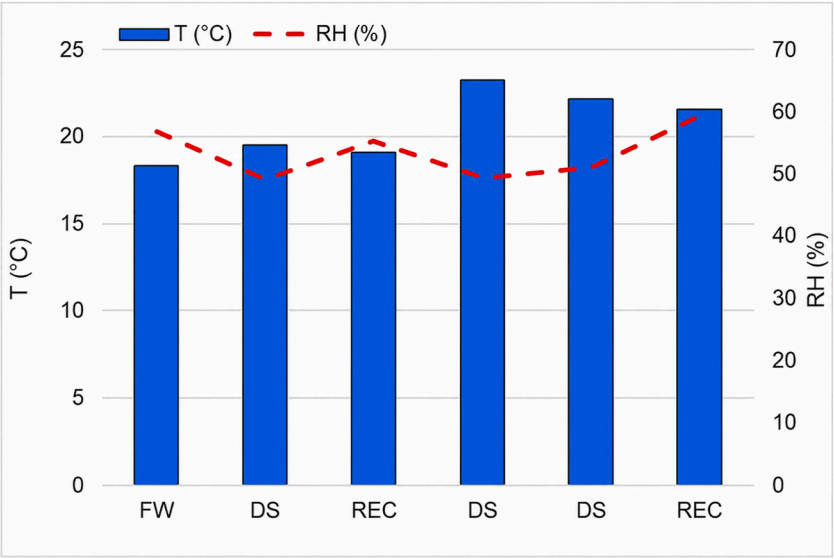

Fig. 2. Climate trend [temperature (°C) and Relative Humidity (RH, %)] during the experimental period.

2.3. Physiological parameters

The leaf gas exchange parameters (net photosynthetic rate (A_N), stomatal conductance (gs), and transpiration rate (E)) using a CO_2/H_2O infrared gas photosynthesis system (LCi, ADC Bioscientific Ltd., Hoddesdon, UK) were measured for every water treatment. Measurements were taken between 09:00 and 13:00.

The chlorophyll *a* fluorescence was evaluated with a modulated chlorophyll fluorimeter OS1-FL (Opti-Sciences Corporation, Tyngsboro, MA, USA). Chlorophyll *a* fluorescence was expressed as the Fv/Fm ratio, where Fm is the maximal fluorescence of the dark-adapted state, Fv is the variable fluorescence, and F0 is the minimum fluorescence.

To determine relative water content, 30 discs (10 mm in diameter) for each replicate were weighed immediately (FW), immersed in distilled water (24 h) and then turgid weight (TW) was obtained. The leaves were then dried at 75 °C for 24 h to obtain dry weight (DW). RWC was determined using the equation: RWC% = [(FW−DW)/(TW−DW)] × 100.

2.4. Determination of Chl and carotenoid concentration

To measure the amount of chlorophyll *a, b*, total and carotenoids, 100 mg of fresh leaf material was crushed, extracted with 5 mL of 99% methanol, and incubated in the dark (24 h at 4 °C). By spectrophotometer device (UV 1900i Spectrophotometer, Shimadzu, Japan) was read optical absorption in wavelengths 665.2 nm, 652.4 nm, and 470 nm. Chlorophyll and carotenoid concentrations were calculated according to the equation reported by Lichtenthaler et al. [29].

$$\text{Chl}a = 16.72A_{665.2} - 9.16A_{652.4}$$

$$\text{Chl}b = 34.09A_{652.4} - 15.28A_{665.2}$$

$$\text{Chl}tot = 1.44A_{665.2} + 24.93A_{652.4}$$

$$Car = \frac{1000A4_{70} - 1.63Chla - 104.96Chlb}{221}$$

2.5. Determination of proline concentration

Proline content was measured by the ninhydrin-acetic acid method according to Bates et al. [30]. Briefly, 1 g of fresh leaf tissue was homogenized in 5 mL of 3% aqueous sulfosalicylic acid and centrifuged at 14,000*g* for 15 min (Neya 10R, REMI, Mumbai, India). Two milliliters of the supernatant were mixed with an equal volume of glacial acetic acid and acid ninhydrin, vortexed, and incubated at 100 °C for 1 h. The reaction was stopped in an ice bath, and the solution was extracted with 4 mL of toluene. The samples were quantified using a spectrophotometer at 525 nm. The toluene was used as a blank (UV 1900i spectrophotometer, Shimadzu, Japan).

2.6. Estimation of lipid peroxidation

Lipid peroxidation was obtained by measuring the amount of malondialdehyde produced by the thiobarbituric acid reaction as reported by Li et al. [31]. Briefly, 0.5 g of sample was homogenized in 1.5 mL of 5% (w/v) trichloroacetic acid. The homogenate was centrifuged at 5,000*g* for 10 min, and the supernatant was then diluted to a final volume of 10 mL. A 2 mL portion of the diluted extract was mixed with an equal volume of 0.67% 2-thiobarbituric acid and incubated in boiling water (100 °C) for 30 min. The mixture was subsequently centrifuged at 5,000*g* for 10 min. Malondialdehyde (MDA) concentration in the aqueous phase was calculated using the following equation: $C\ (\mu mol\ L^{-1}) = 6.45\times(A_{532}-A_{600})-0.56\times A_{450}$.

Lipid peroxidation was expressed as nmol MDA g^{-1} FW.

2.7. Statistical analysis

The experiment used a randomised complete design with three replicates per treatment. The CoStat version 6.311 software was used for statistical analysis. A one and two-way analysis of variance procedure was used. For mean comparisons, Tukey's multiple range test was used at a probability level of 5%. Data were given as mean ± standard error (SE). A multivariate analysis by Principal Components Analysis (PCA) was determined by Minitab® 16 statistics program (Minitab Inc., Philadelphia).

3. Results

Drought treatments significantly affected total dry biomass, and the response varied among species; however, no significant interactions were detected among the experimental factors. The mean effect of drought on total dry biomass showed a 26% reduction in all plants under 40%WC compared with control (100%). Species differed in their response to drought stress, with *D. hybrida* showing the lowest total dry biomass values (Table 1). A similar pattern was observed for epigeous dry biomass, which decreased by 27% under 40%WC. No significant interaction effects were found among the experimental factors. Among the species tested, *P. peltatum* exhibited the greatest reduction in growth under the 40% WC treatment.

The root-to-shoot ratio differed among species, but no significant changes were found among treatments and interactions at the end of the experimental period (Table 1). Higher root/shoot values were observed in *P. hybridum* and *D. hybrida*.

At the end of the experiment, with the increase of stress level (40%WC), a reduction in total leaf area and leaf number was observed. A 29% reduction in leaf area in the 40%WC treatment was detected, and differences were observed among the species (Table 1). The decrease in total leaf area was comparable to the decrement in leaf area, with a reduction of approximately 16% at 40% WC (Table 1).

The response of leaf relative water content (RWC) to the drought stress–rewatering (DS-REC) cycles differed among species (Fig. 3).

Table 1
Species (S) and drought stress (D) effects on total (TB) and epigeous (EB) dry biomass, root/shoot ratio (R/S), total leaf area (TLA), and leaf number (LN) on *P. hybridum*, *P. peltatum,* and *D. hybrida*. The plants were subjected to drought stress and recovery period.

Species/Drought	100%WC	60%WC	40%WC	Mean	S × D
TB (g plant^{-1})					**ns**
P. hybridum	23.70 ± 0.79	22.73 ± 0.76	18.08 ± 1.27	**21.50 ± 0.94a*****	
P. peltatum	19.83 ± 0.72	15.83 ± 0.68	14.12 ± 1.11	**16.60 ± 0.84 b**	
D. hybrida	12.92 ± 1.90	11.44 ± 0.78	9.78 ± 0.26	**11.38 ± 0.98c**	
Mean	**18.82 ± 1.14A*****	**16.67 ± 0.74A**	**13.99 ± 0.88B**		
EB (g plant^{-1})					**ns**
P. hybridum	16.70 ± 0.59	15.99 ± 0.90	12.78 ± 1.09	**15.16 ± 0.86a*****	
P. peltatum	16.94 ± 0.72	12.64 ± 0.67	11.47 ± 1.02	**13.68 ± 0.80a**	
D. hybrida	12.93 ± 1.35	11.44 ± 0.39	6.83 ± 0.26	**10.40 ± 0.67b**	
Mean	**14.30 ± 0.89A*****	**12.36 ± 0.65A**	**10.36 ± 0.79B**		
R/S					**ns**
P. hybridum	0.42 ± 0.04	0.43 ± 0.03	0.42 ± 0.04	**0.43 ± 0.04a*****	
P. peltatum	0.17 ± 0.01	0.25 ± 0.01	0.23 ± 0.01	**0.22 ± 0.01b**	
D. hybrida	0.42 ± 0.04	0.35 ± 0.03	0.43 ± 002	**0.40 ± 0.03a**	
Mean	**0.34 ± 0.03ns**	**0.34 ± 0.02**	**0.36 ± 0.02**		
TLA (cm^2)					**ns**
P. hybridum	941.12 ± 35.00	1025.06 ± 39.19	723.83 ± 32.48	**896.67 ± 35.56a*****	
P. peltatum	822.56 ± 6.74	777.82 ± 7.87	602.46 ± 38.12	**734.15 ± 40.91b**	
D. hybrida	767.00 ± 14.04	660.35 ± 12.95	452.49 ± 10.23	**626.61 ± 12.41c**	
Mean	**843.56 ± 18.59A*****	**821.08 ± 43.34A**	**592.79 ± 26.95 B**		
LN (n°)					**ns**
P. hybridum	72.16 ± 4.00	65.83 ± 3.32	49.33 ± 3.93	**62.44 ± 4.75b*****	
P. peltatum	62.33 ± 2.89	51.01 ± 3.51	53.67 ± 1.64	**55.64 ± 2.68b**	
D. hybrida	321.17 ± 16.00	353.33 ± 12.99	286.17 ± 9.45	**320.22 ± 14.15a**	
Mean	**151.89 ± 7.63*A**	**156.72 ± 6.60A**	**129.72 ± 7.34B**		

*: $p < 0.05$; ***: $p < 0.001$; ns: $p > 0.05$. Data followed by a different letter were significantly different according to Tukey's test. 100%: control; 60%: light drought stress; 40%: severe drought stress. Data are means ± standard error (n = 3). TB = Total dry biomass; EB = Epigeous dry biomass; R/S = Root to shoot ratio; TLA = Total leaf area; LN = Leaf number.

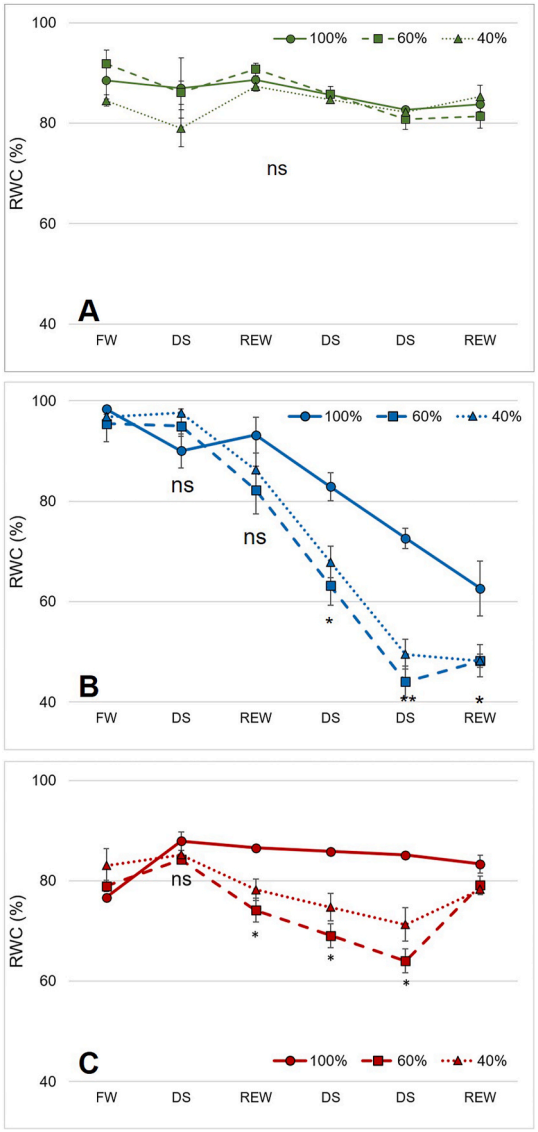

Fig. 3. Relative Water Content (RWC) in *P. hybridum* (A) *P. peltatum* (B) and *D. hybrida* (C) during the experimental period. Plants were irrigated to container capacity (100% WC) or subjected to water stress treatments (60% WC and 40% WC) and subjected to suspension/rewatering treatment [7 days no water (DS), 7 days irrigation (RE**C**), 1**5** days no water (DS2), 7 days irrigation (REC)]. Data are mean ± S.E. (n = **3**). * denote statistical significance from ANOVA at the 0.05 level.

In *P. hybridum* (Fig. 3A), RWC remained stable throughout the experiment, with no significant differences among irrigation treatments. In *P. peltatum* (Fig. 3B), no significant reductions were observed during the first drought cycle (DS) or after the first rewatering (REC). In contrast, during the second drought cycle (DS), RWC significantly decreased by 23% and 39% in plants maintained at 60% WC and by 18% and 31% in plants maintained at 40% WC, compared to the control, at the first and second DS, respectively. Following the second rewatering (REC), RWC partially recovered but remained significantly lower than the control in both deficit irrigation treatments. *D. hybrida* (Fig. 3C) showed a similar response. No significant differences were detected during the first DS, whereas during the second DS, RWC decreased by about 20% and 24% in the 60% WC treatment and by 13% and 16% in the 40% WC treatment, compared with the 100% WC. After the second REC, RWC recovered values not significantly different from the control. The net photosynthesis (An) in the 100%WC was at the start of the experiment (FW), on average, 10.4 μmol CO_2 $m^{-2}s^{-1}$ (*P. hybridum*), 6.6 μmol CO_2 $m^{-2}s^{-1}$ (*P. peltatum*), and 14.1 μmol CO_2 $m^{-2}s^{-1}$ (*D. hybrida*). During the first suspension (DS), the treatment reduced An by 41% in *P. hybridum* in both drought stress treatments (60%WC and 40%WC) in relation to the control plants, whereas in *D. hybrida,* a reduction of 38% was observed, but only in 40%WC. No reduction was observed in *P. peltatum*. During the first recovery (REC), *P. peltatum* and *D. hybrida* showed the same values as the control plants; *P. hybridum* showed values below than control plants (by ~50%). During the second drought event (DS), the drought stress diminished photosynthesis by 56% in *P. hybridum* 40%WC, 80% in *P. peltatum* 60%WC and 40%

WC, and 70% in *D. hybrida* 60%WC and 40%WC. After the second recovery, *P. peltatum* 60% and *D. hybrida* 60% showed no significant difference in relation to the control plants, whereas the 40%WC treatment showed the lowest value compared to the control plants (by 76% and 64% for *P. peltatum* and *D. hybrida,* respectively). *P. hybridum*, indeed, showed a reduction by 70% in 60%WC and 40%WC compared to control plants (Fig. 4A, B, C). The stomatal conductance (gs) in the control plants at the beginning of the experiment (FW) averaged 240 mol H_2O $m^{-2}s^{-1}$ (*P. hybridum*), 160 mol H_2O $m^{-2}s^{-1}$ (*P. peltatum*), and 150 mol H_2O $m^{-2}s^{-1}$ (*D. hybrida*). During the first suspension (DS), gs was reduced by 41% in *P. hybridum* and by 31% in *P. peltatum* in both drought stress treatments (60%WC and 40% WC) compared to the control plants, whereas in *D. hybrida,* a reduction of 38%, was observed, but only in 40%WC treatment. During the first recovery (REC), *P. peltatum* and *D. hybrida* showed the same values as the control plants whereas *P. hybridum* preserved values below those of the 100%WC treatment (by ~50%). During the second drought event (DS), the S-R treatment diminished the stomatal conductance by 56% in *P. hybridum* at 40%WC, 80% in *P. peltatum* at 60%WC and 40%WC, and 70% in *D. hybrida* at 60%WC and 40% WC. After the second recovery, *P. peltatum* 60% and *D. hybrida* 60% showed no significant difference in relation to control plants, while the 40%WC treatment showed the lowest value compared to control plants (by 76% and 64% for *P. peltatum* and *D. hybrida,* respectively). *P. hybridum* showed a reduction of 70% in 60%WC and 40%WC compared to the control plants (Fig. 4D, E, F). Transpiration (E) was not significantly affected by drought-stressed plants during the early water suspension (DS). During the first recovery period, *P. peltatum* and *D. hybrida* plants subjected to drought stress did not show differences compared with control plants. In contrast, *P. hybridum* showed a significant and progressive reduction in plants with a water content of 40%WC beginning in the first DS until the end of the experiment (Fig. 4 G, H, I). During the second drought event, E was reduced by 59% and 45% in *P. peltatum* 60% WC and 40% WC, respectively, and by 28% in *D. hybrida* 40% WC. At the end of the second recovery, all species irrigated at 60%WC restored transpiration values comparable to those of the control plants, with no significant differences detected. In contrast, plants irrigated at 40%WC showed lower values than the control plants (by 23%, 8%, and 61% for *P. hybridum*, *P. peltatum*, and *D. hybrida,* respectively). The chlorophyll *a* fluorescence (Fv/Fm ratio) in *P. peltatum* and *D. hybrida* showed significant differences only in the second DS period (Fig. 5A–C), and at the end of the second re-watering period, the stressed plants (60%WC and 40%WC) showed values similar to those of control plants. In *P. hybridum* (Fig. 5B), D-S treatment had no effect on Fv/Fm during the recovery period (REC).

Fig. 6 illustrates the trend in proline and MDA content in the leaves. Proline content increased during each drought event for all

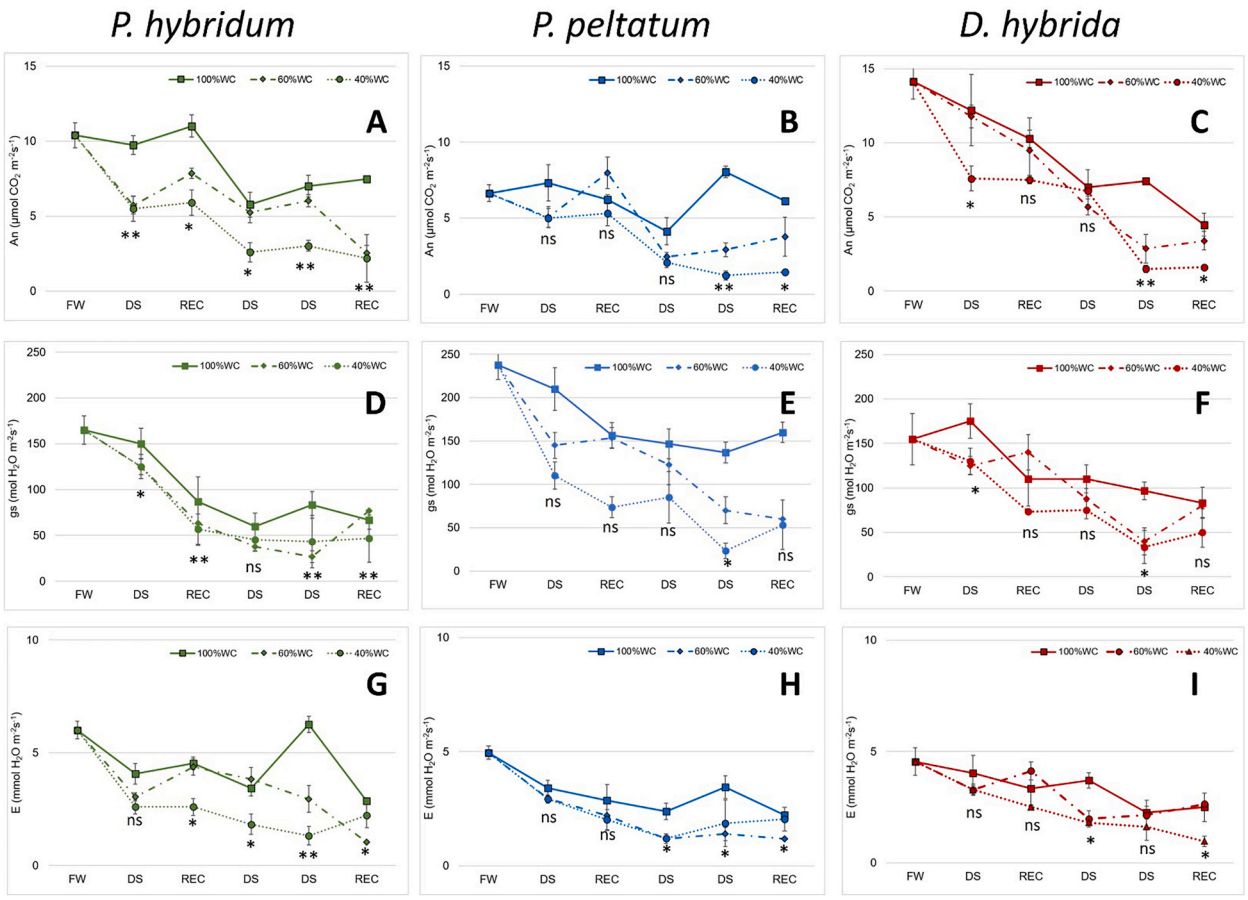

Fig. 4. Net photosynthesis (An), stomatal conductance (gs), and transpiration rate (E) in *P. hybridum* (A, D, and G), *P. peltatum* (B, E, and H) and *D. hybrida* (C, F, and I) during the experimental period. Data are mean ± S.E. (n = 3). *, ** denote statistical significance from ANOVA at the 0.05 and 0.01 levels, respectively.

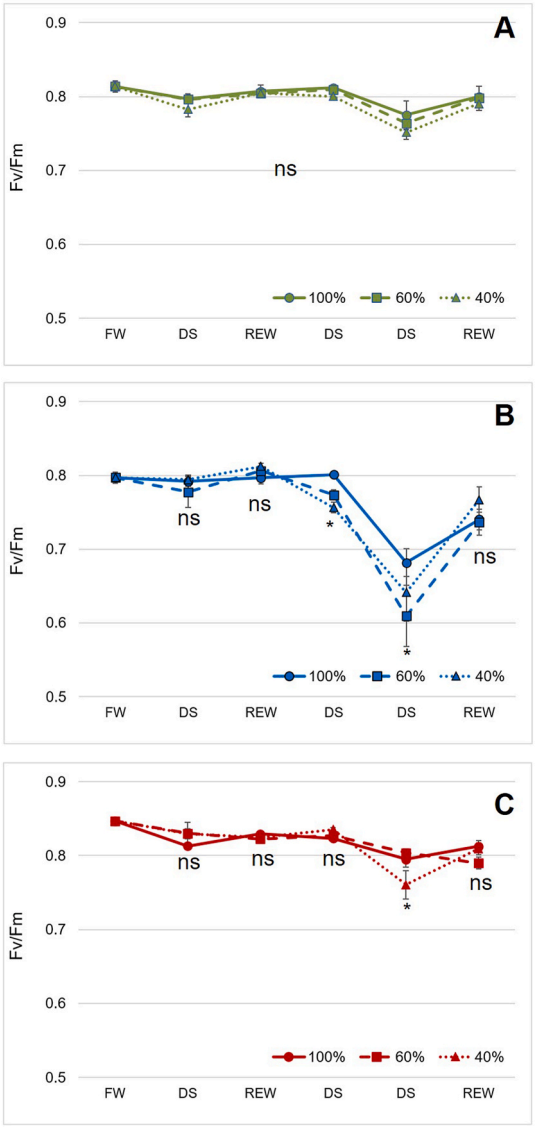

Fig. 5. Maximum quantum efficiency of the PSII (Fv/Fm) in *P. hybridum* (A), *P. peltatum* (B) and *D. hybrida* (C) during the experimental period. Data are mean ± S.E. (n = 3). * denote statistical significance from ANOVA at the 0.05 level.

species in stressed plants compared to control plants. (Fig. 6A, B, C). The highest values were recorded during the second drought event (DS). During the first period of water reduction, the proline content increased by 25% in *P. hybridum* (60%WC and 40% WC) (Fig. 6A) and 10% in *P. peltatum* (60%WC and 40% WC) (Fig. 6B), whereas no significant differences were observed in *D. hybrida* plants (Fig. 6C). During the second period of water reduction, the DS treatment increased proline content by 14% in *P. hybridum* (Fig. 6A), 23% in *P. peltatum* (Fig. 6B), and 30% in *D. hybrida* (Fig. 6C), compared to control plants. After the second recovery period (REC), the DS plants exhibited values similar to those of the control plants (Fig. 6A, B, C).

Overall, MDA tended to increase during the experimental period, in all treatments (Fig. 6D, E, F). Its content was significantly higher during the second water-stress period in the water-stressed treatments (60%WC and 40%WC). At the end of the experimental period, all species showed values similar to those of the 100%WC plants.

No interaction effects were observed among days, species, and irrigation treatments for any of the chlorophyll parameters (Table 2).

The Chl *a*, *b*, and tot contents exhibited different patterns in relation to days and species (Table 2). The chlorophyll contents (Chl*a*, Chl*b*, and Chl tot) did not show significant changes in relation to the water treatment. The effects of these interactions were observed between days and species (Fig. 7). In particular, all species showed a significant reduction in total chlorophyll content as the stress period increased, with the lowest content observed at the end of the trial. Similarly, interaction effects were observed between species and water treatments (Fig. 7). *P. hybridum* had the highest total Chl value compared to the other two species. The *a*/*b* ratio differed

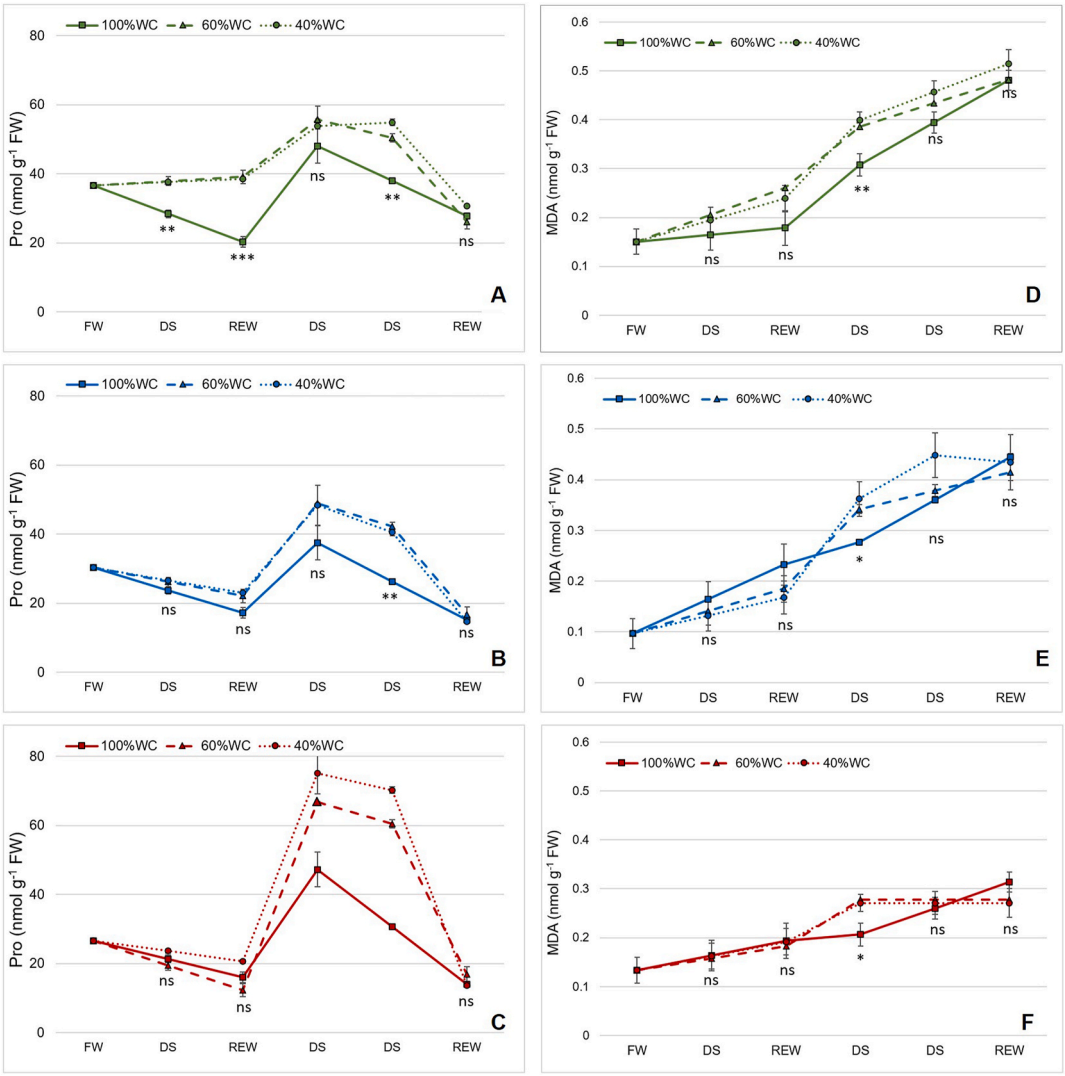

Fig. 6. Contents of Proline and MDA in *P. hybridum* (A,D), *P. peltatum* (B,E) and *D. hybrida* (C,F) during the experimental period. Data are mean ± S. E. (n = 3). *, **, *** denote statistical significance from ANOVA at the 0.05, 0.01 and 0.001 levels, respectively.

according to species. The highest ratio was observed for *P. peltatum*.

The carotenoid content (Car) showed a different trend in relation to day, species, and water treatment (Table 2). As the number of days of irrigation increased, carotenoid content decreased. The highest content was observed in *P. hybridum*. The highest car content was found in the 40%WC treatment (Table 2).

The first four principal components had eigenvalues >1 and together they explained 91.3% of the total variance (Figs. 8 and 9; Tables 3 and 4). PC1 and PC2 described 37.3% and 29.9% of the variance, respectively, and explained the main species separation, while PC3 (17.7%) and PC4 (6.4%) captured further variation related to secondary trait gradients.

PC1 was positively associated with photosynthetic performance and pigment content (Fv/Fm, Chla, Chl tot, LN, R/S, and RWC) and negatively associated with stress and morphological parameters (EB, TB, and MDA). PC2 was mainly driven by negative loadings of leaf area and stress parameters (LA, TB, Pro, and MDA), indicating a stress-related gradient independent of photosynthetic efficiency. PC3 was primarily associated with pigment parameters, showing a strong positive loading for the chlorophyll *a/b* ratio (0.468) and negative loadings for gas exchange (An and E). PC4 was mainly related to stomatal conductance and chlorophyll *b* (gs = −0.425; Chlb = 0.405) (Table 4, Fig. 8).

4. Discussion

Irrigation and water availability in urban and peri-urban areas can be limited to reduce the management costs. The growth and flowering of herbaceous bedding plants in urban areas are hampered because they are often affected by water deficits, with negative

Table 2
Mean effects of days (D), species (S), and treatments (T) on chlorophyll *a, b*, total (Chl*a*, Chl*b*, Chl tot), a/b ratio, and carotenoids (Car).

Days	Species	Treatments	Chl*a* (μg g^{-1})	Chl*b* (μg g^{-1})	Chl tot (μg g^{-1})	a/b ratio	Car (μg g^{-1})
FW			0.746a	0.248a	0.994a	3.079a	0.156a
DS			0.665b	0.221b	0.887b	3.082a	0.140b
REC			0.592c	0.195c	0.787c	3.096a	0.133b
DS			0.500d	0.168d	0.667d	3.106a	0.119c
DS			0.416e	0.139e	0.555e	3.059a	0.100d
REC			0.334f	0.112f	0.446f	3.119a	0.0818e
	P. hybridum		0.702a	0.234a	0.934a	3.055b	0.168a
	P. peltatum		0.372c	0.114c	0.486c	3.319a	0.105b
	D. hybrida		0.553b	0.193b	0.746b	2.897c	0.092c
		100% WC	0.536a	0.185a	0.721a	3.001a	0.117b
		60% WC	0.535a	0.175a	0.710a	3.134a	0.122 ab
		40% WC	0.555a	0.181a	0.736a	3.131a	0.127a
Main effects							
Days			***	***	***	ns	***
Species			***	***	***	***	***
Treatments			ns	ns	ns	ns	***
Interaction							
D x S			***	***	***	*	***
D x T			ns	ns	ns	ns	ns
S x T			**	*	**	ns	**
D x S x T			ns	ns	ns	ns	ns

Values (mean ± se) within each column, followed by the same letter, do not significantly differ at $p < 0.05$ according to Tukey's test; ns = not significant; significant at $p < 0.05$ (*), 0.01 (**), and 0.001 (***). Three biological replicates were used for the analysis (n = 3).

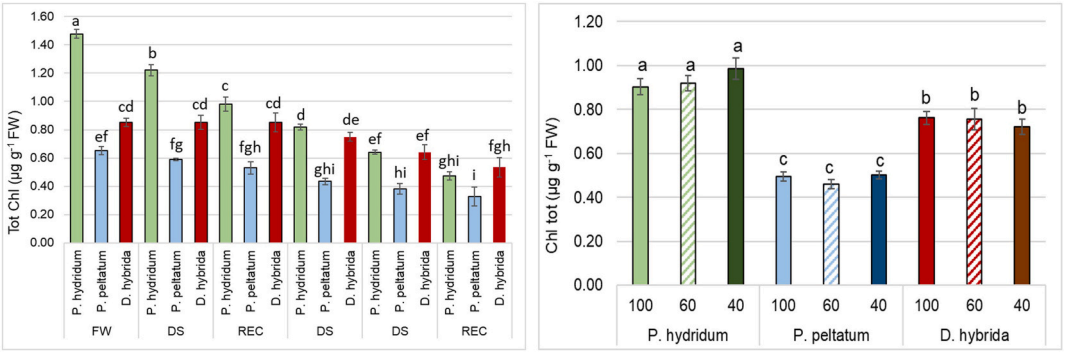

Fig. 7. Interaction effect of *species* × *days* and *species* x *water treatments* on total chlorophyll. Data are means ± standard error (n = 3). According to Tukey's test, different letters denote significant differences between irrigation treatments at each sampling time at $p < 0.05$.

consequences for their aesthetic value [16]. Different studies on ornamental plants have focused on continuous drought stress, whereas our experiment highlights the importance of intermittent drought and recovery, which better reproduces irrigation practices in urban and peri-urban areas.

Annual plants that can avoid drought cannot be used extensively in landscapes with limited water availability because they quickly lose their ornamental value. In contrast, drought-tolerant plants, which can maintain a good visual appearance under conditions of water stress, can be used in landscapes where irrigation is not available or sporadic. Therefore, the choice of plant species is strategic for identifying those that can maintain their aesthetic appearance even under water shortage conditions [32].

A more efficient approach for selecting drought-tolerant plants is to evaluate the physiological and biochemical responses of the plant species to stress [33]. Differences in drought responses of different plants to drought have emerged even within species belonging to the same genus.

Both the response of plants to drought stress and the possibility of recovery when water availability conditions are restored are complex processes that require plants to expend metabolic energy and activate physiological and biochemical processes. These responses depend on the genotype used, age of the plant, and intensity and duration of water stress [16]. To date, choosing the most suitable ornamental species for urban green areas or improving plant tolerance to water stress through breeding is difficult because of the limited information available [32]. The development and survival of plants are often measured to evaluate their tolerance to water stress. Therefore, indirect selection for drought tolerance using physiological or biochemical methods may be an easier and more effective approach. In the present study, we investigated three herbaceous species to understand their adaptation to drought. The tolerance of *Pelargonium* to drought stress is a complex phenomenon, and differences exist between species and cultivars [34]. Severe water stress in *P. peltatum* showed results, and the application of mycorrhiza mitigated the stress [35]. The increase in tolerance was

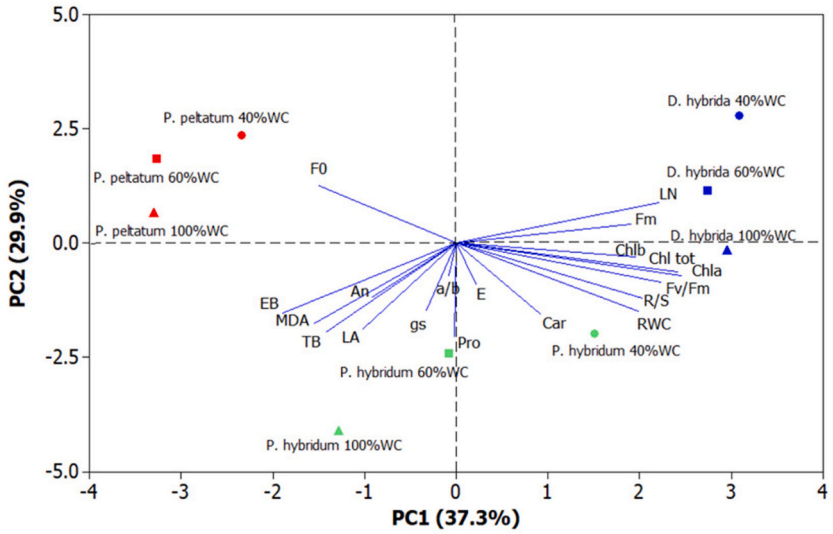

Fig. 8. Biplot graph and scores based on the first two components of morphometric, physiological, and biochemical traits of *P. hybridum, P. peltatum* and *D. hybrida* response to water treatments. EB = epigeous dry biomass; TB = total dry biomass; R/S = root/shoot ratio; LA = leaf area; LN = leaf number; An = net photoynthesis; gs = stomatal conductance; E = transpiration rate; F0 = minimum fluorescence; Fm = maximum fluorescence; RWC = relative water content; Fv/Fm = maximum quantum efficiency of the PSII; Pro = proline; chla = chlorophyll a; chlb = chlorophyll b; chltot = total chlorophyll; Car = carotenoids; a/b = chlorophyll ratio.

associated with improved water uptake, ensured by higher root biomass accumulation and mycorrhization. Our results indicate that significant genotypic variation exists in most physiological and biochemical parameters. The response to water stress can be classified as "low", "moderate" and "severe", based on soil water capacity or water potential [36]; this means that attention is always devoted to

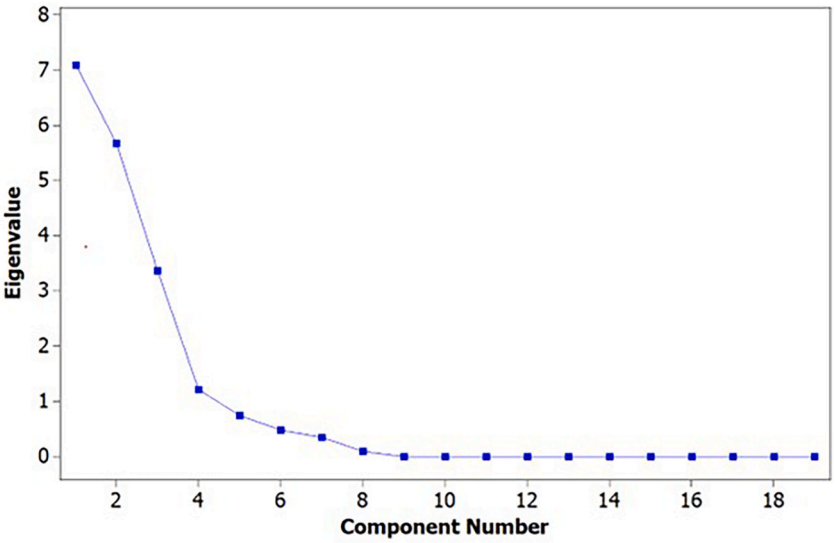

Fig. 9. The graph of the eigenvalue-component number of the scale.

Table 3
Eigenanalysis of the correlation matrix.

PC	Eigenvalue	Proportion	Cumulative
PC1	7.089	0.373	0.373
PC2	5.678	0.299	0.672
PC3	3.369	0.177	0.849
PC4	1.208	0.064	0.913

Table 4
Factor loadings of physiological and biochemical parameters on the first four principal components (PC1, PC2, PC3, and PC4) derived from principal component analysis (PCA).

Variable	PC1	PC2	PC3	PC4
F0	−0.211	0.220	0.180	0.336
Fm	0.269	0.074	0.332	−0.050
Fv/Fm	0.315	−0.152	0.034	−0.306
LA	−0.144	−0.331	−0.018	0.010
LN	0.312	0.154	−0.183	−0.166
EB	−0.267	−0.271	−0.024	0.126
TB	−0.200	−0.342	0.033	0.043
R/S	0.285	−0.212	0.147	−0.246
MDA	−0.218	−0.310	0.109	0.056
Pro	−0.002	−0.362	0.210	0.182
Chla	0.345	−0.126	−0.001	0.198
Chlb	0.276	−0.056	−0.255	0.405
Chl tot	0.340	−0.112	−0.068	0.259
a/b	−0.012	−0.127	0.468	−0.296
Car	0.129	−0.273	0.288	0.275
An	−0.129	−0.209	−0.403	−0.128
E	0.030	−0.158	−0.374	0.129
gs	−0.047	−0.260	−0.258	−0.425
RWC	0.280	−0.262	−0.057	0.042

the physiological state and the level of stress suffered by plant [37]. In our study, intermittent drought was applied to understand the potential stress memory of plants in response to the same type of stress. Abiotic stress memory in plants refers to the physiological and molecular changes that enable plants to respond more effectively to recurring stress after initial exposure [38,39]. This phenomenon is facilitated by processes such as acclimation, priming, and retention of stress-induced epigenetic modifications [40,41]. Interestingly, although the concept of stress memory is increasingly recognised, the mechanisms that maintain this memory are not fully understood. It has been suggested that the memory of stress exposure is associated with epigenetic changes, accumulation of signalling proteins, and transcription factors [41,42]. Moreover, the use of chemical priming agents has been shown to enhance abiotic stress tolerance, suggesting that both natural and synthetic compounds are involved in the establishment of stress memory [43,44].

Based on this information, the responses of the obtained ornamental plants are discussed. During the experiment, as water stress advanced, our plants reduced their ability to assimilate CO_2, and as a result, photosynthesis was reduced. This is related to the increased resistance in the mesophyll and stomatal layers, which leads to a decrease in organ dry weight [45].

As an initial response to water shortage, rapid closure of the stomata is observed, which reduces CO_2 assimilation [46], stomatal conductance (gs), transpiration rate (E), and net photosynthesis (An) [47]. While this response has been widely reported in several ornamental species under drought stress, our results indicate a species-specific recovery pattern. In particular, *D. hybrida* plants when subjected to moderate water stress (60%WC), unlike the other two species, showed photosynthetic activity similar to that of plants with full water content at the end of the second recovery period. This behaviour suggests a higher capacity for physiological recovery following intermittent drought, extending previous studies that mainly focused on continuous water stress conditions.

The decline in leaf RWC during drought stress indicates a progressive loss of plant water status, particularly during the second drought cycle. *P. hybridum* was able to maintain RWC values close to those of well-watered plants throughout the experiment, while *P. peltatum* and *D. hybrida* showed more pronounced reductions under water deficit conditions, suggesting a lower capacity to regulate water balance. Nevertheless, the recovery of RWC after re-watering demonstrates that *P. hybridum* and *D. hybrida* were able to restore their water balance once favourable soil moisture conditions were re-established (Fig. 3A and C).

The higher leaf water content in *D. hybrida*, especially in *P. hybridum,* can maintain metabolic activities and light dissipation [48]. The ability of *P. hybridum* to maintain its relative water content under drought stress conditions is a good indicator of drought tolerance [49]. Some authors have suggested that the RWC is a good index of flower longevity [50,51] and leaf dehydration tolerance in ornamental plants [52,53]. Pourghayoumi et al. [48] found a positive correlation between RWC and total chlorophyll content, suggesting that RWC is responsible for maintaining chlorophyll levels.

Through physiological adaptations (reduced stomatal conductance, net photosynthesis), our study species were able to adapt to repeated drought cycles. One of the aims of this trial was to evaluate changes in the accumulation of protective compounds, including amino acids and carbohydrates [54], in response to drought stress and their effects on increasing drought tolerance of plants.

Plants activate biochemical mechanisms, such as osmotic adjustment, to adapt to water shortages. One mechanism of osmotic adjustment is the accumulation of compatible solutes, such as amino acids and proline [55]. Under drought stress, our results showed that proline accumulated in the leaves of stressed plants of different species in relation to the control condition and that more severe drought stress resulted in an increase in proline accumulation. Our results highlight that in the studied species, proline accumulation correlates with adaptive responses to drought.

Among the studied species, under drought stress conditions, a higher accumulation of proline in *D. hybrida* was recorded, suggesting a higher ability to contrast stress through osmotic adjustment and antioxidant protection. These compatible solutes have been shown to increase stress tolerance in plants by improving osmotic regulation and cell membrane protection [52,56,57]. Other studies have reported that proline accumulation is not associated with drought tolerance [58]; in our experiment, proline levels progressively

increased with stress intensity and decreased after reirrigation, demonstrating the main role of this osmolyte in drought stress adaptation. This trend suggests that proline plays an active role in preventing stress-induced damage.

Pourghayoumi et al. [48] found a negative correlation between RWC and proline accumulation. This finding confirms that the increase in proline is associated with lower water availability, which can be negatively correlated with tissue water retention.

MDA content is widely recognised as a reliable indicator of lipid peroxidation under adverse abiotic stress conditions. Additionally, proline concentration, which functions as an antioxidant and osmoprotectant, typically increases in response to drought stress. Based on this knowledge, our species also accumulated high levels of proline under drought conditions, which alleviated cellular hyperosmolarity and ion imbalance. At the end of the second recovery period (REC), the DS plants (60% and 40% WC) of all species were similar to the control ones.

The photosynthetic pigment content was not affected by drought and recovery stress, whereas a reduction in pigment content was observed in relation to the species and duration of the treatments. Abiotic stresses, such as water stress, affect chlorophyll biosynthesis, resulting in a reduction in chlorophyll concentration. Our results showed that during the recovery phase, plants delayed chlorophyll biosynthesis after water stress. A lower level of chlorophyll reduces the green colour of plants; hence, chlorophyll concentration is a relevant trait for the ornamental value of the plants.

Overall, the photosynthetic changes in the three species demonstrated that photosynthetic parameters, along with antioxidant compounds, are good indicators of crop adaptation to drought conditions.

The experiment focused on short-term physiological and biochemical responses to repeated drought and rewatering cycles and did not evaluate long-term effects on plant growth or ornamental quality. Future studies should include a wider range of ornamental species and assess drought responses under field conditions to validate the applicability of these findings to urban landscaping systems.

5. Conclusion

Results demonstrated that photosynthetic changes were the most affected by antioxidants and osmolytes, such as proline. *P. hybridum* maintained high leaf RWC and photosynthetic efficiency (Fv/Fm) throughout the experiment, although significant reductions in net photosynthesis (An) and transpiration (E) during stress events. *D. hybrida* presented moderate physiological decreases but high values in pigment parameters and proline accumulation, particularly during the second drought stress period. *P. peltatum* showed intermediate responses, with declines in RWC and photosynthesis under severe stress but rapid recovery following rewatering. Considering all parameters, species were classified in their drought tolerance as: *P. hybridum* > *D. hybrida* = *P. peltatum*, highlighting that each species showed distinct adaptations. These results indicated that the combined evaluation of physiological and biochemical parameters under repeated drought and recovery conditions can improve the selection of ornamental species better suited for low-water urban landscapes.

CRediT authorship contribution statement

Luca Leotta: Writing – original draft, Formal analysis, Data curation. **Stefania Toscano:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Agnese Carchiolo:** Formal analysis, Data curation, Writing – original draft. **Antonio Ferrante:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Daniela Romano:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Daniela Romano reports article publishing charges was provided by University of Catania Department of Agriculture Food and Environment. If there are other authors, they 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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