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HOOKER'S OR WARTY BARBERRY? PHYSIOLOGICAL BACKGROUND ANALYSIS FOR CHOOSING THE RIGHT ONE INTO ORNAMENTAL PLANTATIONS ENDANGERED BY DROUGHT

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Abstract

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Barberries as undemanding shrubs with high aesthetic value are often planted in city parks and street greeneries. However, severe urban environment combined with climate change puts pressure on these plants in terms of their ability to cope with drought. In order to avoid plantation fall-offs, a common garden experiment was carried out on the drought tolerance of two Asian barberry species, namely Hooker's barberry (*Berberis hookeri* Lem.) and warty barberry (*Berberis verruculosa* Hemsl. and Wils.). Higher leaf relative water content, postponed but more sensitive stomatal closure (decrease in stomatal conductivity for water) as well as osmotic adjustment (free proline accumulation) and antioxidant defence onset (total antioxidant activity of the hydrophilic phase), and faster photosynthetic pigment decomposition in Hooker's barberry transplants compared to warty barberry, point to better water management and advanced protection of leaf structures in this species under limited soil moisture. Moreover, warty barberry plants with half total leaf area suffered from drought earlier, because of enhanced soil water loss through evaporation. Thus, Hooker's barberry can be taken as more drought tolerant than its counterpart, therefore making it more suitable for plantings in areas that are prone to this environmental constraint.

Keywords: barberry, drought tolerance, leaf anatomy, stomatal conductivity, osmotic adjustment, total antioxidant activity

INTRODUCTION

Urban parks and greenery in general are considered to be very important components of the sustainable development of large human agglomerations from ecological, economic, social and cultural point of view. In social dimensions they provide dust and noise reduction, together with shading and subsequent temperature regulation associated with recreation and health care services (Pietzarka 2016).

Green areas with trees and shrubs counteract so called "urban heat islands" created by concentration of hard surfaces (buildings, roads and pedestrian zones), accumulating and reflecting incoming solar radiation enclosed in a greenhouse of pollutants produced by transportation and industry. They create shadows and transpire, both of which have substantial effect on air temperature and humidity (Wong and Chen 2010).

Barberries are relatively undemanding, easily propagated shrubs of high aesthetic value (Bean, 1970). Hoffman's "List of names of woody plants" identified 360 cultivated barberry species, varieties, forms, hybrids and cultivars, most of them bred from Japanese barberry (*Berberis thunbergii* DC.) (Hofmann 2010). Mlyňany Arboretum presently houses 22 barberry genotypes (Hoťka and Barta 2012). The most abundant species are represented by Chinese barberry (*Berberis julianae* Schneid., 112 individuals) and warty barberry (*Berberis verruculosa* Hemsl. and Wils., 13 individuals) (Hoťka and Konôpková 2009).

Intensifying climate change, which generally amplifies the frequency and duration of extreme weather events, brings prolonged periods of heat and severe drought (Barros 2006). Depending on a tree/shrub's position in an urban area, it can have more-or-less larger cooling demand (Leuzinger *et al.* 2010), thus suffering from a much higher drought than outside the city. Roloff *et al.* (2009) modelled a list of woody plant species suitable for urban environment in Central Europe under predicted climate change. The species were native to middle Asia and the western parts of North America.

The question of urban woody plant replacement by drought tolerant species is highly relevant not only from the city greenery stabilization point of view but also because of drought-caused changes in their aesthetic value. The ornamental plants' look is markedly affected by leaf wilting, colour defects and precocious fall-off, lower vegetative growth, phenological anomalies, decreased flower and fruit load and in extreme cases by branch/plant die-back (Augé *et al.* 2003; Cameron *et al.*, 2008; Zollinger *et al.* 2006). This necessarily means that the plants become very unattractive to the general public.

Studies investigating the responses of barberries to drought stress are lacking. The works that deal with this issue usually compare different woody plant species including barberries co-occupying specific extreme biotopes (Peri *et al.* 2011; Laferrière 1992; Kulikov and Domanskaya 1972) or numerous ornamental shrubs in urban parks (Tan 2013; Xu *et al.* 2012; Jiang *et al.* 2011). Furthermore, most of the studies have been conducted on Japanese barberry's variety Atropurpurea.

The aim of this study was to test the drought tolerance of two attractive but less frequently cultivated Asian barberry species, namely Hooker's (*Berberis hookeri* Lem.) and warty barberry (*Berberis verruculosa* Hemsl. and Wils.). More specifically we aimed to obtain a detailed description of their physiological responses to water deficit, as measured by various leaf and root characteristics, and the subsequent development of recommendations for horticultural practice.

MATERIALS AND METHODS

Plant material and cultivation

Shoot cuttings of two different 25–30 years old barberry species, namely Hooker's barberry (*Berberis hookerii* Lem.), native to the Himalayas, and warty barberry (*Berberis verruculosa* Hemsl. and Wils.), native to China-Sichuan, were collected in June 2013 from the shrubs, both of which were located in shady stands of Mlyňany Arboretum. The cuttings were rooted in organo-mineral soil substrate (garden compost:gravel [<4 mm] = 4:1 in plastic pots of 9 cm side) after being treated with 1 % indole-3-acetic acid (IAA, Rhizopon A, Rhizopon, Netherland). At the end of August 2014 (after vegetative growth completion, as usual for drought appearance in the Middle Europe), one-year-old transplants (12 individuals per genotype) were transferred from the nursery into controlled cultivation conditions: photosynthetically active radiation (PAR) 100 $\mu mol.m^2.s^{-1}$, photoperiod 16/8 hours, and temperature 27/20 °C. The transplants were acclimated for approximately 5 weeks. Plants were not allowed to dry out and were watered regularly.

Dehydration cycle, measurements and analyses

After acclimatization, three weeks lasting dehydration cycle was imposed to half of individuals (6 repetitions) by water withholding. The second half, represented by control plants, was analysed just before treatment. Following parameters of water regime were recorded before (day 0), in the course (day 6, 10 and 14) and at the end of the dehydration cycle (day 20) from each individual:

Relative water content (RWC) was calculated according to the formula described in the work of Slavík (1965):

$$RWC = ((W_{act}-W_{dw})/(W_{sat}-W_{dw}))*100\ (\%),$$

where:
W_{act} .....actual leaf weight (g),
W_{sat} .....weight of water saturated leaf (g),
W_{dw} .....leaf dry weight (g).

Stomatal conductivity (g_s) on the abaxial leaf side was measured at 10:00 a.m. (3 hours after light switched on) in four replicates per each plant using a porometer SC-1 (Decagon Devices Inc., USA).

For stomatal density (SD) and aperture length (SAL) determination offprints of leaves were prepared using transparent nail lacker and adhesive tape, and studied using a light microscope (Motic BA310) in a software environment for image analysis (Motic Images Plus 2.0 ML). Leaf (LT) and upper cuticle thickness (CT) were measured from leaf section preparates (Němec, 1962).

In free proline (^{100}Pro) analysis we followed the procedure of Bates *et al.* (1973). Approximately 0.5 g leaf material was homogenized in 10 ml of 3 % sulfosalicylic acid. After homogenate filtration, 2 ml ninhydrine solution (1.25 g ninhydrine in

30 ml cold acetic acid and 20 ml 6 M phosphoric acid) and another 2 ml cold acetic acid were added and the reaction mixture was incubated at 95 °C for one hour. The reaction was then stopped by transferring into ice bath, and thorough shaking with 4 ml toluene enabled chromophore extraction. Absorbance was subsequently measured at 520 nm. Concentrations were calculated on RWC 100 % level (^{100}Pro).

Chlorophyll *a* and *b* (Chl *a*, Chl *b*) as well as total carotenoid (Car) concentrations were determined using the method of Šesták and Čatský (1966). Leaf segments were homogenized using a mortar and pestle in the presence of sea sand, $MgCO_3$ and 100 % acetone. After evaporation of all liquid, the powder was quantitatively transferred into 80 % acetone and filtered using a vacuum pump. Thereafter, filtrate absorbance was measured at wavelengths 470, 647 and 663 nm, respectively. Photosynthetic pigment concentrations were calculated according to Lichtenthaler (1987):

$$\text{chl . a} = (12.25\ A_{663} - 2.79\ A_{647})\ T$$
$$\text{chl. b} = (21.5\ A_{647} - 5.1\ A_{663})\ T$$
$$\text{Car} = ((1000.A_{470} - (1.82\ \text{chl.a} + 85.02\ \text{chl . b}))/198)T,$$

where:
A .....filtrate absorbance at respective wavelength,
T.....cuvette thickness (cm).
Pigment concentrations were expressed in $mg.g^{-1}$ sample dry weight.

Total antioxidant activity (TAA) analysis was conducted according to Pellegrini *et al.* (1999). First we prepared leaf water extracts as follows: approximately 0.1 g leaf material was homogenized with sea sand and 7 ml deionized water, and centrifuged at 6000 rpm for 15 min. 10 µl supernatant was then added to 1 ml 80 µM solution of cationic radical of diammonium salt of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{+\cdot}$), after 2 min absorbance of the reaction mixture at 734 nm measured and calculated as follows:

$$\Delta = (A734_c - A734_s) / A734_c,$$

where:
Δ ..... relative portion of reduced radical,
$A734_c$ ... absorbance of control (water) at 734 nm,
$A734_s$ ... absorbance of sample at 734 nm.
Δ was expressed in nmol Trollox antioxidant activity equivalent (TEAC) per g dry sample weight.

At the end of experiment total leaf area (A) using specific leaf area (SLA, Šesták and Čatský, 1966) and dry root weight (W_R) were determined.

$$SLA = A/W\ (dm^2g^{-1}),$$

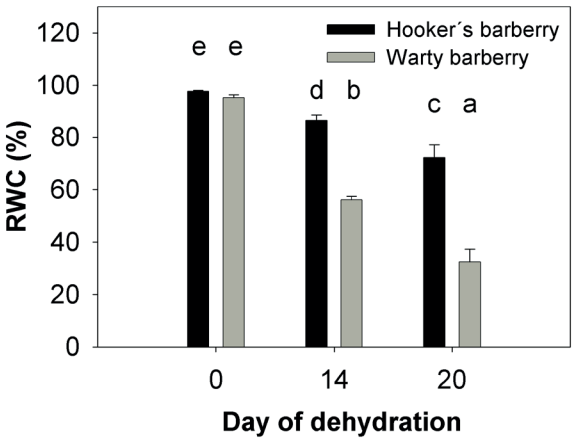
where:
A .....leaf area (dm^2),
W .....leaf dry weight (g).
Then we calculated A/W_R.

Statistical analysis

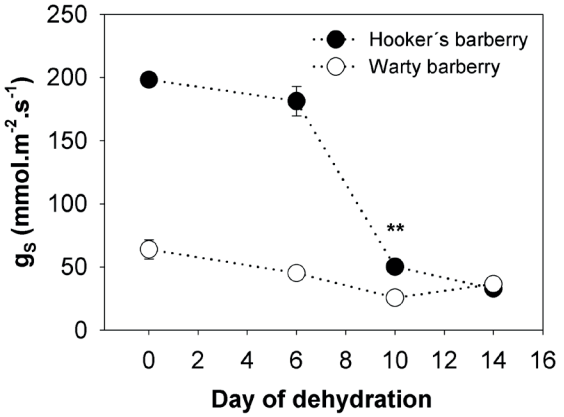
The data was submitted to analysis of variance (ANOVA) using Statgraphics Plus v. 4.1 software. LSD tests at $P \leq 0.05$ were performed to RWC, g_S, SD, SAL, LT, CT, ^{100}Pro, TAA, Chl.*a*, Chl.*b*, Car, A, W_R and A/W_R. Relationships between RWC and respective parameters were described by regression analyses.

RESULTS

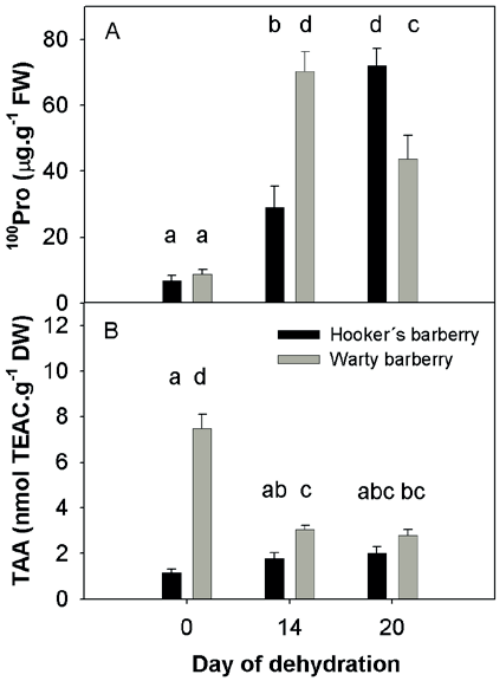
Responses of the studied barberries to excluded irrigation were species specific. Leaf relative water content in warty barberry decreased much faster over time than in Hooker's barberry (Fig. 1). After 14 days of dehydration, the former showed RWC approx. 56 % and the latter 86 %. On the 20th day the difference between them increased even more.



1: *Leaf relative water content (RWC) in tested barberry species under controlled dehydration. Letters indicate statistically significant difference at P ≤ 0.05.*



2: *Stomatal conductance (g_S) of lower leaf side in the studied barberry species during the dehydration cycle. Double asterisk indicates statistically significant difference at $P \leq 0.05$.*



3: *Free proline accumulation (^{100}Pro, A) and total antioxidant activity (TAA, B) in barberry leaves as influenced by continuous dehydration. Letters indicate statistically significant difference at $P \leq 0.05$.*

At the start of the dehydration cycle, stomatal conductivities in Hooker's barberry and warty barberry were 200 and 60 mmol.m^2.s^{-1} (Fig. 2), respectively. The dynamics of the stomatal closure in these barberry genotypes was markedly different. After 6 days of dehydration, Hooker's barberry exhibited only 10 % lower g_S values but after 10 days this decreased by 75 %. Additional four days of dehydration caused a further 10 % reduction in stomatal conductivity. On the other hand, the decrease in g_S for warty barberry during the dehydration cycle was more gradual (approx. 30 %). However, in this species stomata closed 4 days earlier than in Hooker's barberry.

The studied species differed in stomatal density and stomatal aperture length (Tab. I). Hooker's barberry showed 50 % less stomata than warty barberry but it had significantly larger SAL (ca. 3 µm) compared to the latter species. On the other hand, we did not observed any difference in cuticle and whole leaf section thickness between the two species.

Fourteen days of dehydration caused more than a four-fold increase in free proline concentration at 100 % RWC (Fig. 3A) in Hooker's barberry leaves (to approx. 29 µmol.g^{-1} FW) and an eight-fold increase in warty barberry (to approx. 70 µmol.g^{-1} FW). However, after an additional 6 days of dehydration

I: *Stomatal density (SD) and aperture length (SAL) as well as cuticle (CT) and mesophyll thickness (MT) in mature leaves from studied barberry species. Letters indicate statistically significant difference at P ≤ 0.05.*

Species	SD (mm^{-2})	SAL (µm)	CT (µm)	MT (µm)
Berberis hookerii **Lem.**	*204 ± 9a	24.4 ± 0.4b	36.1 ± 1.2a	341.9 ± 7.0a
Berberis verruculosa **Hemsl. and Wils.**	305 ± 16b	21.5 ± 0.3a	30.7 ± 1.2a	344.3 ± 11.1a

*Average ±SE

II: *Leaf photosynthetic pigments in studied barberry species during dehydration cycle. Letters indicate statistically significant difference at P ≤ 0.05.*

Species/Dehydration level	mg.g^{-1} DW		
	Chlorophyll *a*	Chlorophyll *b*	Carotenoids
Berberis hookerii **Lem.**			
control	*1.757 ± 0.336 ab	0.869 ± 0.077 bc	1.397 ± 0.108 bc
14th day of dehydration	2.131 ± 0.222 abc	0.737 ± 0.045 abc	1.393 ± 0.094 bc
20th day of dehydration	1.369 ± 0.290 a	0.477 ± 0.127 a	0.788 ± 0.233 a
Berberis verruculosa **Hemsl. and Wils.**			
control	2.386 ± 0.158 bc	0.788 ± 0.022 bc	1.697 ± 0.057 c
14th day of dehydration	2.775 ± 0.324 c	0.862 ± 0.110 c	1.666 ± 0.154 c
20th day of dehydration	1.609 ± 0.225 ab	0.592 ± 0.085 ab	0.995 ± 0.134 ab

*Average ±SE

III: *Total leaf area (A), root dry weight (W_R) and their ratio in respective barberry species at the end of dehydration cycle. Letters indicate statistically significant difference at P ≤ 0.05.*

Species	A (dm^2)	W_R (g)	A/W_R
Berberis hookerii **Lem.**	*1.679 ± 0.076 b	1.078 ± 0.136 b	1.466 ± 0.085 a
Berberis verruculosa **Hemsl. and Wils.**	0.910 ± 0.044 a	0.502 ± 0.075 a	1.825 ± 0.201 a

*Average ±SE

IV: *Correlation coefficients (r) and linear regression slopes (a) for relation of RWC vs. g_S, Pro, TAA, Chl.a, Chl.b or Car in both barberry species.*

Parameter	B. hookerii		B. verruculosa	
	r	a	r	a
g_S	0.889**	11.923	0.787**	0.714
^{100}Pro	–0.777**	–1.289	–0.610**	–0.596
TAA	–0.378*	–0.015	0.872**	0.068
chl.*a*	0.590**	0.024	0.467*	0.011
chl.*b*	0.878**	0.014	0.430*	0.003
car	0.866**	0.025	0.702**	0.010
A/W_R	–0.017	–0.0002	–0.947**	–0.030

Note: ** – strong (1 > r ≥ 0.5) and * – moderate (0.5 > r ≥ 0.3) linear regression.

free proline in the former species increased by a similar amount again but in the latter species it decreased by almost 38 %.

Leaf total antioxidant activity exhibited only a minor increase with an increase in drought stress for Hooker's barberry (Fig. 3B). The opposite was true for warty barberry where it decreased markedly from approx. 7.5 at the beginning to 2.8 µmol TEAC.$^{-1}$ DW at the end of the dehydration cycle.

A significant decrease in photosynthetic pigments concentration was found after 20 days of dehydration (Tab. II). Chlorophyll *a*, chlorophyll *b* and total carotenoid concentration fell by 1.15, 0.27 and 0.67 mg.g^{-1} DW, respectively in warty barberry, and total carotenoid content was 0.6 mg.g^{-1} DW lower in Hooker's barberry. In general, the former exhibited higher values than its counterpart.

Hooker's barberry produced a significantly larger total leaf area and root dry mass compared to warty barberry (almost twice as much) (Tab. III). However, we found no statistically significant difference in their ratios (ca. 1.6).

Correlation analysis (Tab. IV) between RWC and g_S showed a strong ($r \approx 0.8 – 0.9$) positive linear relationship in both barberry species,

respectively. However, in Hooker's barberry the slope of the relationship was much larger than in warty barberry (11.923 and 0.714, respectively). Conversely, the relationship between RWC and ^{100}Pro was negative for both species (correlation coefficients for Hooker's and warty barberry were –0.777 and –0.610, respectively). The rate of change for free proline concentration in Hooker's barberry was double that of warty barberry (slope –1.289 compared to –0.596). TAA was negatively correlated with RWC in Hooker's barberry (moderately strong correlation) but positively correlated in warty barberry (strong correlation). In the case of photosynthetic pigments there were consistently stronger linear correlations in Hooker's than warty barberry. For chlorophyll *a* concentration in Hooker's barberry we found r = 0.59, for chlorophyll *b* concentration r = 0.878 and for total carotenoids it was 0.866. Relative water content was strongly dependent on A/W_R ratio only in warty barberry (r = –0.947).

DISCUSSION

Drought tolerance is crucial for stabilization of greenery aesthetic functions (Cameron *et al.*, 2008). The two studied barberry species differed substantially in response to increasing dehydration, showing different drought tolerance. The following attributes indicate that Hooker's barberry has better water management and increased protection of leaf structures under limited soil moisture compared to warty barberry: higher leaf relative water content, postponed but more sensitive stomatal closure (decrease of conductivity for water) as well as onset of osmotic adjustment (free proline accumulation), and antioxidant defence (total antioxidant activity of the hydrophilic phase) combined with faster photosynthetic pigment decomposition. Thus, it can be regarded as more drought tolerant than its counterpart.

Loewenstein and Pallardy (1998), describing relationships between predawn/midday leaf water potential and stomatal conductivity, showed different patterns of water loss control in black walnut (*Juglans nigra* L.), sugar maple (*Acer saccharum* Marsh.) and white oak (*Quercus alba* L.) under soil drying. The highest stomatal sensitivity was observed in black walnut, which is a drought-sensitive species. The drought tolerance of olive trees (*Olea europaea* L.) is associated with effective regulation of transpiration, osmo-protectant accumulation, antioxidant enzymes stimulation as well as intense root growth (Sofo *et al.* 2008). Most of listed attributes was found in Hooker's barberry, as well. Ennajeh *et al.* (2010) specified leaf-anatomical attributes connected with the drought-tolerant olive cultivar 'Chemlali': increased thickness of the palisade and spongy parenchyma as well as upper and lower epidermis, enlarged stomatal and trichome density, and decreased leaf area. According to Xu and Zhou (2008), moderate water deficit had a positive effect on stomatal number in the perennial grass *Leymus chinensis* (Trin.) Tzvel, but more severe deficits led to a reduction in stomatal number. On the other hand, stomatal size decreased with water deficit. The work of Kosma *et al.* (2009) revealed an inductive effect of water stress on cuticle thickness and impermeability caused by enhanced overproduction of waxy alkanes.

Since our analysis took place after vegetative growth resumed, we could only evaluate species differences. Although leaf and cuticle thickness showed substantial similarities, stomatal density was one third lower in Hooker's barberry than in warty barberry, however the size of stomata were ca. 3 µm larger in the latter species. In contrast to Xu and Zhou (2008), we did not find a positive correlation between stomatal density and stomatal conductivity.

Grapevine leaves and roots subjected to long-term drought in the study of Król *et al.* (2014) exhibited lower total antioxidant activity of the hydrophilic phase than in watered plants. We observed similar situation in our study. Leaf TAA in warty barberry was high in the first part of the dehydration cycle but decreased significantly with stress development, demonstrating a different functional strategy and considerable sensitivity of this protective mechanism compared to Hooker's barberry with continuously growing TAA.

Early leaf senescence represents an effective mechanism that enables plants to survive under severe drought (Munné-Bosch and Alegre 2004). Liu *et al.* (2011), analysing responses of six woody plant species to rising drought level, described the onset of chlorophyll and carotenoid breakdown at mild stress, when free proline accumulation and antioxidant defence started to increase. Similarly to our work and that of Król *et al.* (2014), severe drought caused a decrease in these parameters.

When considering that leaf transpiratory area and root system volume in Hooker's barberry plants are double that of warty barberry, our results on water relations and associated metabolic parameters are unexpected. Although their ratios were almost the same, representing an important measure of drought acclimation ability (Achten *et al.* 2010; Lloret *et al.* 1999), the limited water supply should theoretically be exploited faster by the more robust Hooker's barberry. We suppose that in pots with warty barberry plants large portion of the soil water was lost by evaporation, therefore they earlier suffered from drought. But what is more important, slower acclimatory responses concerning protective mechanisms against drought stress move this species to the second place. Thus, our recommendation is as follows: Hooker's barberry should be chosen instead of warty barberry for sites that are prone to or potentially threatened by drought.

CONCLUSION

1. Higher leaf relative water content, postponed but more sensitive stomatal closure (decrease in stomatal conductivity for water) as well as osmotic adjustment (free proline accumulation) and antioxidant defence onset (total antioxidant activity of the hydrophilic phase), and faster photosynthetic pigment decomposition in Hooker's barberry transplants compared to warty barberry, point to better water management and advanced protection of leaf structures in this species under limited soil moisture.
2. Thus, Hooker's barberry can be taken as more drought tolerant than its counterpart, therefore making it more suitable for plantings in areas that are prone to this environmental constraint.

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