

# Morpho-anatomical and physiological traits of two *Bougainvillea* genotypes trained to two shapes under deficit irrigation

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## Abstract

**Key message** The analysis of the growth behaviour, wood anatomical, and eco-physiological traits of two *Bougainvillea* genotypes, trained to two shapes and subjected to different irrigation regimes, fully supports the idea that structural properties interact with cultural practices (i.e., training) in determining the adaptive capability of plants, hence their productivity and survival.

**Abstract** *Bougainvillea* species are cultivated for landscaping in the arid Mediterranean region. In *Bougainvillea* spp., secondary xylem and phloem are formed by successive cambia leading to a peculiar stem anatomy which could favour water storage and plant adaptation under drought conditions. To achieve sustainable production of *Bougainvillea* ornamental shrubs, it is crucial to understand how different genotypes respond to deficit irrigation and how cultural practices, such as canopy training, may interact with morpho-anatomical traits in modifying the plants' ability to withstand water deficit. A greenhouse experiment was conducted to determine the effects of deficit irrigation on plant growth, ecophysiology, and branch anatomy in two *Bougainvillea* genotypes [*B. × buttiana* 'Rosenka', *B.* 'Lindleyana' (= *B.* 'Aurantiaca')]

trained to globe and pyramid shapes. Irrigation treatments were based on daily evapotranspiration (ET): control (C, 100 % ET) or deficit irrigation (DI, 25 % ET). The two genotypes exhibited morphological adaptations to cope with water deficit, including reductions in dry weight, leaf number, and lamina size. In both genotypes, the DI-induced increase in stomatal resistance was accompanied by a decrease in stomata size. Water deficit triggered adjustments in wood anatomical functional traits, also depending on canopy shape and genotype, favouring either water conduction efficiency or safety against embolism. The occurrence of a safer hydraulic system in the pyramid-trained plants suggests a better control of water transport, thus supporting better growth performance under DI conditions compared to globe-trained plants. Such differences, induced by different canopy-shape trainings, should be considered in the management of DI.

**Keywords** Leaf water potential · Quantitative wood anatomy · Stomata · Stomatal resistance · Successive cambia · Water stress

## Introduction

In the recent decades, there has been increasing interest in adopting strategies for water saving in horticulture, due to water scarcity and the competition for water resources with other sectors (e.g., urban, industrial, and environmental) (WWAP 2014). Nevertheless, the general perception is that water use in horticulture is often wasteful and highly inefficient (Hsiao et al. 2007).

Whereas water requirements of food crops are well known, little information is available on those of ornamental species (Grant et al. 2012). Among the factors

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affecting growth and quality of ornamental plants in pre- and post-production, water availability is a key factor. In many areas of potted ornamental plant production, water quantity and quality is often a limiting factor, especially because nursery plants use more water per unit of cultivated land than other agricultural crops (e.g., field and horticultural) (Beeson 2004). At present, there is considerable pressure on the ornamental plant industry to produce crops more efficiently and to reduce irrigation water volumes in the face of possible government regulations on water use. Deficit irrigation (DI), in which the crop is supplied with less water than the volume required throughout the whole growth period, may be used to control excessive vegetative growth, mainly in fruit trees (Egea et al. 2010) and vegetables (Rouphael et al. 2008; Casa and Rouphael 2014), whereas its application to ornamental crops has to date received limited interest.

In the production of some Mediterranean ornamental shrubs, DI strategies have been applied to enhance water use efficiency (WUE) and to improve plant quality by reducing excessive vigour and promoting a more compact habit (Cameron et al. 2006, 2008). At present, the detection of the level and duration of water stress to modulate growth habit and plant shape are a critical point (Álvarez et al. 2009). Indeed, numerous experimental trials on ornamental plants have demonstrated that plant quality declines under severe DI (Hansen and Petersen 2004; Silber et al. 2007; Bernal et al. 2011).

The genus *Bougainvillea* includes 18 species of flowering plants and belongs to the family of *Nyctaginaceae*, native to South America (Kobayashi et al. 2007). Potted *Bougainvillea* plants are a significant part of the Italian ornamental production due to the high demand of national and European markets (ISMEA 2015). *Bougainvillea* species are reported to be tolerant to water stress (Cella Pizarro and Bisigato 2010); the response to the application of different levels of DI is influenced by both the genotype and the shape the plants are trained to (Cirillo et al. 2014). In general, the different shapes of ornamental plants in the nursery contribute to match with the increasing demand for diversified products (Kobayashi et al. 2007). Since plant architecture affects the whole hydraulic functioning of plants, it is likely that training techniques exert control over plant growth responses to water availability. *Bougainvillea* plants have a peculiar stem and branch anatomy, whereby secondary xylem and phloem are produced by successive cambia (Carlquist 2007; Chew 2010; Robert et al. 2011). It is recognised that the large amount of phloem produced by successive cambia can play an important role in water and photosynthate storage, and in mechanisms of embolism repair, thus priming adaptation under conditions of changing soil water availability or frequent drought (Robert et al. 2011, 2014). Although the general stem

anatomical organisation is species-specific, wood functional quantitative traits, such as the number and size of conducting elements, can be severely affected by both environmental conditions and growth habit (De Micco et al. 2008; Beeckman 2016). Several models (e.g., da Vinci's rule and the West, Brown, and Enquist–WBE model) (McCulloh and Sperry 2005) have been proposed to explain patterns of hydraulic architecture and their implications for the efficiency and safety of water transport against embolism, in relation to various plant types and habits. The relationships between vessel diameter and possible environmental drivers (e.g., water availability and temperature) have been explored over the past 50 years, leading to the consideration that, plant size being equal, under stressful conditions (e.g., drought) the selection would favour narrow, slowly transporting but embolism-resistant vessels (De Micco et al. 2008; Carlquist 2012). Olson et al. (2014), taking into account the principles of the hydraulic optimality (HO) models, and using data from 256 species with different habits and from various habitats, recently demonstrated that the main driver for global variation in mean vessel diameter is plant size which explains 63 % of the variation. Analysing 28 *Eucalyptus* species, Pfautsch et al. (2016) uncoupled the effect of plant height and environment, showing the strong relationship of vessel diameter with climate but not with stem diameter. The analysed *Eucalyptus* species showed long-term adaptation of hydraulic architecture to water availability (following the rule of narrower vessels formed under conditions of limiting water availability), but scarce plasticity when environmental conditions changed rapidly (Pfautsch et al. 2016). The relative weight of different environmental drivers for variations in vessel size is likely species-specific. In addition to the total stem or branch length, the general architecture of the above-ground organs needs to be considered. Indeed, the crown structure, which under cultivation is designed through specific training techniques, is known to affect water transport, carbon assimilation, and partitioning, because it affects light interception (Zimmermann 1978; Willaume et al. 2004; Souza et al. 2011). The ability to modify quantitative wood traits is responsible for the adaptive capability of a species under stressful conditions. Although general trends in mean vessel diameter and wood density are by far the most frequently analysed in response to water deficit, it is recognised that different combinations of various anatomical parameters (e.g., vessel size and frequency, and vessel grouping) are of adaptive value more than a single trait in the tradeoff between efficiency and safety of water transport (De Micco et al. 2008; Carlquist 2012).

Starting from the above considerations, the aim of this study was to assess growth, leaf water potentials, stomata traits, twig anatomy, and functional quantitative wood

traits of two *Bougainvillea* genotypes, commonly found on the market, trained to two canopy shapes, and cultivated under full and deficit irrigation regimes. The two genotypes were selected on the basis of the previous experimental results that indicated significant differences in plant growth parameters (e.g., total dry biomass and leaf area) and in the capacity of increasing water use efficiency (WUE) under deficit irrigation (Cirillo et al. 2014, 2015). We aimed to verify whether the different growth behaviours under different irrigation conditions can be ascribed to a different plasticity of hydraulic anatomical traits. We hypothesise that the training of the two *Bougainvillea* genotypes to the two canopy shapes ultimately triggers different hydraulic responses of the plants to water stress, thus affecting their growth performances under conditions of DI. If the hypothesis is confirmed, the possible influence of the different canopy-shape trainings on the hydraulic anatomical plasticity of plants should be taken into account in the management of DI.

## Materials and methods

### Plant material, experimental conditions, and irrigation treatments

The experiment was conducted from March to October 2011 in a 265 m<sup>2</sup> glass-zinc-coated steel greenhouse at the Experimental Station of the University of Naples Federico II, Southern Italy (43°31'N, 14°58'E; 60 m a.s.l.). Plants were grown under a 50 % black shading net. The greenhouse was maintained at a daytime temperature between 16 and 26 °C and day/night relative humidity of 50/88 %.

Two-year-old plants of two potted *Bougainvillea* genotypes, *B. × buttiana* 'Rosenka' and *B. 'Lindleyana'* (= *B. 'Aurantiaca'*) (names according to the Royal Horticultural Society, London, UK), were obtained from a commercial grower (Vivai Torsanlorenzo, Ardea, Rome, Italy) and transplanted, at the beginning of March, into pots (d 20 cm, h 18 cm) containing 4.5 L of peat moss. The pots were placed on three troughs (180 cm wide and 7 m long) with a plant density of 6 plants/m<sup>2</sup>. Plants were trained to two canopy shapes (S): pyramid (Pyr) and globe (Glo) (Fig. 1a, b). The pyramid-shaped *Bougainvillea* plants were grown as a vine through a tutor and pruned by trimming the exceeding shoots, whereas the globe shape was obtained by regular pruning based on new shoot thinning and cut-back.

The irrigation treatments consisted of: (a) control (C), in which the substrate moisture was maintained close to container capacity and irrigation was applied when 20 % of the water was leached, and (b) deficit irrigation (DI), in which plants were watered with 25 % of the amount of water supplied in the C treatment. The electrical

conductivity of the supplied water was 0.6 dS/m. The levels of water recovery were obtained using 4 and 1 emitter/s per plant, for the C and DI treatments, respectively, at a flow rate of 2 L/h. Plants were fertigated with a nutrient solution containing the following macro- and micronutrients: 1.45 mM N-NO<sub>3</sub>, 2.66 mM N-NH<sub>4</sub>, 4.36 mM N-ureic, 1.41 mM P, 4.24 mM K, 5.34 µM Fe, 3.45 µM Mn, 0.84 µM Cu, 0.83 µM Zn, 37 µM B, and 2.08 µM Mo.

In summary, the treatments were defined by a factorial combination of two irrigation treatments based on daily water use [100 %, control (C) or 25 %, deficit irrigation (DI)], two *Bougainvillea* genotypes [*B. × buttiana* 'Rosenka' (BxbR) or *B. 'Lindleyana'* (= *B. 'Aurantiaca'*) (Ba)], and two canopy shapes [pyramid (Pyr) or globe (Glo)]. The treatments were arranged in a randomised complete block design with three replicates of 15 plants each.

### Leaf water potential measurement and growth analysis

At full blooming (171 days after transplanting—DAT), stomatal resistance ( $r_s$ ) to water vapour (s cm<sup>-1</sup>) was measured between 11:00 and 13:00 h, on the youngest fully expanded leaf (three per replication and nine plants per treatment), with a diffusion porometer (AP-4, Delta-T Devices, Cambridge, UK). The water potential components of leaves were measured on the same leaves on the same date, using a dew-point psychrometer (WP4, Decagon Devices, Pullman, WA, USA). Leaf water potential ( $\Psi_l$ ) was measured at midday. The osmotic potential ( $\Psi_\pi$ ) was measured on frozen/thawed leaf samples, and the pressure potential ( $\Psi_p$ ) was estimated as the difference between  $\Psi_l$  and  $\Psi_\pi$ , assuming that a matric potential equals 0.

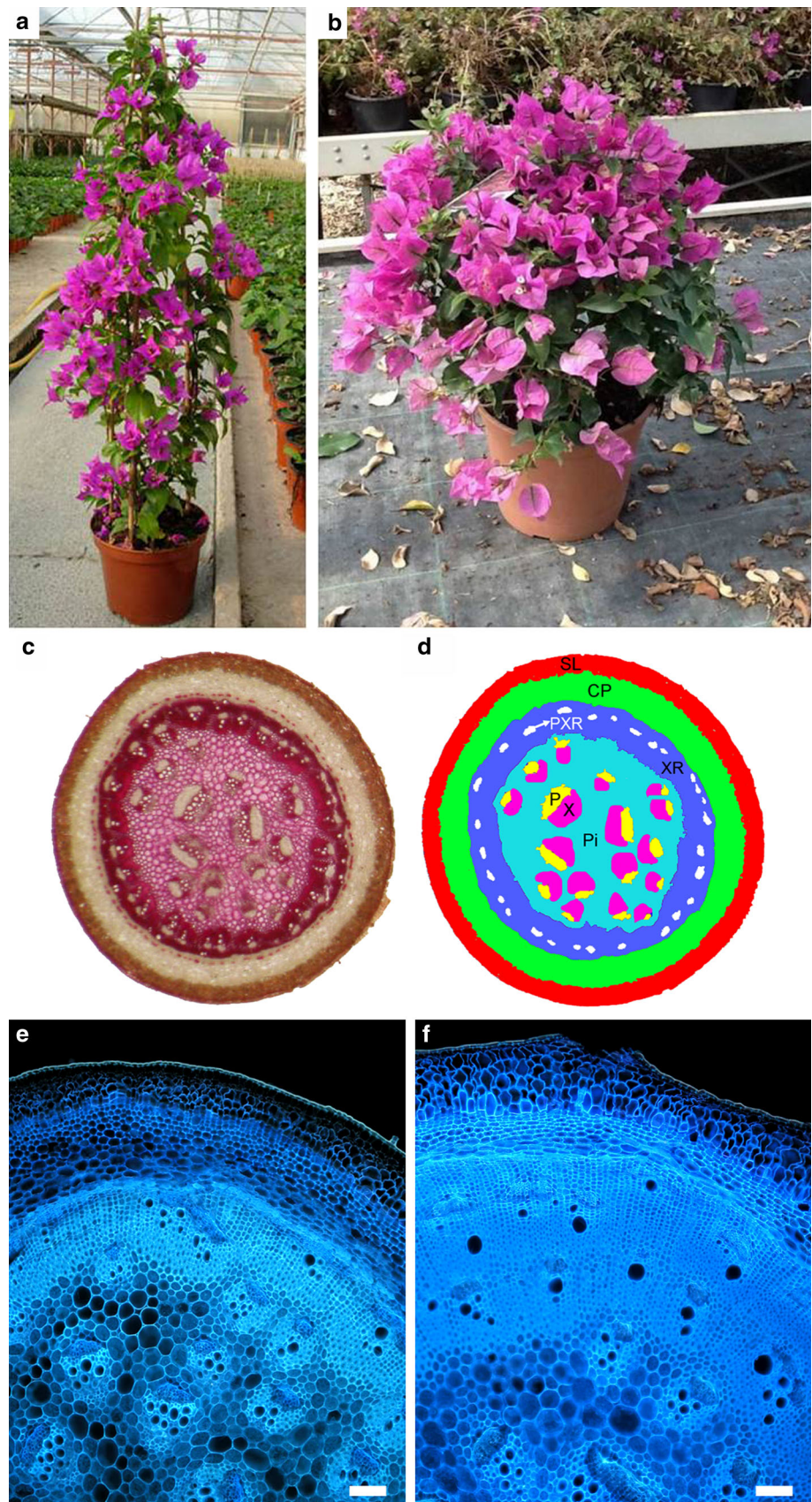
At the end of the production cycle (225 DAT), four plants per replicate were sampled. Shoot biomass (leaves and stems) and flowers were dried to constant weight (DW) in a forced-air oven at 80 °C for 72 h. Leaf area (LA) was measured by means of an electronic area meter (Li-Cor 3000, Lincoln, NE, USA). The number of leaves and the number of flowers per plant were also counted.

### Microscopy analysis

At the end of the experiment, three fully expanded leaves and three one-year-old twigs were harvested from three plants per treatment. A sub-sample of harvested leaves, cut in the median region of the lamina, was immediately fixed in FAA (5 ml 40 % formaldehyde, 5 ml glacial acetic acid, and 90 ml 50 % ethanol) for the characterisation of stomata traits. A sub-sample (2 cm long) of harvested twigs, cut



**Fig. 1** *Bougainvillea* plants trained to pyramid (a) and globe (b) shapes. Scheme of a cross section of a twig showing the analysed regions (c, d): suberised epidermal and sub-epidermal layers (SL), cortical parenchyma (CP), xylem ring including vessels and conjunctive tissue (XR), phloem of the vascular bundles embedded in XR (PXR), pith (Pi), phloem of the vascular bundles in pith (P), and xylem of the vascular bundles in pith (X). Fluorescence microscopy views of stem cross sections of *B. × buttiana* ‘Rosenka’ (BxbR) (e) and *B. ‘Lindleyana’* (= *B. ‘Aurantiaca’*) (Ba) (f), showing the overall distribution of tissues. Bars 50  $\mu$ m



at their base at a distance of 5 mm from the branching junction, was fixed in FAA as well.

#### *Stomata characterisation*

To analyse stomata frequency and size, the abaxial epidermis of the leaf lamina was carefully peeled off with a pointed tweezer, in a region of the lamina not including the main vein. The epidermis strip was flattened on a glass slide and mounted with mineral oil for microscopy. Three film strips from each leaf were observed under a transmitted light microscope (BX60, Olympus, Hamburg, Germany). Digital images of the epidermis were collected by means of a camera (CAMEDIA C4040, Olympus), avoiding veins.

#### *Twig anatomy*

Semi-thin cross sections (15 µm thick) were cut from subsamples of the collected twigs (as reported above) by means of a sliding microtome (Swiss Federal Institute for Forest, Snow, and Landscape Research WSL, Birmensdorf, Switzerland) and divided into two groups. The first group of sections was devoted to the quantification of tissues: more specifically, sections were stained with safranin O and astra blue (Vasquez-Cooz and Meyer 2002; De Micco and Aronne 2007), or with a saturated solution of phloroglucinol in 20 % HCl (Jensen 1962), to distinguish between lignified and un-lignified cell walls. After being mounted with mineral oil, sections were observed under a dissection microscope (SZX9, Olympus). The second group of sections, not stained, was mounted with mineral oil for fluorescence and observed under an epi-fluorescence microscope (BX60, Olympus) equipped with a Mercury lamp, bandpass filter 330–385 nm, dichromatic mirror 400 nm and above, and barrier filter 420 nm and above. Such settings allow the auto-fluorescence of lignin to be detected (Fukuzawa 1992; Ruzin 1999), also maximising the contrast for subsequent digital image analysis and easing the quantification of xylem properties. Digital images of stained and unstained sections were collected as reported above. Radial and tangential longitudinal sections were also cut and analysed through fluorescence microscopy to verify the presence of imperforate tracheary elements.

#### *Digital image analysis*

All digital images were analysed with the AnalySIS 3.2 (Olympus) software program. Stomata were counted, and their frequency (number of stomata per surface unit,  $\text{n/mm}^2$ ) was calculated. The length (guard-cell length, pole to pole) and width (guard-cell width in the median position) of ten stomata per image were measured.

The cross-sectional area of twigs (twig cross section, TCS) as well as the area occupied by each tissue was quantified. Preliminary light and fluorescence microscopy observations of TCS in both species confirmed the previous observations on *Bougainvillea* spp. (Chew 2010) and helped defining the parameters for measurement. In particular, TCS showed numerous vascular bundles, part of which is embedded in the pith, the others being enclosed in a complete ring of thick-walled lignified cells referred to as conjunctive tissue following Carlquist (2007) (Fig. 1c–f). The xylem ring was surrounded by a cortical parenchyma made of many layers of cells, a multilayered sub-epidermal phellem of suberised cells, and an epidermis covered by a cuticle. Based on this description, the following regions were considered: suberised epidermal and sub-epidermal layers (SL), cortical parenchyma (CP), xylem ring, including lignified cells (either vessels or conjunctive tissue) (XR), phloem of the vascular bundles embedded in XR (PXR), pith (Pi), phloem of the vascular bundles in pith (P), and xylem of the vascular bundles in pith (X) (Fig. 1d).

The following anatomical features were quantified in the pith:

- Number of vascular bundles (NB in Pi);
- Number of vessels per bundle: counted in five bundles per twig (NVB);
- Vessel lumen area (VLA in Pi): measured in all vessels of five bundles per twig (corresponding to a minimum number of 20 vessels measured per twig).

The following anatomical features were quantified in the region of the xylem ring:

- Vessel frequency (VF in XR): measured as the number of vessels occurring in a given area (Wheeler 1986). This measurement was determined by counting all vessels individually in two selected regions per image. The regions were selected by dividing the whole circumference into eight equal slices and choosing two not-consecutive slices of the best technical quality, also avoiding reaction wood;
- Vessel grouping (VG in XR): calculated as the proportion of grouped vessels in relation to the total number of vessels in two selected regions per image as described above;
- Vessel lumen area (VLA in XR): measured in all vessels of the two selected regions per twig (corresponding to a minimum number of 20 vessels measured per twig).

Frequency distribution of the vessels in classes of lumen area was calculated in both vascular bundles in pith and in the xylem ring to hypothesise how different distributions of vessel lumen area are related to hydraulic properties.

## Statistical analysis

All data were statistically analysed by the three-way analysis of variance (ANOVA) using the SPSS software package (SPSS 13.0 for Windows). Whenever the two-way interaction was significant, a one-way ANOVA was performed. Duncan's multiple range test was performed at  $p \leq 0.05$  on each of the significant variables measured. Shapiro–Wilk and Kolmogorov–Smirnov tests were performed to check for normality. Percent data were transformed through the arcsine function before statistical analysis.

## Results

### Biomass production, partitioning, and growth parameters

Except for flower dry weight, the plant tissue biomass and plant growth parameters decreased in response to water stress (Table 1). Leaf, flower, total biomass dry weight, and final leaf area were significantly affected by both the genotype (G)  $\times$  irrigation (I) and genotype (G)  $\times$  shape (S) interactions, while the stem dry weight and the number of leaves per plant were only influenced by the G  $\times$  I interaction (Table 1). When averaged over

shape treatments (G  $\times$  I interaction), the lowest values of total, and leaf dry mass, the number of leaves and final leaf area per plant were recorded in *B. 'Aurantiaca'* (Ba) under deficit irrigation (DI) conditions (Fig. 2a–d). The same applied to stem dry mass, whereas the lowest flower dry mass values were observed in Ba under both control and DI conditions (Fig. S1a, b). Irrespective of the irrigation treatment (G  $\times$  S interaction), the highest total dry mass values were recorded in *B.  $\times$  buttiana* 'Rosenka' (BxbR) trained to a pyramid shape, whereas the highest values of leaf dry mass were measured in globe-trained Ba (Fig. 2e, f). Ba genotype showed the lowest values of leaf number and total leaf area in globe-trained and pyramid-trained plants, respectively (Fig. 2g, h). Finally, the lowest stem and flower dry mass were observed in Ba trained to a globe and pyramid shape, respectively (Fig. S1c, d).

### Stomatal resistance and leaf water potential

The  $r_s$  increased by 60.5 % under DI, whereas the opposite trend was observed for both  $\Psi_1$  and  $\Psi_\pi$  with an increase of 70.1 and 54.4 % (Table 2).  $\Psi_1$  and  $\Psi_\pi$  were significantly influenced by the G  $\times$  S interaction (Table 2). When averaged over irrigation treatment, the least negative water ( $\Psi_1$ ) and osmotic potential ( $\Psi_\pi$ ) values were observed in Ba trained to a pyramid shape (Fig. 3a, b).

**Table 1** Effects of genotype, irrigation treatment, and shape on dry weight (DW) production and partitioning, number of leaves ( $n.$ ) and total leaf area ( $\text{cm}^2$ ) per plant of potted *Bougainvillea* plants

	Leaf (g DW/plant)	Stem (g DW/plant)	Flower (g DM/plant)	Total biomass (g DW/plant)	Leaves ( $n.$ /plant)	Leaf area ( $\text{cm}^2$ /plant)
Genotype (G)						
BxbR	4.7	42.6	12.6	60.0	76.5	440.8
Ba	5.2	30.7	5.6	41.5	50.6	322.1
Irrigation (I)						
C	6.1	42.5	9.7	58.3	73.8	447.8
DI	3.8	30.8	8.6	43.2	53.2	315.2
Shape (S)						
Pyr	4.6	47.6	8.6	60.8	68.1	379.0
Glo	5.3	25.6	9.6	40.7	59.0	384.0
G	NS	***	***	***	***	***
I	***	***	NS	***	***	***
S	NS	***	NS	***	*	NS
G $\times$ I	***	**	***	*	**	*
G $\times$ S	***	NS	***	***	NS	**
I $\times$ S	NS	NS	NS	NS	NS	NS
G $\times$ I $\times$ S	NS	NS	NS	NS	NS	NS

NS not significant

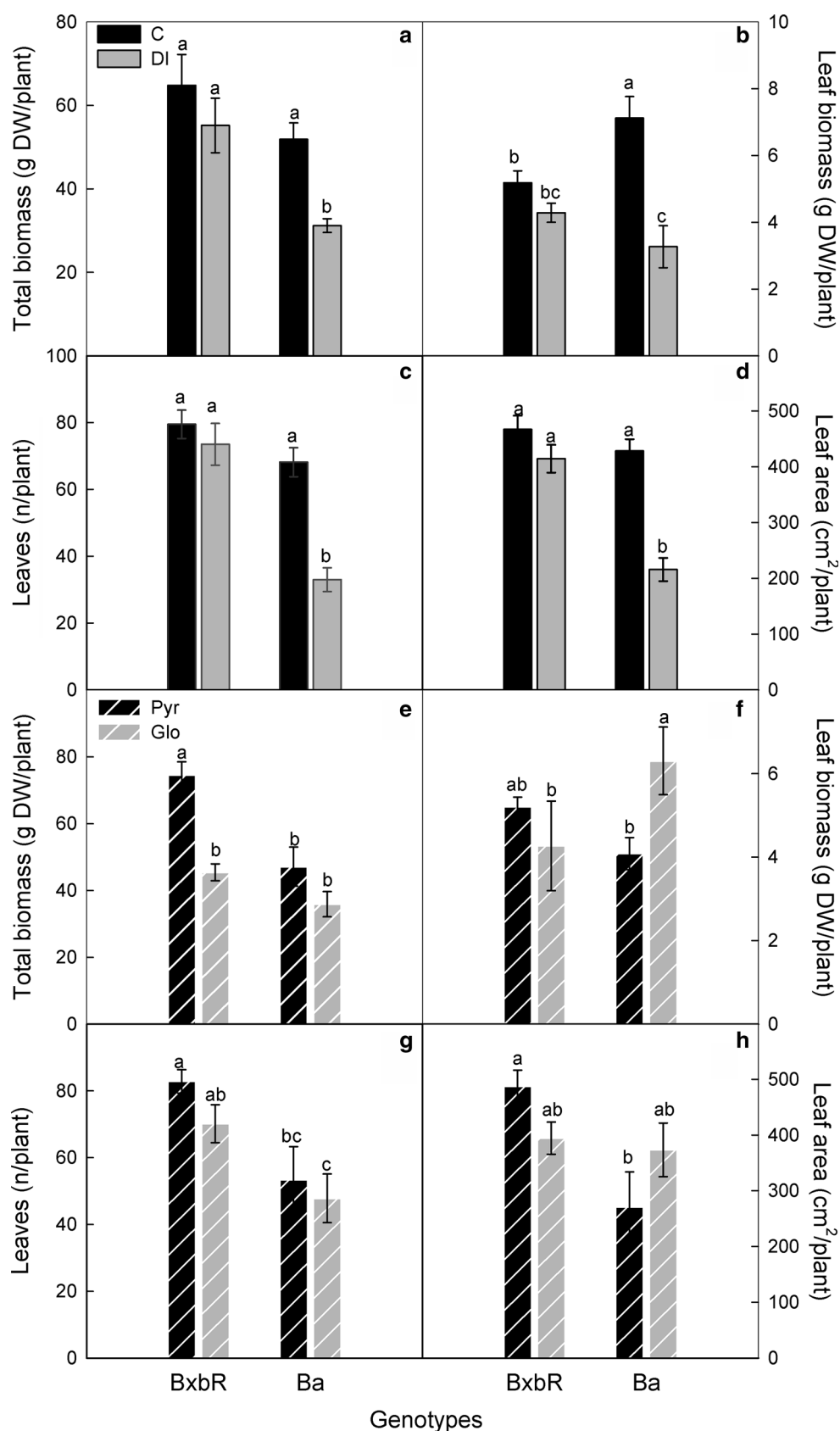
\* significant at  $p \leq 0.05$

\*\* significant at  $p \leq 0.01$

\*\*\* significant at  $p \leq 0.001$



**Fig. 2** Effect of the interaction between genotype [BxbR = *B. × buttiana* ‘Rosenka’; Ba = *B. ‘Lindleyana*’ (= *B. ‘Aurantiaca*’)] and irrigation treatment (C control, DI deficit irrigation) (a–d), and between genotype and canopy shape (Pyr pyramid, Glo globe) (e–h) on: plant total biomass (a, e), leaf biomass (b, f), leaf number (c, g), and leaf area (d, h) of the two potted *Bougainvillea* genotypes. Mean values and standard errors are shown. Different letters correspond to significantly different values ( $p \leq 0.05$ )



**Table 2** Effects of genotype, irrigation treatment, and shape on stomatal resistance ( $r_s$ ), leaf water potential ( $\Psi_l$ ), leaf osmotic potential ( $\Psi_\pi$ ), leaf turgor potential ( $\Psi_p$ ), stomata frequency, length (L) and width (W) of stomata guard cells of potted *Bougainvillea* plants

	$r_s$ (s/cm)	$\Psi_l$ (MPa)	$\Psi_\pi$ (MPa)	$\Psi_p$ (MPa)	Stomata frequency ( $n/mm^2$ )	L ( $\mu m$ )	W ( $\mu m$ )
Genotype (G)							
BxbR	1.413	−1.510	−1.796	0.286	230.9	30.2	11.1
Ba	1.743	−1.627	−1.914	0.290	197.5	31.1	10.9
Irrigation (I)							
C	0.893	−1.163	−1.458	0.296	215.2	32.2	11.5
DI	2.263	−1.979	−2.252	0.280	213.2	29.1	10.5
Shape (S)							
Pyr	1.663	−1.503	−1.786	0.285	197.0	30.8	11.3
Glo	1.493	−1.633	−1.924	0.291	231.3	30.6	10.7
G	NS	NS	NS	NS	***	***	***
I	***	***	***	NS	NS	***	***
S	NS	NS	NS	NS	***	NS	***
G × I	NS	NS	NS	NS	**	NS	NS
G × S	NS	*	*	NS	***	*	***
I × S	NS	NS	NS	NS	*	**	NS
G × I × S	NS	NS	NS	NS	NS	**	NS

NS not significant

\* significant at  $p \leq 0.05$ \*\* significant at  $p \leq 0.01$ \*\*\* significant at  $p \leq 0.001$ 

### Stomata traits

Stomata frequency was influenced by the  $G \times I$ ,  $I \times S$ , and  $G \times S$  interactions (Table 2). Specifically, this parameter was generally higher in BxbR than in Ba with no significant differences between the shapes (Fig. 3c, d). However, it was increased in globe-trained plants in comparison with the pyramid shape, with significant differences in Ba genotype, irrespective of the irrigation treatment (Fig. 3c–e).

The length of stomata guard cells was significantly affected by the  $G \times I \times S$ ,  $G \times S$ , and  $I \times S$  interactions, while the width was only affected by the  $G \times S$  interaction (Table 2). Smaller stomata (reduced length and width of guard cells) were found in DI plants than in control plants, irrespective of genotype and shape (Table 2). Under DI, stomata length was reduced in Ba globe-shaped plants compared to pyramid-shaped ones, whereas it was unaffected by shape in BxbR (Fig. S2).

### Twig anatomy

Light and fluorescence microscopy observations of the cross sections of one-year-old twigs of both species trained to the two canopy shapes showed that the overall anatomical structure is not affected by either the genotype

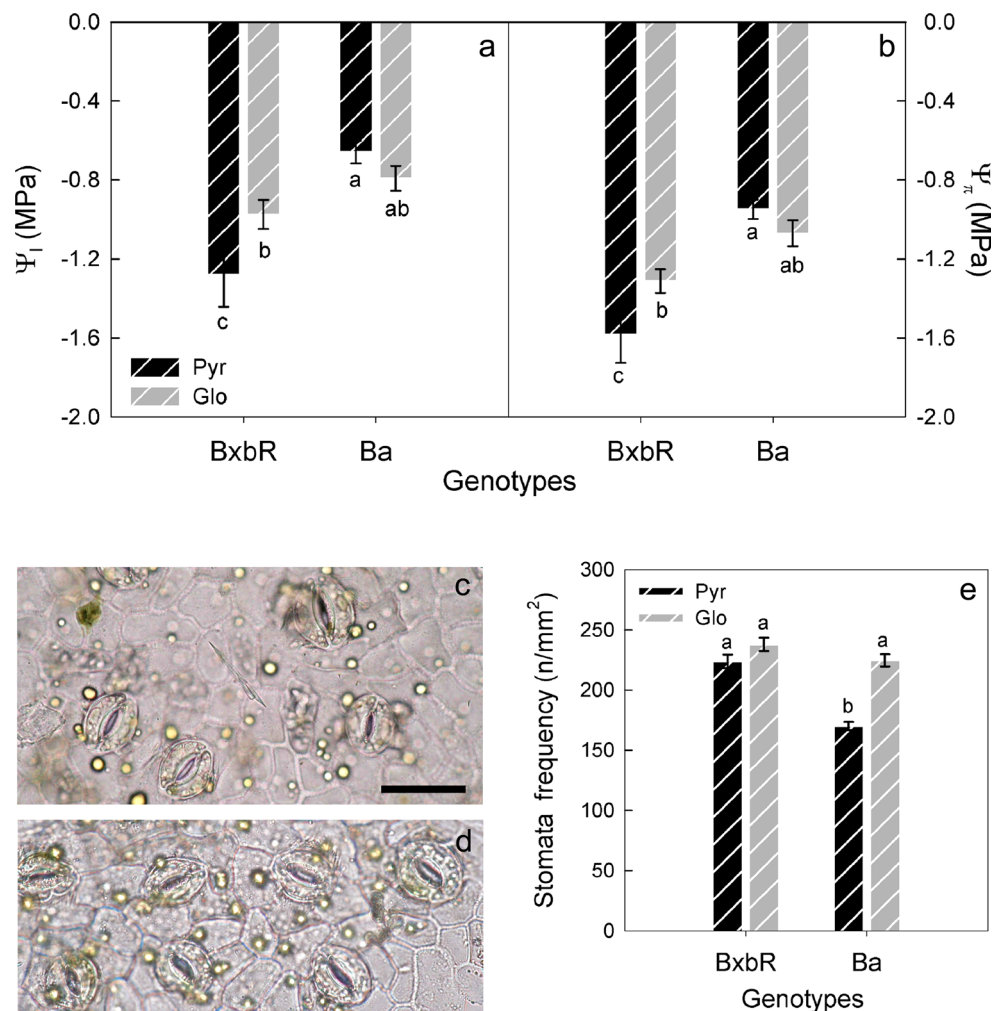
or the canopy shape (Fig. 1e, f). Phloroglucinol staining and UV microscopy showed that both the background of the conjunctive cells at the xylem ring level and pith cells are characterised by lignified cell walls.

The twig cross section (TCS) was significantly affected by the  $G \times S$  interaction (Table 3). This parameter in BxbR plants trained to a pyramid shape was  $19.42 \text{ mm}^2$ , a value significantly higher than that measured in twigs of the same genotype trained to a globe ( $7.50 \text{ mm}^2$ ) and of Ba grown in both pyramid ( $7.15 \text{ mm}^2$ ) and globe ( $5.72 \text{ mm}^2$ ) shapes. The area of all analysed tissues but phloem in the xylem ring (PXR) was affected by the  $G \times S$  interaction (Table 3). The relative proportion of each tissue over the TCS was unaffected by genotype, irrigation treatment, and canopy shape (Table 3).

As regards the water transport system, the number of vascular bundles in the pith (NB in Pi) was affected by the sole canopy shape, where pyramid training determined the formation of a significantly higher number of bundles per twig (Table 3). The number of vessels per vascular bundle in the pith (NVB) was significantly affected by the  $G \times I \times S$  interaction, with the highest values found in BxbR plants trained to a pyramid shape, under full irrigation conditions (Fig. 4a). However, the pyramid-shape training induced the formation of narrower vessels in both genotypes (with significant differences in BxbR), in



**Fig. 3** Effect of the interaction between genotype [BxbR = *B. × buttiana* ‘Rosenka’; Ba = *B. ‘Lindleyana’* (= *B. ‘Aurantiaca’*)] and canopy shape (Pyr pyramid, Glo globe) on leaf water potential (**a**;  $\Psi_l$ ), leaf osmotic potential (**b**;  $\Psi_\pi$ ) and stomata frequency (**e**) of the two potted *Bougainvillea* genotypes. Stomata traits (**c–e**): light microscopy views of epidermis of leaves of *B. × buttiana* ‘Rosenka’ (BxbR) trained to pyramid (**c**) and globe (**d**) shape under deficit irrigation (DI). Images are at the same magnification. Bar 50  $\mu\text{m}$ . Mean values and standard errors are shown. Different letters correspond to significantly different values ( $p \leq 0.05$ )



comparison with the globe-shape training, irrespective of the irrigation treatment (Fig. 4b). Moreover, in the xylem ring, vessel frequency (VF in XR) was affected by the I  $\times$  S interaction (Table 3). The vessel frequency of twigs of plants trained to a pyramid shape under full irrigation conditions was 80.69 n/mm<sup>2</sup>, a value significantly higher than that of twigs of globe-trained *Bougainvillea* grown under the same watering conditions (60.53 n/mm<sup>2</sup>). Vessel grouping (VG) and vessel lumen area (VLA) in the xylem ring were affected by the G  $\times$  S interaction (Table 3). The lowest values of VG were observed in BxbR pyramid-trained plants (Fig. 4c); the narrowest VLA values were found in BxbR pyramid-shaped plants and Ba globe-shaped plants (Fig. 4d). The distribution of vessels in classes of lumen size in both vascular bundles of pith (Fig. 5a, c, e) and the xylem ring (Fig. 5b, d, f) was similar in two genotypes (Fig. 5a, b), with most of the water flow relying on vessels with lumen size up to 600 and 800  $\mu\text{m}^2$  in the two regions of the twig, respectively. Interestingly, classes of vessels with lumen size exceeding 1000  $\mu\text{m}^2$  were less represented in the pith than in the xylem ring suggesting

safer water transport in the first. Moreover, in pith, in classes up to 300  $\mu\text{m}^2$ , the frequency of vessels was higher in control than in DI plants, while in classes of larger vessel lumen area, water-stressed plants showed higher values than controls (Fig. 5c). The opposite trend was observed in the xylem ring: in classes up to 300  $\mu\text{m}^2$ , the frequency of vessels was higher in DI than in control plants, while in classes of larger vessel lumen area, the control showed higher values than DI plants (Fig. 5d). In both twig regions, the pyramid shape increased the frequency of vessels in the classes of narrow lumen compared with globe-trained plants (Fig. 5e, f).

## Discussion

Results from the combined analysis of the growth behaviour and hydraulic anatomical traits of the two *Bougainvillea* genotypes trained to two canopy shapes and subjected to full and deficit irrigation support the hypothesis that structural properties play a major role in

**Table 3** Effects of genotype, irrigation treatment, and shape of potted *Bougainvillea* plants on the area of twig cross section (TCS), on the area occupied by each tissue (SL, suberized epidermal and sub-epidermal layers; CP, cortical parenchyma; XR, xylem ring; PXR, phloem in XR; Pi, pith; X, xylem of the vascular bundles in pith; P, phloem of the vascular bundles in XR), and on xylem functional traits of twigs (NB in Pi, number of bundles in pith; NVB, number of vessel per bundle; VLA in Pi, vessel lumen area in bundles in pith; VF in XR, vessel frequency in XR; VG in XR, vessel grouping in XR; VLA in XR, vessel lumen area in XR)

Genotype (G)	TCS (10 <sup>6</sup> μm <sup>2</sup> )	SL (10 <sup>6</sup> μm <sup>2</sup> )	CP (10 <sup>6</sup> μm <sup>2</sup> )	XR (10 <sup>6</sup> μm <sup>2</sup> )	PXR (10 <sup>6</sup> μm <sup>2</sup> )	Pi (10 <sup>6</sup> μm <sup>2</sup> )	X (10 <sup>6</sup> μm <sup>2</sup> )	P (10 <sup>6</sup> μm <sup>2</sup> )	NB in Pi (n./twig)	NVB (n./bundle)	VLA in Pi (μm <sup>2</sup> )	VF in XR (n./mm <sup>2</sup> )	VG in XR (%)	VLA in XR (μm <sup>2</sup> )
BxbR	13.5	2.5 (18.6)	3.3 (24.5)	3.9 (28.9)	0.3 (2.2)	2.4 (17.8)	0.65 (4.8)	0.42 (3.1)	18.7	7.9	245.8	65.7	80.9	385.8
Ba	6.4	1.2 (18.5)	1.6 (24.6)	1.9 (29.7)	0.2 (3.1)	1.2 (18.5)	0.29 (4.5)	0.11 (1.7)	17.2	7.2	236.4	79.9	85.0	371.8
Irrigation (I)														
C	9.8	1.8 (18.3)	2.4 (24.3)	2.9 (29.6)	0.2 (2.0)	1.8 (18.3)	0.44 (4.5)	0.32 (3.2)	17.3	7.5	223.3	67.5	78.8	331.1
DI	10.0	1.9 (19.0)	2.4 (24.0)	3.0 (30.0)	0.2 (2.0)	1.8 (18.0)	0.50 (5.0)	0.21 (2.0)	18.6	7.6	260.2	78.1	86.8	433.3
Shape (S)														
Pyr	13.3	2.3 (17.3)	3.2 (24.1)	3.9 (29.3)	0.3 (2.3)	2.5 (18.8)	0.65 (4.9)	0.42 (3.2)	19.3	7.7	220.2	78.1	79.2	373.0
Glo	6.6	1.3 (19.7)	1.6 (24.2)	2.0 (30.3)	0.2 (3.0)	1.1 (16.7)	0.29 (4.4)	0.11 (1.7)	16.6	7.4	263.6	67.5	86.4	384.7
G	***	***	***	***	***	***	***	*	NS	*	*	**	NS	NS
I	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	***	NS	*	***
S	***	***	***	***	**	***	***	*	*	NS	***	*	NS	NS
G × I	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*	NS	NS	NS
G × S	***	***	***	*	NS	***	***	*	NS	*	**	NS	**	***
I × S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	***	*	NS	NS
G × I × S	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS

The incidence of each tissue (as percentage) over the total twig cross-sectional area is reported in parenthesis

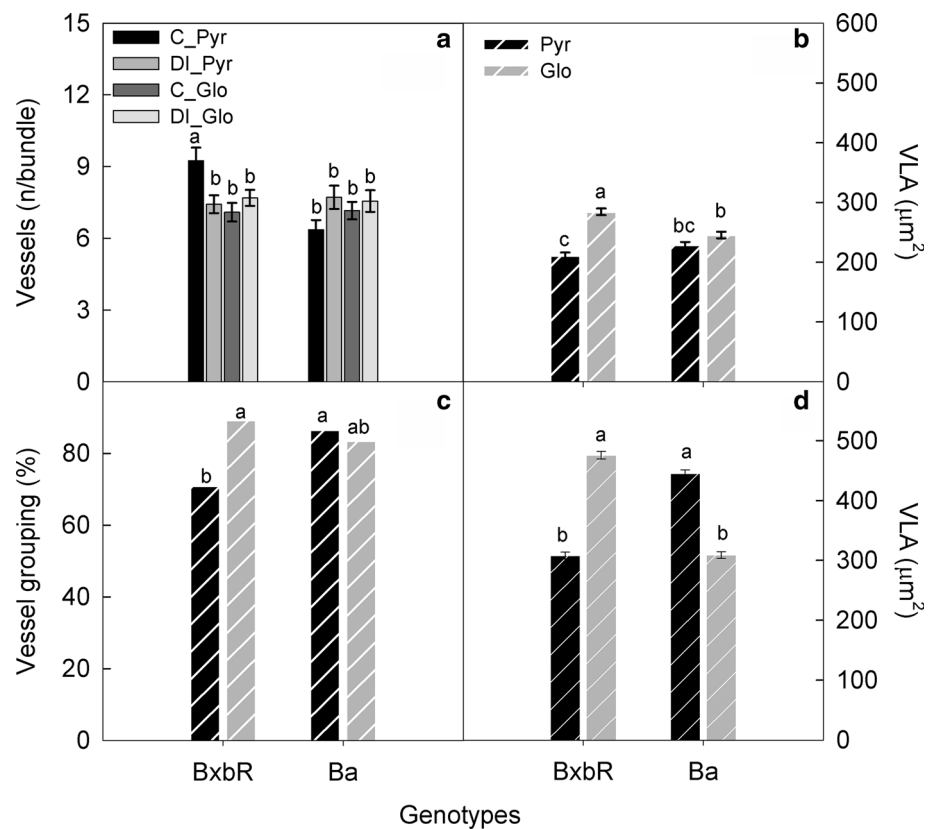
NS not significant

\* significant at  $p \leq 0.05$

\*\* significant at  $p \leq 0.01$

\*\*\* significant at  $p \leq 0.001$

**Fig. 4** Effect of the interaction among genotype [BxbR = *B. × buttiana* ‘Rosenka’; Ba = *B. ‘Lindleyana’* (= *B. ‘Aurantiaca’*)], canopy shape (Pyr pyramid, Glo globe), and irrigation treatment (C control, DI deficit irrigation) on the number of vessels per bundle in pith (a) and effect of the interaction between canopy shape and genotype on lumen area of vessels (VLA) in pith (b) in the two *Bougainvillea* genotypes. Effect of the interaction between canopy shape and genotype on vessel grouping (c) and lumen area of vessels (VLA) in the xylem ring (d) in the two *Bougainvillea* genotypes. Mean values and standard errors are shown. Different letters correspond to significantly different values ( $p \leq 0.05$ )

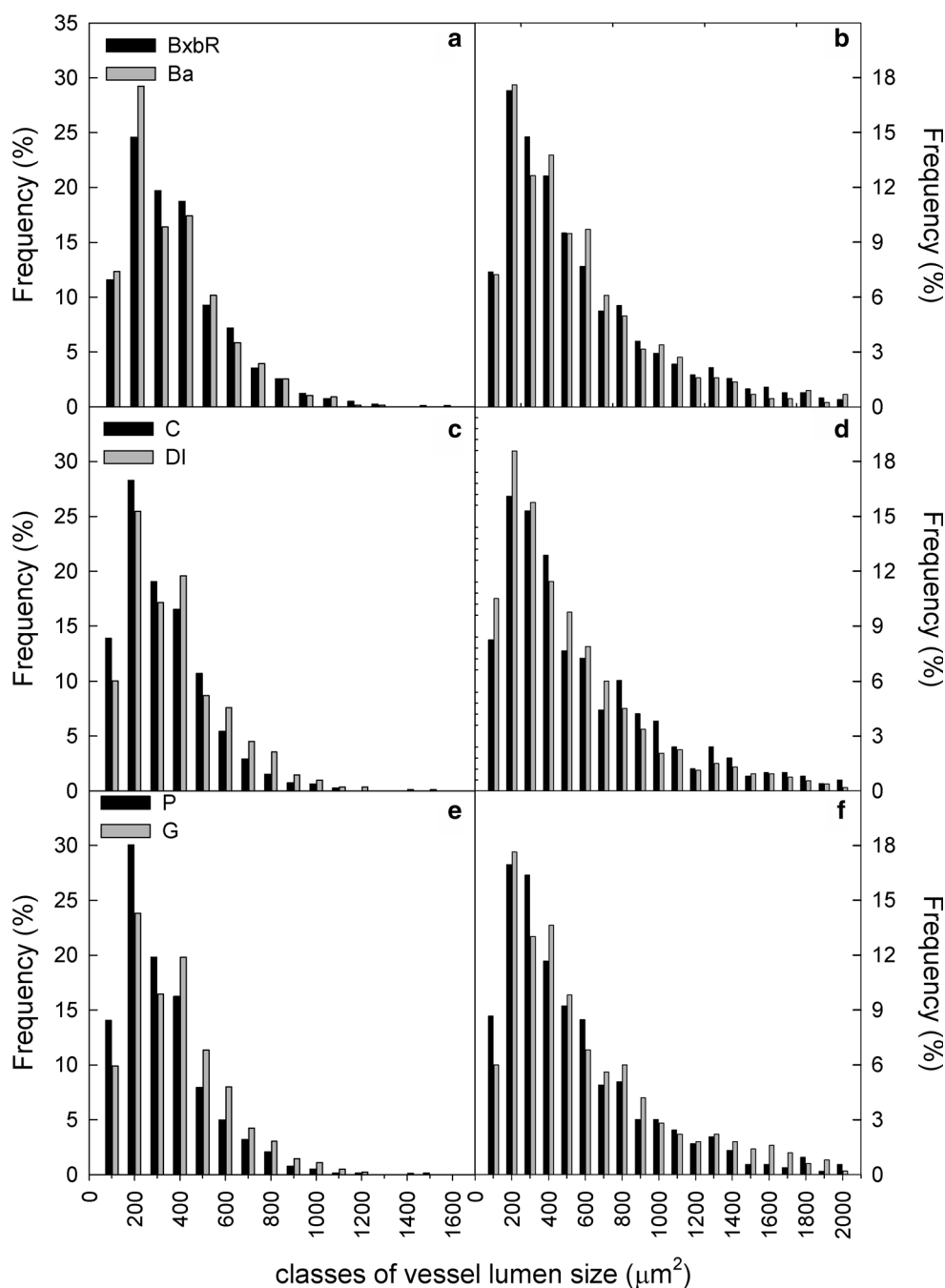


determining the adaptive capability of plants, hence their productivity and survival. The process of water transport is not only subjected to molecular-biochemical-physiological regulation, like all metabolic processes, but is also strictly controlled by the anatomical traits of the vascular pathway up to the leaves. Indeed, structural properties ultimately influence the water flow rate, gas exchanges, and biomass accumulation in both optimal and stressful watering conditions (Brodribb 2009). Our findings indicate that the two genotypes of *Bougainvillea* have several morphological adaptations to cope with imposed water deficit, including the reduction in leaf number, leaf lamina size, and dry weight, which are in agreement with previous studies on several genotypes of *Bougainvillea* spp. (Cirillo et al. 2014), *Callistemon* spp. (Álvarez and Sánchez-Blanco 2013), and other woody ornamentals (Cameron et al. 2006). Indeed, the minimisation of the evaporative surface area is a stress-avoidance mechanism which can be achieved through various strategies as reported in ornamental plants, cultivated under water stress conditions, as well as in wild shrub species, growing in drought-prone environments (Álvarez et al. 2011; De Micco and Aronne 2012a; Cirillo et al. 2014; Toscano et al. 2014). In our study, the imposed deficit irrigation treatment severely decreased shoot dry mass (leaf and stem biomass), probably reducing water losses significantly (Sánchez-Blanco et al. 2009; Álvarez et al. 2011; Toscano et al. 2014),

irrespective of canopy shape (Table 1, Fig. 2, S1). *B. ‘Aurantiaca’* was reported to be very sensitive to water stress irrespective of the canopy shape (Cirillo et al. 2014). We showed that morphological responses to water stress varied between canopy shapes and genotypes. In both genotypes, the lowest biomass values were recorded in globe-trained *Bougainvillea* plants, due to the frequent shoot cut-back applied during the cultivation cycle.

In both *Bougainvillea* genotypes, DI induced substantial increase in stomatal resistance (Table 2). This is an adaptive mechanism to control transpiration, as reported in several cultivated species, including *Bougainvillea* spp. (Franco et al. 2006; Scheiber and Beeson 2007; Niu et al. 2008; Álvarez et al. 2011; Álvarez and Sánchez-Blanco 2013; Cirillo et al. 2014). In both analysed genotypes, the increase in stomatal resistance induced by water deficit was accompanied by the decrease in stomata size, but not by modifications in stomata frequency (Table 2). Smaller stomata are known to have faster dynamic characteristics than larger ones, thus allowing a better control of water loss and regulation of water use efficiency under conditions of reduced water availability (Drake et al. 2013). Quick stomata control in *Bougainvillea* plants growing under DI would also reduce the risk of xylem embolism, since prompt stomata closure, under water stress conditions, is a strategy to avoid xylem hydraulic failure (Meinzer et al. 2009).

**Fig. 5** Distribution of vessels in classes of lumen area in the vascular bundles of pith (a, c, e) and xylem ring (b, d, f) depending on genotype (a, b) [BxbR = *B. × buttiana* ‘Rosenka’; Ba = *B. B.* ‘Lindleyana’ (= *B.* ‘Aurantiaca’)], irrigation treatment (c, d) (C control, DI deficit irrigation), and canopy shape (Pyr pyramid, Glo globe) (e, f)



In most plants undergoing stressful conditions, stomata closure is consequent to water potential adjustment (Ludlow 1980). Under DI conditions, both *Bougainvillea* genotypes showed lowering of leaf water potential and leaf osmotic potential (Table 2), confirming the correlation between leaf water potentials and stomatal resistance (Chaves et al. 2002; Sánchez-Blanco et al. 2009). The reduction in leaf water potential under water stress conditions found in this study is consistent with the previous studies on *Pittosporum* and *Viburnum* (Toscano et al.

2014), roses (Niu et al. 2008), *Callistemon* (Álvarez et al. 2011), and *Bougainvillea* (Cirillo et al. 2014).

Water deficit is responsible not only for modifications in biomass allocation, but also for other permanent changes: indeed, differences in stem diameter have been observed in several ornamental shrubs and trees (Corcuera et al. 2004; Ugolini et al. 2014). In our experiment, basal stem diameter was not affected by irrigation treatment but was dependent on the genotype and canopy shape (Table 3). In pyramid-trained plants, a total leaf area similar to that of



globe-trained plants is sustained by a twofold sapwood area (Tables 1 and 3), leading to double Huber value (Zimmermann 2002). The presence of higher Huber value in pyramid-trained plants suggests better adaptation to water deficit conditions, in line with ecological trends indicating a higher sapwood/leaf area ratio in plants of dry habitats compared to those of humid conditions (Zimmermann 2002; Carter and White 2009). Although absolute values of stem size may change according to different environmental factors, such as temperature and water availability, it seems that allocation and proportion among different tissues are quite well-conserved features and are maintained irrespective of the genotype, irrigation level, and canopy shape (Table 3). This suggests that the peculiar stem anatomy of *Bougainvillea* spp. (Figure 1) guarantees large volumes devoted to living water-storage tissues (i.e., cortical parenchyma, phloem, and pith) (Robert et al. 2014), suberised water-saving tissues (i.e., epidermal and sub-epidermal cell layers) (De Micco and Aronne 2012b), and multiple function parenchyma, including hydraulic, storage, and defence tissues (i.e., cortical parenchyma and pith) (Morris et al. 2016). However, any morpho-anatomical adjustments to cope with changing factors do not rely on the increase in such water-storage or water-saving tissues, but on the modification of quantitative xylem anatomical traits. Actually, xylem traits are regulated not only by gene expression and signalling biological molecules, as every process from cell division in meristems towards cell differentiation (Schuetz et al. 2013), but also by physical drivers (e.g., turgor pressure and cell-wall constraints) during cell differentiation (Braidwood et al. 2013).

It is generally reported that water stress induces the formation of narrower vessels as a consequence of reduced turgor-driven enlargement and as a mechanism to reduce the cavitation risk (Hacke et al. 2006). However, many species which have adapted to cope with frequent fluctuations in water availability have also evolved strategies of hydraulic transport based on the modification of xylem functional traits (Beeckman 2016; De Micco et al. 2016). These strategies can favour water transport efficiency, under favourable conditions, while maintaining safety against embolism, during drought events (De Micco et al. 2008). Although efficient water transport mainly relies on vessel size, in studies to analyse the ability of plants to cope with water scarcity, mean vessel size is not the most appropriate parameter: it needs to be accompanied by information about the overall distribution of all vessels in size classes. Indeed, the occurrence of even a few large vessels can ensure rapid water transport when water is available, but the presence of a ground tissue of narrow vessels is crucial to allow water transport when large vessels are embolised due to water deficit (De Micco and Aronne 2009). Within this context, the two *Bougainvillea* genotypes appear to respond to water

deficit by increasing mean vessel size to achieve rapid water transport when water is available, while maintaining safety under DI, especially at the xylem ring level, due to increased vessel frequency and grouping (Table 3). High vessel frequency is a feature promoting resistance to water stress: it is a sort of redundancy to increase the probability that a sufficient part of vessels would remain active in water transport under embolism-inducing conditions (Carlquist 1989). The functional meaning of vessel grouping is still controversial, since there is evidence to support both its benefits (e.g., redundancy, easier removal of cavitation, and increasing permeability of intervessel pit membranes) and drawbacks (e.g., increased risk of embolism spreading) under water-limiting conditions (Brodersen et al. 2013; von Arx et al. 2013). Quantitative data from the literature are insufficient to unravel this question and we refrain from entering the debate here. Vessel distribution in classes of lumen size also suggests that vessels belonging to narrower classes of lumen size in the xylem ring are higher in DI plants than in controls, while the opposite is true in pith (Fig. 5). This suggests that under deficit irrigation, vessels in the xylem ring would be mainly pushed to maintain safety, while those in pith vascular bundles would be primed to increase their size to favour efficiency. Vessels in vascular bundles occurring in the pith are able to afford this increase in vessel size without increasing the risk of embolisms because they reach maximum values of lumen size almost half those of xylem ring vessels. Of course, vessels in pith and in the xylem ring have different timings of formation. Hence, environmental factors and the metabolic status of the plant may have exerted a different control of xylogenesis in the two compartments. Irrespective of the irrigation regime, *Bougainvillea* plants trained to a pyramid shape have a safer hydraulic system because of the occurrence of narrower vessels (especially at the pith level) and higher redundancy than globe-trained plants (Table 3; Figs. 4, 5). The occurrence of narrower vessels in pyramid- than globe-trained plants appears to conflict with the longer main stem typical of the pyramid shape, which should support wider vessels compared to globe canopies, according to the hydraulic optimality models that predict wider vessels in taller plants (Olson et al. 2014). However, it fits well with the reduced need for vessel widening towards the plant base to overcome drops in conductivity, thanks to the occurrence of a few branch junctions which are common sites of decreased conduit diameter (Tyree and Alexander 1993; Olson et al. 2014). Apart from these mechanistic models, other wood traits play an important role in the control of water transport, such as morphology and size of intervessel pits which are strictly related to vulnerability to embolism (Wheeler et al. 2005).

Both *Bougainvillea* genotypes modify growth behaviour and anatomical hydraulic properties depending on the canopy-shape training and irrigation level. In addition,

under DI conditions, their vascular system is designed to benefit rapidly from the irrigation episodes by quickly absorbing water through wide vessels, still maintaining wood traits promoting safety. The occurrence of a safer hydraulic system in the pyramid-trained plants suggests better control of efficiency and safety of water transport, thus supporting better growth performance even under DI conditions compared to globe-trained plants. Our results also suggest that *B. × buttiana* ‘Rosenka’ is adapted better than *B. ‘Aurantiaca’* to DI. Overall, this study highlights the importance of taking into account the anatomy of the hydraulic pathway to understand the growth behaviour and adaptive capability of ornamental shrubs, to regulate cultivation practices (e.g., pruning and training), including species and cultivar selection.

**Author contribution statement** SDP designed the study and coordinated the experiment; VDM, AB, and RC carried out sampling; CC and RC performed growth analysis; VDM and AB performed microscopy and digital image analyses; CC and VDM performed statistical analyses; YR contributed to the analysis tools; CC, VDM, and YR wrote the main part of the manuscript; all authors contributed to manuscript revision, read, and approved the submitted version.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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