Can anthocyanin presence ameliorate the photosynthetic performance of *Prunus* saplings subjected to polyethylene glycol-simulated water stress?

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Abstract

The aim was the evaluation of the biochemical and physiological responses of green- (GP) and red-leafed (RP) *Prunus cerasifera* mature leaves to 20 d of polyethylene glycol (PEG 6000)-induced water stress in order to elucidate a possible ameliorative role exerted by anthocyanins. At 10 d, the anthocyanin content remained unchanged in RP water-stressed leaves. Photosynthetic rate was lower in GP than that of RP (83.4 *vs*. 76.5%, respectively), paralleled by a higher degree of photoinhibition (F_v/F_m) in GP leaves. Leaves of GP accounted for higher content of soluble sugars at 10 d, when RP only showed a slight sucrose increase. At 20 d of stress, both morphs recovered their F_v/F_m values, suggesting the ability of both genotypes to adjust their photosynthetic metabolism under conditions of water stress. In conclusion, besides the sunscreen role served by anthocyanins, the carbon sink by these flavonoids might have further prevented sugar accumulation and the consequent sugar-promoted feedback regulation of photosynthesis in drought-stressed red leaves.

Additional key words: chlorophyll *a* fluorescence; drought; photosystem II; sugar metabolism.

Introduction

Trees represent a good indicator of urban ecosystem status and provide multiple benefits in urban environments (Millennium Ecosystem Assessment 2005). Trees are well known to provide important ecosystem services, such as air pollutant removal, carbon sequestration, urban heat island mitigation (Tyrväinen *et al.* 2005), as well as other socio-economic benefits; for example, they can improve human mental and physical health due to their aesthetic value (Turner-Skoff and Cavender 2019). Environmental factors (temperature, light, water availability, and pollution) and edaphic conditions (texture, structure, and underground obstacles) strongly influence the performances of tree species in urban areas (Núñez-Florez *et al.* 2019).

Drought is one of the main constraints for trees in Mediterranean cities (Sjöman *et al.* 2018). Under water-limiting conditions, different strategies are adopted by leaves to reduce water losses, which include leaf movement, stomata closure, osmotic adjustment, and enhancement of antioxidant systems (Fang and Xiong 2015). Sugar metabolism is also affected in leaves by drought;

an increased content of sugars usually occurs during the first stages of water stress (Pinheiro *et al.* 2001, Pinheiro and Chaves 2011). This transient sugar accumulation perturbs the overall leaf carbon balance and increases the sensitivity of leaves to photoinhibition due to possible feedback regulation of photosynthesis (Krapp and Stitt 1995, Adams *et al.* 2013).

Prunus cerasifera Ehrh. var. *pissardii* plants have a high aesthetic value due to the permanent red color of their leaves, which is attributable to the presence of anthocyanins. Foliar anthocyanins, with their sunscreen role, can also contribute to a better tolerance when red-leafed plants are subjected to abiotic constraints (Chalker-Scott 1999, Karageorgou and Manetas 2006, Winefield *et al.* 2009, Landi *et al.* 2013, 2015). Under stress conditions, the ability of anthocyanins to intercept a proportion of supernumerary photons striking chloroplast can ameliorate the plant performance, thereby reducing the risk of photoinhibition (Steyn *et al.* 2002, Manetas *et al.* 2003, Zhang *et al.* 2010, Landi *et al.* 2014).

Beside their sunscreen prerogative, anthocyanin and sugar metabolism are strictly interconnected, and the sugar-

promoted enhancement of anthocyanin contents has been demonstrated *in planta* (Solfanelli *et al.* 2006, Das *et al.* 2012). Therefore, it has been postulated that anthocyanins might offer additional photoprotective functions, such as a sugar-buffering role, which contribute in ameliorating plant performance under stress conditions (Landi *et al.* 2015, Gould *et al.* 2018). Recently, a dynamic interplay between anthocyanin biosynthesis, photosynthesis, and sugar metabolism was observed in red-leafed *Prunus* seedlings during the leaf ontogenesis (Lo Piccolo *et al.* 2018). The authors observed that anthocyanin biosynthesis maintained an efficient carbon-sink strength in young and senescent leaves, avoiding possible negative effects related to excessive leaf sugar accumulation.

In the urban environment, water stress represents a crucial factor for tree survival for different reasons. Climate change promotes more extended and frequent period of drought (Dai 2011, 2013), with a parallel rise in temperatures and heatwaves, thus increasing the urban heat island effect (Grimm *et al.* 2008). According to these considerations, the selection of urban trees should be focused on stress resistance and climate adaptation, in addition to the aesthetic appeal (Pauleit 2003). To date, only a few studies have reported the effects of environmental stresses in the urban environment on red-leafed tree species (*e.g.*, Napoli *et al.* 2016) and only a research work focused on the effect of drought in *P. cerasifera* var. *pissardii* in Mediterranean area (Kyparissis *et al.* 2007). The aim of the present work was the comparison of the leaf response of *P. cerasifera* var. *pissardii* (with red leaves) and *P. cerasifera* clone 29C (with green leaves) to a short-term polyethylene glycol (PEG 6000)-induced drought. The attempt was to clarify whether the red morph was more tolerant than the green counterpart and to describe a possible role of anthocyanins in water-limiting conditions.

Materials and methods

Plant material: One-hundred four-year-old *P. cerasifera* saplings (clone 29C, GP; var. *pissardii*, RP) were purchased from an Italian nursery (*Vivai Battistini*, Cesena, Italy) and after a homogeneous selection for size and shape, were grafted onto Myrabolan 29C rootstock in November 2016. For detail of cultivation, *see* Lo Piccolo *et al.* (2018). Saplings were maintained under greenhouse conditions until March 2018, when they were transferred to open field conditions at the Department of Agriculture, Food and Environment, University of Pisa, Italy (43°42'N, 10°25'E). The experiments started the 3 July and three sampling dates were chosen (0, 10, 20 d after the start of the experiment). During the cultivation period, minimum and maximum air temperatures were 21.2 ± 1.0 and $30.6 \pm 1.1°C$, respectively. Plants were kept well-watered and fertilised before the imposition of PEG 6000 treatment, which was utilised to simulate the drought condition.

Fifty plants were treated with PEG 6000 solution (150 g L^{-1}) for 20 d; the other fifty were used as controls and were well irrigated with tap water. All control and treated plants were watered every day (with water or PEG 6000 solution, respectively) through an automated system in order to maintain field capacity.

At each sampling date (0, 10, and 20 d after the beginning of the simulated water stress), fully expanded leaves were used for physiological analyses. Leaves homogeneous to those used for physiological analysis were collected and immediately frozen in liquid nitrogen and stored at –80°C until biochemical analysis. All utilised chemicals were purchased from *Sigma-Aldrich*, Milan, Italy.

Water potential: The predawn water potential (ψ_w) was determined in a fully expanded leaf per plant ($n = 4$) by a Scholander pressure chamber (model *600*, *PMS Instrument*, Albany, OR, USA).

Leaf gas-exchange and chlorophyll (Chl) *a* fluorescence analysis: Gas-exchange parameters were determined in fully expanded leaves ($n = 5$) from 10:00 to 14:00 h at ambient light and CO_2 concentration [on average 1,800 μmol(photon) m^{-2} s^{-1} and 390 μmol(CO_2) mol^{-1}]. Net assimilation of photosynthesis (P_N) and stomatal conductance (g_s) were measured. Instantaneous water-user efficiency (WUE) was calculated according to Medrano *et al.* (2015) as net assimilation of photosynthesis/ transpiration (P_N/E) ratio. For the analyses, a portable infrared gas analyser (*Li-Cor 6400*, *Li-Cor Inc.*, Lincoln, NE, USA) was used.

Chl fluorescence parameters were measured in fully expanded leaves ($n = 5$) using a *PAM-2000* fluorometer (*Walz*, Effeltrich, Germany). Dark-adapted leaves (30 min) were initially exposed to a weak, modulated measuring beam [< 0.2 μmol(photon) m^{-2} s^{-1}], followed by the exposure to a flash of saturated white light [about 15,000 μmol(photon) m^{-2} s^{-1} and a duration of 800 ms]. In this way, the determination of minimal and maximal Chl fluorescence (F_0 and F_m, respectively) was determined, and potential quantum yield of PSII photochemistry (F_v/F_m) was calculated. When actinic light is given [500 μmol(photon) m^{-2} s^{-1}], the photosynthetic light reactions starts and a steady-state fluorescence yield (F_s) is reached. The effective photochemical yield of PSII (Φ_{PSII}) was calculated as ($F_m' - F_s)/F_m'$, where F_m' is the maximum level of fluorescence yield in the light induced by a saturation pulse over imposed to the actinic light (Genty *et al.* 1989). The nonphotochemical quenching (NPQ) was calculated according to the equation NPQ = $F_m/F_m' - 1$ of Bilger and Björkman (1990). The quantum yield of nonregulated energy loss and quantum yield of nonphotochemical quenching (Φ_{NO} and Φ_{NPQ}, respectively) were calculated as F_s/F_m' and ($F_m' - F_s)/(F_s - F_m)$, respectively (Klughammer and Schreiber 2008).

Anthocyanin content: Anthocyanins were extracted as described in Landi *et al.* (2014) in acidified methanol (1.5% HCl, v/v) overnight at room temperature ($n = 3$), and the absorbance at 535 nm was determined using an *Ultrospec 2100 Pro* UV-VIS spectrophotometer (*GE Healthcare Ltd.*, Chicago, IL). Absorbance values were converted to cyanidin-3-O-glucoside equivalents (molar extinction coefficient of 34,300 M cm^{-1} and molecular mass of 484.3 g mol^{-1}) and expressed as mg g^{-1}(DM).

Soluble sugar, sorbitol, and starch content: Sugar and polyol quantifications ($n = 3$) were conducted according to Yusof *et al.* (2016) and Sotelo *et al.* (2014) with some modifications, as reported in Lo Piccolo *et al.* (2018). Glucose and fructose content represent the nontranslocated sugars (NT), sucrose and sorbitol the translocated sugars (T), the sum of NT and T sugars represents the total sugar (TOT). Then, the ratios between T/NT, T/TOT, and NT/TOT were calculated.

Statistical analysis: Data were analysed by two-way analysis of variance (*ANOVA*) using PEG treatment and sampling date as the variability factors, followed by *Fisher*'s least significant difference (LSD) *post-hoc* test ($p=0.05$). For Chl fluorescence parameters, a one-way *ANOVA* test was applied, using the sampling date as variability factor. Before *ANOVA*, the assumption of homogeneity of variances was tested using *Bartlett*'s test. All statistical analyses were conducted using *GraphPad* (*GraphPad*, La Jolla, CA, USA).

Results and discussion

Leaf water potential: Values of ψ_w remained constant in well-watered saplings until the end of the experiment (about –0.5 MPa), whereas a strong reduction was observed in leaves of water-stressed saplings (about –1.3 MPa; Fig. 1). The reduction was recorded after 10 d of water stress, and ψ_w values remained unchanged after further 10 d of stress. The decrease of water potential in *Prunus* species subjected to water stress has been already reported (Escobar-Gutiérrez *et al.* 1998, Kyparissis *et al.* 2007, Mellisho *et al.* 2011, Jiménez *et al.* 2013) although in this study the decrease of water potential ψ_w occurred earlier than that in previous research, likely due to the severity of the treatment imposed in our experiments.

Leaf gas exchange, Chl fluorescence, and anthocyanin content: Ten days after the PEG treatment, P_N declined severely in both GP and RP leaves, but with a higher amplitude in GP than RP leaves (83.4 and 76.5%, respectively). A drastic constraint of g_s values was also observed in both the morphs (Fig. 2). The parallel decline of these two parameters suggests a strong influence of stomatal limitation on CO_2 assimilation rate, and it is

supportive for previous finding highlighting that stomatal closure represents one of the first response to water limitation (Flexas and Diaz-Espejo 2015). The pattern of P_N and g_s was also maintained in RP morphs at the end of the experiment, in which P_N and g_s values remained similar to those detected at 10 d (Fig. 2*B,D*). Conversely, a slight increase in P_N, associated with no significant changes in g_s, was detected in GP morphs at 20 d as compared to the values measured at 10 d (Fig. 2*A,C*). The different behaviour in P_N and g_s detected in GP and RP morphs at 20 d (compared to values obtained at 10 d) highlighted that biochemical limitations were also responsible for photosynthesis impairment.

In water-stress conditions, plants need to optimize carbon and water utilization in relation to stomatal aperture (Manzoni *et al.* 2011). An increase in WUE value was only detected in RP leaves at the end of the treatments. This parameter has been interpreted as a measure of carbon gain per unit of water loss (Singh and Reddy 2011). The improvement of WUE could play a vital role for plants experiencing water withholding in crops, and therefore also in the urban environment (McCarthy *et al.* 2011, Singh and Reddy 2011) (Fig. 2*E,F*).

Chl fluorescence parameters highlight that both the *Prunus* morphs resulted photoinhibited (*i.e.*, a reduction of F_v/F_m) 10 d after the start of water stress, with lower values in GP (0.67) than that of RP morphs (0.73). In control plants of both morphs, no differences in Chl fluorescence parameters were reported during the experimental period (data not shown). Both stressed GP and RP leaves recovered completely the values of F_v/F_m at the end of the experiment (0.74 and 0.77, respectively; Table 1). Similarly, Φ_{PSII} decreased after 10 d more strongly in GP than that in RP (45 and 28% in GP and RP, respectively) and did not further decrease after 20 d of the treatment in GP whilst in RP leaves values of Φ_{PSII} underwent a further decrement (40%; Table 1).

Drought-induced photoinhibition is a common response among plant species, including *Prunus* spp. (Kyparissis *et al.* 2007, Pérez-Jiménez *et al.* 2017). The decline of photosynthetic yield under water stress might be partially related to a decreased photochemical PSII activity (Govindjee 2004, Foyer 2018), which generates a surplus of excitation energy in thylakoid membranes not efficiently tunnelled to nonphotochemical dissipative mechanisms
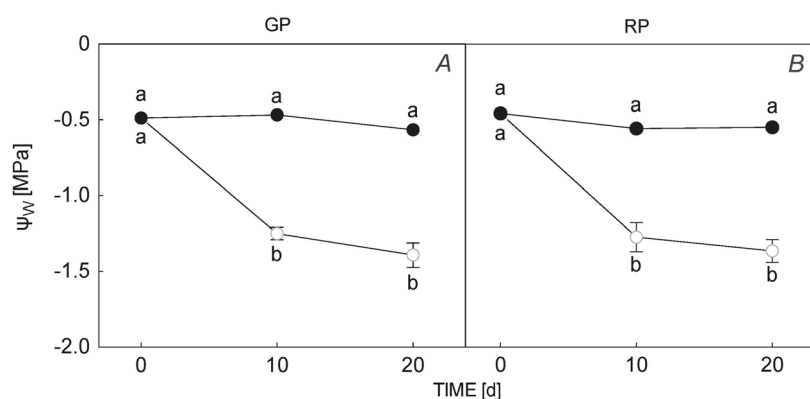

Fig. 1. Predawn water potential (Ψ_w) of leaves of two morphs of *Prunus cerasifera* saplings with green (GP) or red (RP) leaves subjected to PEG 6000-induced water stress (*open circle*) or tap-water (control; *closed circle*). Each point is the mean of four replicates (± standard deviation). Data are subjected to two-way *ANOVA* with PEG treatment and sampling date as sources of variation. Means flanked by *the same letter* are not statistically different for $p=0.05$ after *Fisher*'s least significant difference *post-hoc* test.
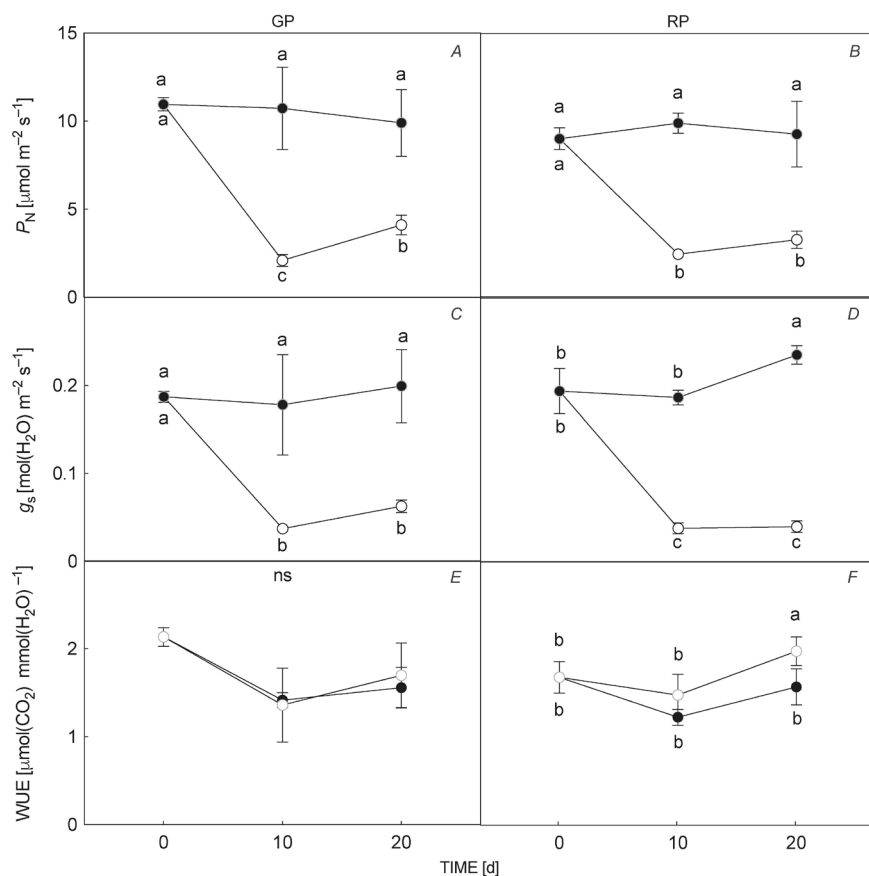
Fig. 2. Gas-exchange parameters of leaves of two morphs of *Prunus cerasifera* saplings with green (GP) or red (RP) leaves subjected to PEG 6000-induced water stress (*open circle*) or tap-water (control; *closed circle*). Each point is the mean of five replicates (± standard deviation). Data are subjected to two-way *ANOVA* with PEG treatment and sampling date as sources of variation. Means flanked by *the same letter* are not statistically different for *p*=0.05 after *Fisher*'s least significant difference *post-hoc* test. The absence of letters indicates nonsignificant interactions between the two factors. g_s – stomatal conductance; P_N – net photosynthetic rate; WUE – water-use efficiency (= P_N/E).

Table 1. Potential quantum yield of PSII photochemistry (F_v/F_m), effective quantum yield of PSII photochemistry (Φ_{PSII}), quantum yield of nonregulated energy loss (Φ_{NO}), quantum yield of nonphotochemical quenching (Φ_{NPQ}), and nonphotochemical quenching (NPQ) in leaves of two morphs of *Prunus cerasifera* saplings with green (GP) or red (RP) leaves subjected to PEG 6000-induced water stress. Data of 0 d represent the start of the experiment. Each point is the mean of five replicates (± standard deviation). Data are subjected to one-way *ANOVA* with sampling date as sources of variation. Means flanked by *the same letter* are not statistically different for *p*=0.05 after *Fisher*'s least significant difference *post-hoc* test.

| | GP | | | RP | | |
	0 d	10 d	20 d	0 d	10 d	20 d
F_v/F_m	0.77 ± 0.03^a	0.67 ± 0.05^b	0.74 ± 0.01^a	0.78 ± 0.01^a	0.73 ± 0.02^b	0.77 ± 0.09^a
Φ_{PSII}	0.40 ± 0.04^a	0.22 ± 0.03^b	0.24 ± 0.04^b	0.43 ± 0.04^a	0.31 ± 0.02^b	0.26 ± 0.01^c
Φ_{NO}	0.21 ± 0.01	0.23 ± 0.02	0.23 ± 0.02	0.23 ± 0.01	0.24 ± 0.01	0.22 ± 0.01
Φ_{NPQ}	0.38 ± 0.03^b	0.54 ± 0.03^a	0.52 ± 0.03^a	0.33 ± 0.05^c	0.44 ± 0.01^b	0.51 ± 0.02^a
NPQ	1.77 ± 0.08^b	2.29 ± 0.31^a	2.22 ± 0.12^a	1.44 ± 0.24^c	1.87 ± 0.06^b	2.23 ± 0.20^a

(Ruban 2016). Results of the present experiments showed no changes in Φ_{NO} during the water stress in both GP and RP, whereas the increment of Φ_{NPQ} values highlight that both morphs were able to dissipate the excessive excitation energy through regulated processes (Hendrickson *et al.* 2004). Indeed, in both morphs, NPQ and Φ_{NPQ} increased significantly, but in RP the increase of both parameters paralleled the water stress duration, whereas in GP both increased after 10 d and remained unchanged at 20 d (Table 1). However, both morphs recovered completely from photoinhibition at the end of the experiment, which is supportive for the ability of plants, after a first phase

of stress and metabolic perturbation, to adjust their metabolism in condition of reduced water availability. Similar results were also demonstrated in both green- and red-leafed *Prunus* plants during a typical Mediterranean summer (Kyparissis *et al.* 2007).

The anthocyanin content decreased in control RP leaves 10 d after the beginning of the experiment, whereas it remained constant in stressed RP leaves. The anthocyanin content was below the instrument detectability in GP leaves. Of note, at 20 d, anthocyanin content drastically decreased in stressed RP whereas it remained unchanged in controls (compared to 10 d; Fig. 3).

Anthocyanins are usually believed to ensure a photo-protective function in leaves (Hughes *et al.* 2005, Gould *et al.* 2018, Moustaka *et al.* 2018). These pigments, absorbing principally the yellow–green wavebands and a proportion of blue and red light, reduce the photons that would otherwise strike the chloroplast, thereby alleviating excessive excitation pressure to PSII (Harborne 1958, Tattini *et al.* 2017). This sunscreen prerogative might explain the F_v/F_m decrease at a lower extent in RP compared to GP stressed morphs observed at 10 d, and can also explain the constitutive lower level of NPQ in RP control leaves respect to GP, a common feature of red-leafed species (Manetas *et al.* 2002, Hatier *et al.* 2013, Hughes *et al.* 2014, Landi *et al.* 2014). Leaves belonging to both stressed morphs increased the level of NPQ to dissipate the excessive radiation (10 d) showing higher NPQ values in GP than in RP leaves (2.29 and 1.87, respectively), when the anthocyanin content remained unchanged under water stress. Higher values of Φ_{NPQ} detected in stressed GP than RP at 10 d (+42 and +33%, respectively in comparison to their relative controls), demonstrated that GP morphs necessitated to dissipate more excitation energy than the anthocyanin-equipped RP morph, in which the mesophyll cells are partially shaded by the anthocyanin layer, thereby reducing the burden of light to chloroplast. Supportively for this sunscreen (photoprotective) role, leaves of RP morphs necessitated to further increase NPQ and Φ_{NPQ} at 20 d (19 and 16%, respectively) in respect to values detected at 10 d, when the anthocyanin content was strongly reduced. This also explains the further decrease in Φ_{PSII} values at 20 d in RP and then the lack of a partial P_N recovery at 20 d as observed in GP leaves.

The gas exchange, Chl fluorescence, and anthocyanin analyses are supportive for a photoprotective role of antho-cyanins and also highlighted a strict interplay between these colourful flavonoids and carotenoids in leaf photo-protection (Hughes *et al.* 2007, 2012; Lo Piccolo *et al.* 2018).
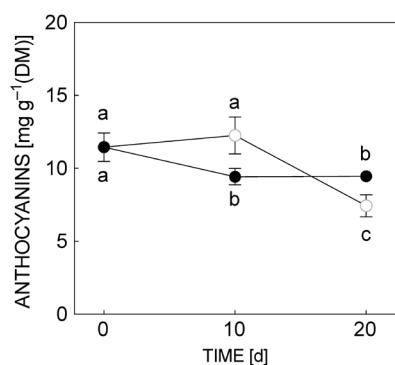

Fig. 3. Anthocyanin content in leaves of *Prunus cerasifera* var. *pissardii* saplings leaves subjected to PEG 6000-induced water stress (*open circle*) or tap-water (control; *closed circle*). Each point is the mean of three replicates (± standard deviation). Data are subjected to two-way *ANOVA* with PEG treatment and sampling date as sources of variation. Means flanked by *the same letter* are not statistically different for *p*=0.05 after *Fisher*'s least significant difference *post-hoc* test.

Sugar analysis: After 10 d of water stress, a sharp increase in soluble sugars (glucose, fructose, and sucrose) was observed in leaves of GP (Fig. 4*A,C,E*), whilst only a slight increase in sucrose was observed in RP morphs (Fig. 4*F*). However, after further 10 d of stress, the differences in sucrose content between control and stressed leaves of GP were less pronounced, and fructose content was similar in control and stressed leaves (Fig. 4*A–F*). At the end of the experiment (20 d), the sucrose content in RP stressed leaves was similar to control plants (Fig. 4*F*). In both morphs, a strong increase in sorbitol was observed and its content in GP at 20 d of treatment was higher than the value recorded at 10 d, whereas in RP leaves at 20 d (Fig. 4*G,H*) it was unchanged when compared to values found at 10 d. Sorbitol has been reported to be the main translocating sugar in Rosaceae family (Bieleski and Redgwell 1980) and the increase in sorbitol content is a common response in *Prunus* plants under drought (Lo Bianco *et al.* 2000, Rieger *et al.* 2003). It has been reported that sorbitol plays a key role in leaf osmotic adjustment during drought stress in cherry (Ranney *et al.* 1991) and apple (Wang and Stutte 1992). Drought-induced perturbation of sugar metabolism was also evident in leaves of both the morphs by the analyses of starch patterns. Starch content decreased in both 10- and 20-d stressed RP and GP leaves when compared with their relative controls, but a stronger decline of starch amount was detected in GP than that in RP leaves after 20 d of treatment (Fig. 4*I,J*).

Besides the classic assumption that anthocyanins serve a sunscreen function in leaves, anthocyanin synthesis further represents an 'expensive' carbon sink (Gould 2004), which, under stress, might contribute to reduce sugar accumulation when plants experience an imbalance between light burden to chloroplast and the rate of carbon-assimilating pathway (Steyn *et al.* 2002, Landi *et al.* 2015, Gould *et al.* 2018, Lo Piccolo *et al.* 2018). Sugar-promoted anthocyanin accumulation was previously demonstrated by Solfanelli *et al.* (2006), therefore, it has been hypothesized that their biosynthesis limits the accumulation of foliar sugars under stress (Landi *et al.* 2015). In this way, anthocyanin synthesis might represent a further mechanism by which these pigments prevent photoinhibition, caused by sugar-promoted feedback regulation of photosynthesis (Adams *et al.* 2013, Eveland and Jackson 2012). The lower reduction in carbon assimilation detected in RP compared to GP, followed by a lower soluble sugar (sucrose, glucose, and fructose) increase observed at 10 d of water stress is supportive for this hypothesis (Figs. 2–4). In addition, the strong anthocyanin content reduction found in RP stressed leaves at 20 d of water stress (Fig. 3) is likely attributable to a conservative strategy of RP stressed leaves, in which the production of T is prevalent, as shown by values of T/NT (Table 2). On the contrary, the lack of the alternative carbon sink of anthocyanins in GP leaves was likely the reason of a further increase of sorbitol, as an attempt to contrast the accumulation of glucose and fructose, which are well-known feedback inhibitors of photosynthesis.

The lower content of sugars in RP *vs.* GP leaves in the first stages of the stress (10 d) can be related to two main factors: the biosynthesis of anthocyanins and the higher
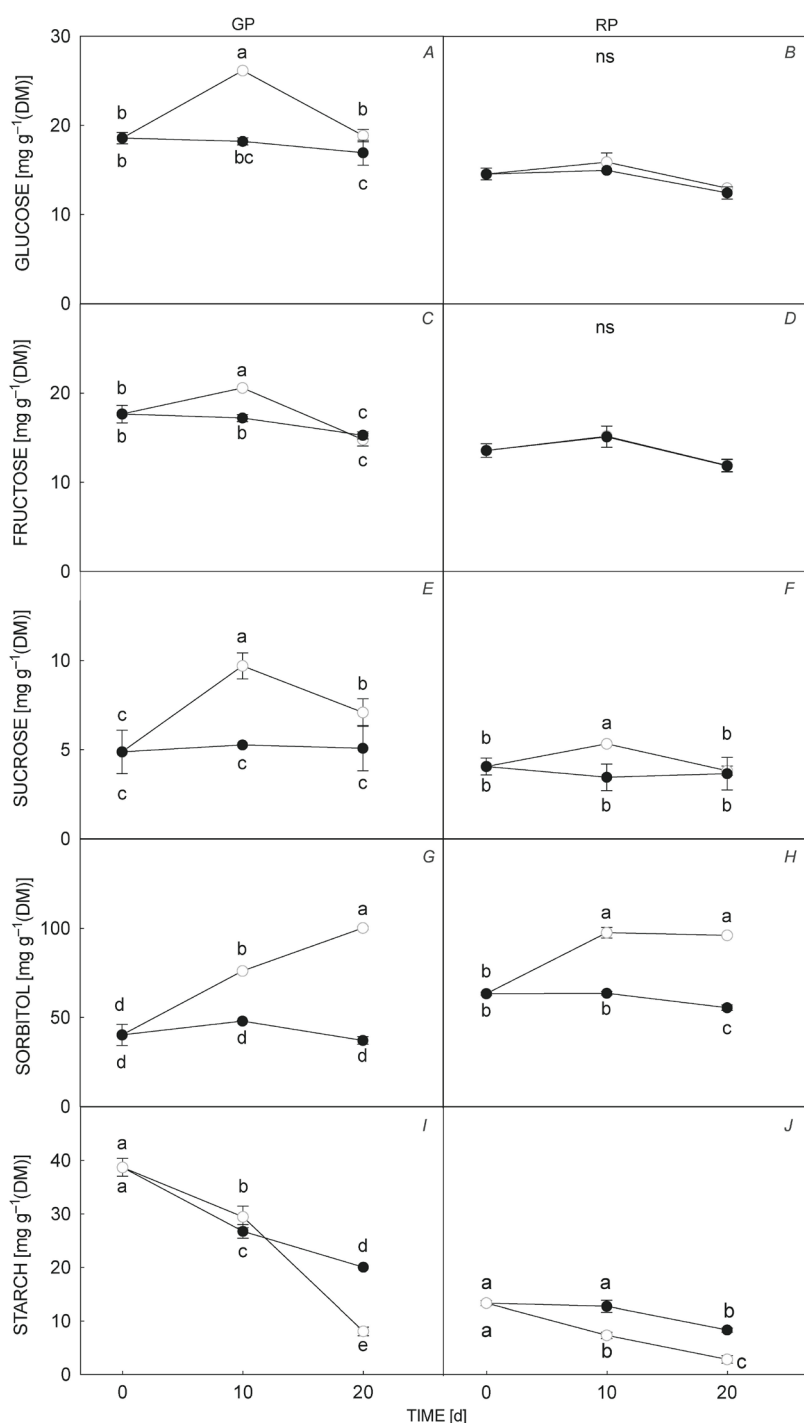
Fig. 4. Sugars content in leaves of two morphs of *Prunus cerasifera* saplings with green (GP) or red (RP) leaves subjected to PEG 6000-induced water stress (*open circle*) or tap-water (control; *closed circle*). Each point is the mean of three replicates ($\pm$ standard deviation). Data are subjected to two-way *ANOVA* with PEG treatment and sampling date as sources of variation. Means flanked by *the same letter* are not statistically different for $p=0.05$ after *Fisher*'s least significant difference *post-hoc* test. The absence of letters indicates no significant interactions between the two factors.

constitutive ability of RP than GP leaves to promote the biosynthesis of T instead of NT sugars, as revealed by the T/NT ratio (1.97 in GP *vs.* 3.38 in RP leaves; $p<0.01$) and T/TOT (0.62 in GP *vs.* 0.72 in RP leaves; $p<0.05$) (Table 2). This induced a twofold beneficial effect in RP saplings; firstly, it contributed in limiting sugar-induced photoinhibition, and secondly, this could also favor the supply of sugar to younger tissues, which were not completely developed in their photosynthetic apparatus.

Conclusion: Results of the present experiment suggest that anthocyanins serve a photoprotective function in *Prunus* leaves when plants are subjected to water stress. The photoprotection exerted by these pigments consists of both their sunscreen and sugar-buffering capacity. In the first case, their ability to absorb a proportion of sunlight reduces the burden of energy to the chloroplast. At the same time, the anthocyanin pathway represents a consistent carbon sink which reduces the probability of feedback regulation of photosynthesis. RP saplings also

Table 2. Translocated on nontranslocated sugar ratio (T/NT), nontranslocated on total sugar ratio (NT/TOT), and translocated on total sugar ratio (T/TOT), in leaves of two morphs of *Prunus cerasifera* with green (GP) or red (RP) leaves subjected to PEG 6000-induced water stress (treated) or tap-water (control). Each point is the mean of three replicates (± standard deviation). Data are subjected to two-way *ANOVA* with PEG treatment and sampling date as sources of variation. Means flanked by *the same letter* are not statistically different for *p*=0.05 after *Fisher*'s least significant difference *post-hoc* test. The absence of letters indicates no significant interaction between sources of variation.

		GP			RP		
		0 d	10 d	20 d	0	10 d	20 d
T/NT	Control	1.24 ± 0.08^d	1.50 ± 0.21^c	1.31 ± 0.20^c	2.39 ± 0.10^c	2.23 ± 0.04^c	2.43 ± 0.26^b
	Treated	1.24 ± 0.08^d	1.97 ± 0.10^b	2.69 ± 0.04^a	2.39 ± 0.10^c	3.38 ± 0.02^b	3.68 ± 0.22^a
NT/TOT	Control	0.44 ± 0.03^a	0.40 ± 0.01^a	0.43 ± 0.03^a	0.29 ± 0.04	0.31 ± 0.03	0.29 ± 0.02
	Treated	0.44 ± 0.03^a	0.38 ± 0.05^{ab}	0.34 ± 0.02^b	0.29 ± 0.04	0.28 ± 0.01	0.25 ± 0.05
T/TOT	Control	0.55 ± 0.03^b	0.60 ± 0.03^b	0.57 ± 0.03^b	0.70 ± 0.04	0.69 ± 0.06	0.71 ± 0.03
	Treated	0.55 ± 0.03^b	0.62 ± 0.04^{ab}	0.66 ± 0.04^a	0.70 ± 0.04	0.72 ± 0.03	0.74 ± 0.06

demonstrated a higher constitutive ability to allocate assimilated carbon to T than NT sugars. In RP leaves, both these features can contribute in limiting sugar-promoted feedback regulation of photosynthesis, which represents another factor responsible for photoinhibition under water stress. This study also highlights the importance to investigate the role of anthocyanins as sugar buffer and the consequences of their presence for leaf photosynthesis in plants under stress deeply.

As an applicative result, we offer the evidence that presence of anthocyanins makes the difference in terms of drought responses between red *vs.* green leaves, at least during a first phase of stress. In a constrained environment such as Mediterranean cities, these features have to be considered for the selection of tree species in the urban environment.

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