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Screening for drought resistance among ornamental maples (*Acer* sp.). A field experiment in juvenile plants

Abstract: Urban greenery is often affected by adverse environment (including inadequate water availability) of human agglomerations. Besides high aesthetic value, maples (*Acer* sp.) with elevated resistance to air/soil pollution, have become common components of ornamental plantations in Europe. Because of high theoretical (representativeness for species evaluation) and practical (planting age) relevancy, morphological and metabolic reactions of juvenile plants of eight maple species to one month long summer drought were studied in the experimental field to estimate the species drought resistance. In spite of marked growth differences, none from tested species showed a significant decrease in leaf relative water content indicating stress. However, in some of them activation of protective mechanisms was observed, what points to the past stress sensing. *A. negundo* with *A. tataricum* bet on transpiration area reduction (leaf shedding) and *A. buergerianum*, *A. palmatum* and *A. saccharinum* on osmotic adjustment (increased free proline concentration). Increase in stomatal index and/or reduction in specific leaf area, observed almost exclusively in species with continuous growth, had limited effect on the plant hydration status. Relative trunk growth was particularly affected in the fast growing species. Taking into account the decisive protective mechanisms onset and trunk growth stability, species can be ordered in respect of drought resistance as follows: *A. campestre*, *A. monspessulanum*, *A. platanoides*, *A. tataricum*, *A. negundo*, *A. saccharinum*, *A. palmatum* and *A. buergerianum*.

Keywords: urban greenery, maples (*Acer* sp.), drought resistance, osmotic adjustment, stomatal and leaf morphology, growth

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Introduction

Urbanization shows a quickly escalating trend at a planetary level (Lüttge & Buckeridge 2020) and this trend will continue with the increase of population (Ferrini et al., 2014), which is according to UN-Habitat estimated to result in 60% of the world population living in urban areas in 2030 (Murshed & Yusuf Saadat, 2018). These estimates suggest, that climate change will be intensified (Chapman et al., 2017; Murshed & Yusuf Saadat, 2018).

Trees in the urban environment are crucial tools in the process of climate change mitigation through their ecosystem services (Locosselli et al., 2019) on a local and also regional scale (Sieghardt et al., 2005). They help to enhance urban sustainability and also improve human health (Salmond et al., 2016). Urban green spaces are capable to reduce air pollution (Tong et al., 2016; Sicard et al., 2018), regulate the air temperature (Doick & Hutchings, 2013), provide shade, absorb and scatter solar radiation, raise atmospheric humidity (Ferrini et al., 2014), store and sequester carbon (Nowak & Crane, 2002; Fares et al., 2017), manage stormwater (Berland et al., 2017), provide windbreaks (Lüttge & Buckeridge, 2020), reduce traffic noise (Samara & Tsitsoni, 2011) and they also contribute to aesthetics perceiving (Salmond et al., 2016).

The environmental conditions in urban areas differ from natural growth conditions and the survival and vitality of urban vegetation are affected by various constraints (Sieghardt et al., 2005). Stress factors affecting urban trees growth and survival comprise limited rooting space, air pollution, light heterogeneity, heat, poor quality, compaction and sometimes low availability of nutrient minerals in the soil, salinity, limited water availability, but also biotic factors such as pathogens (Sæbø et al., 2003; Ferrini et al., 2014; Lüttge & Buckeridge, 2020). However, criteria for the species selection used in the urban environment are often based on aesthetics, rather than on the tolerance to stresses which are common in built-up areas (Ferrini et al., 2014).

One of the most important abiotic stresses, that negatively affects plant growth and development, is drought (Salehi-Lisar et al., 2016). In a broad sense, drought is the consequence of low precipitation and high evaporation (Larcher, 2003). However, drought can be also caused by soil factors, such as flooding, high salinity, and low temperature – in this case, it is known as physiological drought (Salehi-Lisar et al., 2012).

Urban plants experience an „urban heat island" (UHI) effect (Fares et al., 2017), a phenomenon when the air temperature in urban areas is higher than in the surrounding rural environment (Önder & Akay, 2014). Higher temperatures accelerate water loss by evapotranspiration (Fares et al., 2017), moreover reduced soil moisture is also caused by surface runoff and poor water retention due to high coverage of impermeable surfaces (Czaja et al., 2020). Soil sealing in many cases even prevents water from reaching the root system (Fares et al., 2017).

For the survival of drought conditions, plants have developed various drought resistance mechanisms (Salehi-Lisar et al., 2016). The two main strategies to cope with water deficit are known as drought avoidance and drought tolerance (Salehi-Lisar et al., 2012; Bacelar et al., 2012; Fares et al., 2017). Drought avoidance is the ability of a plant to maintain high water potential under drought, usually through morphological changes (reduced stomatal conductance, root system development, decreased leaf area, etc.) (Salehi-Lisar et al., 2012). Modulations of number and size of stomata allow plants to adjust their stomatal pore area as a response to surrounding environment, finally affecting their maximum and minimum gas exchange (Bertolino et al., 2019). Drought tolerance is the capacity to maintain physiological and metabolic processes during decreasing water potential (Fares et al., 2017), which could be achieved through the regulation of genes and metabolic pathways (Fang & Xiong, 2015). For example, maintaining high turgor by an osmotic adjustment is regarded as an important drought tolerance mechanism (Luo, 2010; Bacelar et al., 2012). Responses of plants to water stress range from molecular to whole plant level and depend on duration and intensity of stress, but also upon plant species and its development stage (Salehi-Lisar et al., 2012; Salehi-Lisar et al., 2016).

A wide range of species were used in urban areas of central and North-Western Europe, however, only 3-5 genera usually accounted for 50-70% of all street trees planted. Among the most popular were *Tilia, Acer, Platanus, Aesculus, Quercus* and *Fraxinus*. In European parks and woodlands, the species varied according to climate, although with prevalence of *Tilia, Quercus, Acer, Alnus, Fraxinus* and *Fagus* (Pauleit et al., 2002). Genus *Acer* consists of deciduous and also evergreen species, which are found in various climate and landscape types, from dry steppes to moist and rich forests (Sjöman et al., 2015). The species of this genus have moderate requirements for mineral elements and are noticeably tolerant to high pollution, which could be reasons why are they so popular in the urban environment (Mitrović et al., 2006). There are also several remarkable species with various aesthetic properties such as autumn colors or attractive bark (van Gelderen et al., 2010).

In the most of recent works, authors studied different aspects of maple drought resistance in seedlings (e.g. Carón et al., 2015; Guo et al., 2013; Piper & Fajardo, 2016) or adults (e.g. Tissier et al.,

2004; Lens et al., 2011; Gillner et al., 2014). Only Stratópoulos et al. (2019) deal with juvenile field maple (*Acer campestre* L.) plants. Since conflicting results come from studies on aging effect in tree drought resistance (e.g. Li et al., 2012; Lucas-Borja et al., 2021; Unawong et al., 2022), a representative tree age seems to be an important issue in the species evaluation process. We suppose that juvenile plants, fully manifesting species differences, could meet this requirement. Moreover, trees has usually been planted in this age. Therefore, we studied physiological reactions in juvenile individuals of eight maple species to summer drought in natural conditions of experimental field and provided species drought resistance ranking as a recommendation for gardening practice.

Material and Methods

Study site and biological material

Six individuals of one year old seedlings (from own seed material) of eight maple species were planted in the experimental field of the Mlyňany Arboretum IFE SAS in 2014 in spacing of 3 times 3 m (east-west row orientation). Tree individuals were organized following assumed final height in order to prevent shading each other, and simultaneously distributed in different parts of the field to exclude soil heterogeneity effect. During the first three summers, seedlings were watered two times a week to avoid deep drought stress. For the proper crown formation, lateral branches at heights below 1.5 m were removed. Area beneath the trees was mowed regularly.

The studied species were: trident maple (*Acer buergerianum* Miq.; BUE), coming from mountain forests of eastern China, Korea and Japan (Krüssmann, 1960), field maple (*Acer campestre* L.; CAM), growing in warm oak forests and forest-steppes of Slovakia (Dostál & Červenka, 1991), Montpellier maple (*Acer monspessulanum* L.; MON) from rocky slopes of Mediterranean to Caucasus mountains (Krüssmann, 1960), boxelder (*Acer negundo* L.; NEG), an alluvial tree species originating in eastern North-America (Krüssmann, 1960), palm maple (*Acer palmatum* Thunb.; PAL), a species of submontane and montane regions of China, Japan and Korea (Slavík, 2010), Norway maple (*Acer platanoides* L.; PLA), naturally growing in oak and lower beech forests of Slovakia (Bertová, 1984), silver maple (*Acer saccharinum* L.; SAC), from eastern North-American lowlands (Slavík, 2010) and Tatar maple (*Acer tataricum* L.; TAT), an associate of the field maple in Slovakia (Slavík, 2010).

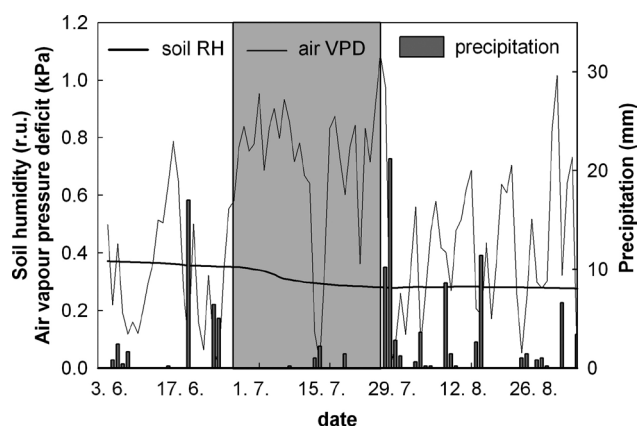Dynamics of related environmental factors was recorded. Daily average temperature (T_{ave}), daily

Fig. 1. Dynamics of precipitation (P), air vapour pressure deficit (VPD), and soil relative humidity at 40 cm depth (SRH) in the experimental field of the Mlyňany Arboretum IFE SAS during the summer drought period (grey area) in 2019

average relative humidity (RH_{ave}), and daily precipitation (P) were recorded from the beginning of June to the end of August 2019. Subsequently, the average daily vapor pressure deficit (VPD) was calculated.

$$VPD = (610.7 \times 10^{(7.5 \times T_{ave} / (237.3 + T_{ave}))} / 1000) \times ((100 - RH_{ave}) / 100) \quad (kPa)$$

Relative soil moisture at the depth of 40 cm (SRH) was measured with the soil moisture sensor 10HS (Decagon Devices Inc., USA) connected to a datalogger (MicroLog V3A, EMS Brno, Czech Republic). Data related to the soil/air humididy are presented in Fig. 1. According to the work of Polláková (2018), in the area of the experimental field, luvic chernozem is indicated.

Treatment and physiological measurements

For description of the experimental trees, their height (h; Suunto PM-5/1520, Suunto, Finland) and trunk diameter at 1 m height ($d_{1.0}$; defined from perimeter measured by tailor meter) was determined in early spring 2019 (Table 1). Then, at the beginning (26th June 2019) and the end (26th July 2019) of the regular summer dry period (before and after it), expanded leaves from the apical zone of each tree were collected at the noon (1:00 p.m.) for determination of relative water content (RWC), specific leaf area (SLA), free proline concentration (PRO), stomatal index (SI) and stomatal area (SA). Simultaneously, total tree leaf area (A) in each tree was estimated, and after resuming of the tree vegetative growth in 2019 and 2020 (end of June), trunk diameter at 1 m height ($d_{1.0}$) was determined for the relative trunk growh (RTG) calculation.

Table 1. Tree height (h) and trunk diameter at 1 m height ($d_{1.0}$) in eight maple species cultivated in the field for six years. Letters indicate statistical difference between species at the confidence level P≤0.05

Species	h (m)	$d_{1.0}$ (cm)
BUE	3.62±0.18b	3.3±0.3b
CAM	2.89±0.16b	3.1±0.3b
MON	3.00±0.06b	2.8±0.2b
NEG	5.32±0.35d	6.4±0.4c
PAL	1.40±0.16a	0.4±0.1a
PLA	4.54±0.34c	4.0±0.4b
SAC	6.30±0.34e	6.6±1.0c
TAT	4.11±0.38c	3.6±0.4b

Abbreviations: BUE – *Acer buergerianum* Miq., CAM – *A. campestre* L., MON – *A. monspessulanum* L., NEG – *A. negundo* L., PAL – *A. palmatum* Thunb., PLA – *A. platanoides* L., SAC – *A. saccharinum* L., TAT – *A. tataricum* L.

Relative water content was calculated according to the formula:

$$RWC = ((W_{act} - W_{dry}) / (W_{sat} - W_{dry})) \times 100 \quad (\%)$$

where: W_{act} – actual weight of a leaf segment, W_{dry} – leaf segment dry weight, W_{sat} – weight of the water-saturated leaf segment.

In the specific leaf area calculation, we followed this formula:

$$SLA = A_{act} / W_{dry} \quad (dm^2 \cdot g^{-1})$$

where: A_{act} – fresh leaf segment area as determined by scanner and Image J software.

The total leaf area of a tree was estimated using this multiplication:

$$A = A_{leaf} \times l \times b \quad (m^2)$$

where: A_{leaf} – area of an average leaf, l – number of leaves on a branch, b – number of branches in a tree.

Relative trunk growth was calculated as follows:

$$RTG = (d_{2020} - d_{2019}) / d_{2019}$$

where: d – tree trunk diameter at 1.0 m height in the studied years.

Free proline determination

Approximately 0.05 g of leaf material was homogenized in 6 ml of 3% sulfosalicylic acid. After 15 min centrifugation at 6000 rpm, 2 ml ninhydrin solution (1.25 g ninhydrin in 30 ml cold acetic acid and 20 ml 6 M phosphoric acid) and 2 ml cold acetic acid were added to 2 ml supernatant and the reaction mixture was incubated at 95 °C for one hour. The reaction was stopped by submerging into the ice bath, and thorough shaking with 4 ml toluene enabled chromophore extraction. Subsequently, the absorbance of the upper layer at 520 nm was measured against toluene (Bates et al., 1973).

Stomatal index and area determination

Abaxial side of the leaves, was covered by a layer of clear nail polish. After dessication, this layer was transferred using a transparent duct tape on a microscope slide. Individual slides with leaf imprints were observed under the light microscope Carl Zeiss Axiostar Plus (Carl Zeiss GmbH., Germany) at the total magnification of 400) and photos of two selected areas were taken with a digital camera Canon Power Shot G7 (Canon Inc., Japan), with a zoom setting the photographed area to 205 times 275 µm. After that, epidermal cells (excluding vein cells) and stomata were counted using ImageJ software. From the obtained data, value of the stomatal index (SI) was calculated:

$$SI = (number\ of\ stomata / (number\ of\ stomata + number\ of\ epidermal\ cells)) \times 100 \quad (\%)$$

Furthermore, length and width of stomata were measured in each photo using the ImageJ software (15 measurements per photo, 30 per slide). Based on these measurements, stomatal area (SA) was determined as the area of an ellipse:

$$SA = a / 2 \times b / 2 \times \pi \quad (µm^2)$$

where: a/2 and b/2 being half lengths of the long and short axes of the guard cells (Herrera & Cuberos, 1990).

Statistical analysis

Results are means±standard errors (SEs) of 6 replicates (n = 6), each from an individual plant. Data on RWC, PRO, SI, SA, SLA, A, h, and $d_{1.0}$ and RTG were submitted to one-way ANOVA in the Statgraphics Plus v. 4.0 software environment. Comparison of means was performed using Duncan's multiple range test. Different letters indicate statistically significant difference between species at the confidence level of 95%. Asterisk describes statistically significant change caused by the summer dry period at the confidence level 95%, double asterisk – 99% and triple asterisk – at the confidence level 99.9%. Thereafter, regression analysis was applied to relations between differences of RWC and PRO, SI, SLA, A and RTG as well as between PRO and SLA or RTG, for each maple species (Pearson's correlation coefficient, r).

Results

We observed significant differences in relative water content (RWC) across analyzed species (P ≤ 0.05; Fig. 2A). The highest percentual value of RWC before the drought was achieved by PAL (96.3 ± 0.4%) followed by MON (95.8±0.2%), while the lowest RWC was found in SAC (92.3±0.3%) and CAM (92.6±0.3%). The decrease of RWC due to the period of summer drought was statistically significant in each species (P ≤ 0.001). Half of the species, namely BUE, MON, PAL, SAC, reached RWC of approximately 90%. A little lower percentage was observed in NEG (88.8±0.9%) and TAT (88.3 ± 0.6%), and even lower by PLA (86.7±0.5%). Noticeably, the lowest RWC after the drought was measured in leaves of CAM with 82±0.7%. The analysis of control samples showed significant differences across species in free proline content (PRO; P ≤ 0.05; Fig. 2B), with the highest concentration in CAM (5.53±0.51 µg/g). The rest of the analyzed species reached significantly lower concentrations, minimal in SAC (2.41±0.14 µg/g) and TAT (2.55±0.29 µg/g). For most of the species, summer drought did not significantly affect the PRO concentrations. However, two species showed a significant increase in PRO, namely BUE ca. 2.4-fold increase (P ≤ 0.01) and SAC ca. 1.9-fold (P ≤ 0.05), resulting in 9.60±1.59 µg/g and 4.66±0.68 µg/g, respectively. Increase in PAL was insignificant.

Across the maple species, significant variability in stomatal index (SI; Fig. 3A) was observed (P ≤ 0.05). NEG and PAL showed the highest values of SI, around 20% both. Stomatal index values in BUE and TAT were also high, but they lagged behind
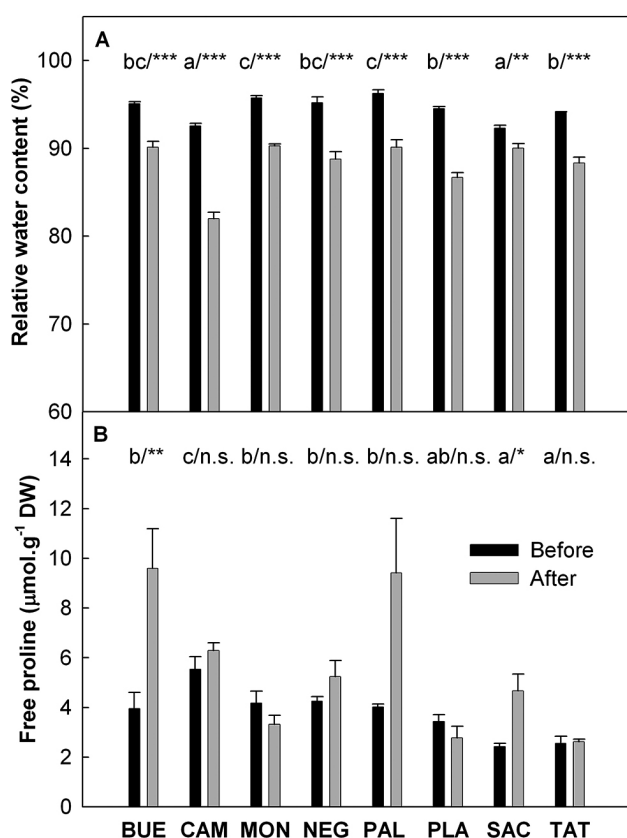

Fig. 2. Relative water content (A) and free proline concentration (B) in the top expanded leaf of juvenile seedlings of the eight studied maple species before and after the summer drought period in 2019. Letters indicate statistical difference between species before the dry period at the confidence level P≤0.05, n.s. – non significant difference and * – statistical difference between sampling terms (before and after the dry period) for each species at the same confidence level, ** – that at the confidence level P≤0.01 and *** – that at the confidence level P≤0.001

Abbreviations: BUE – *Acer buergerianum* Miq., CAM – *A. campestre* L., MON – *A. monspessulanum* L., NEG – *A. negundo* L., PAL – *A. palmatum* Thunb., PLA – *A. platanoides* L., SAC – *A. saccharinum* L., TAT – *A. tataricum* L.
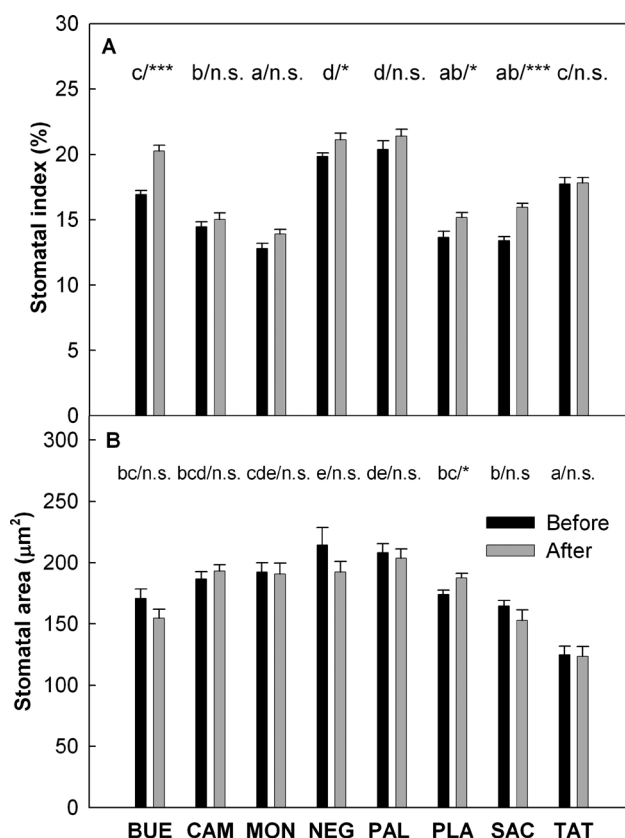

Fig. 3. Stomatal index (A) and area (B) in the abaxial side of the top expended leaves of the eight studied maple species as modified by the summer drought period in 2019. Letters indicate statistical difference between species before the dry period at the confidence level P≤0.05, n.s. – non significant difference and * – statistical difference between sampling terms (before and after the dry period) for each species at the same confidence level and *** – that at the confidence level P≤0.001

Abbreviations: BUE – *Acer buergerianum* Miq., CAM – *A. campestre* L., MON – *A. monspessulanum* L., NEG – *A. negundo* L., PAL – *A. palmatum* Thunb., PLA – *A. platanoides* L., SAC – *A. saccharinum* L., TAT – *A. tataricum* L.

previously mentioned species by 3%. The stomatal indices of CAM, PLA, and SAC were noticeably lower, in the range of 13.4–14.5%. The lowest SI value was measured for MON (below 13%). The drought period had a significant impact on the stomatal index in half of the observed species, responding with SI increase. The highest percentual increase was recorded for BUE, by about 3.3% (P ≤ 0.001). The appreciable increase was also detected in SAC (2.5%; P ≤ 0.001), PLA (1.5%; P ≤ 0.05), and NEG (1.3%; P ≤ 0.05). There were shown measurable differences in stomatal area (SA) across studied species (P ≤ 0.05), with NEG and PAL reaching the greatest values, 214.6±13.9 and 208±7.6 μm², respectively (Fig. 3B). On the contrary, the smallest stomata belonged to TAT (124.7±7.1 μm²). Stomatal area of the rest species ranged in 170–190 μm². Consequences of the

dry period were significant only in stomata of PLA, being larger by 13.6 μm².

Collected data showed, that of specific leaf area (SLA; Fig. 4A) was dominated by PAL, high above other species (P ≤ 0.05) with a value of 2.43±0.08 dm²/g, followed by NEG with 1.82±0.06 dm²/g. On the contrary, the lowest SLA was showed by SAC and TAT (1.31±0.08 dm²/g and 1.35±0.01 dm²/g). Changes due to the period of drought were statistically significant in half of the studied species. A decrease in SLA was noted in BUE by 0.29 dm²/g, and MON by 0.18 dm²/g (P ≤ 0.05), however in NEG and TAT drought caused the opposite effect – significant increase of SLA by 0.40 dm²/g (P ≤ 0.05) and 0.30 dm²/g (P ≤ 0.01), respectively. The analysis of studied species revealed variation in estimated total leaf area (A; P ≤ 0.05; Fig. 4B), with the highest values in SAC (15.50±5.06 m²). Closest to this value, however, with a remarkable difference, were PLA (11.74±1.69 m²) and NEG (11.03±3.30 m²). Noticeably lower leaf area was found in BUE (8.22±2.66 m²), followed by even lower values in TAT (5.15±0.34 m²), CAM (3.78±0.94 m²), and MON (3.53±0.14 m²). The lowest value was reached by PAL, with only 0.37±0.17 m². These values did not show any significant changes after the drought period, although obvious leaf shedding was observed in NEG and TAT.

Concerning the relative trunk growth (RTG; Fig. 5), the greatest values were achieved by PAL (0.369±0.053) and CAM (0.214±0.029) – significant higher (P ≤ 0.05) than in BUE, MON, PLA, SAC
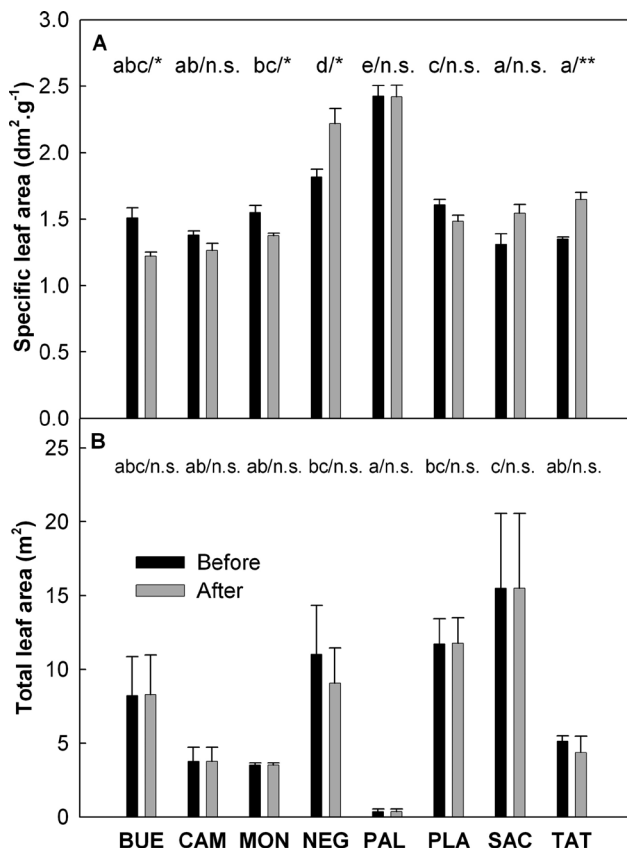

Fig. 4. Specific leaf area (A) and total leaf area (B) in the studied maple species before and after the summer drought period in 2019. Letters indicate statistical difference between species before the dry period at the confidence level P≤0.05, n.s. – non significant difference and * – statistical difference between sampling terms (before and after the dry period) for each species at the same confidence level and ** – that at the confidence level P≤0.01

Abbreviations: BUE – *Acer buergerianum* Miq., CAM – *A. campestre* L., MON – *A. monspessulanum* L., NEG – *A. negundo* L., PAL – *A. palmatum* Thunb., PLA – *A. platanoides* L., SAC – *A. saccharinum* L., TAT – *A. tataricum* L.

Fig. 5. Relative trunk growth at 1.0 m height in the eight ornamental maple species in 2020 as affected by the summer drought period in 2019 (RTG = $(d_{2020} - d_{2019})$ / d_{2019}, where d is the tree trunk diameter measured at the end of June). Letters indicate statistical difference between species at the confidence level P≤0.05

Abbreviations: BUE – *Acer buergerianum* Miq., CAM – *A. campestre* L., MON – *A. monspessulanum* L., NEG – *A. negundo* L., PAL – *A. palmatum* Thunb., PLA – *A. platanoides* L., SAC – *A. saccharinum* L., TAT – *A. tataricum* L.
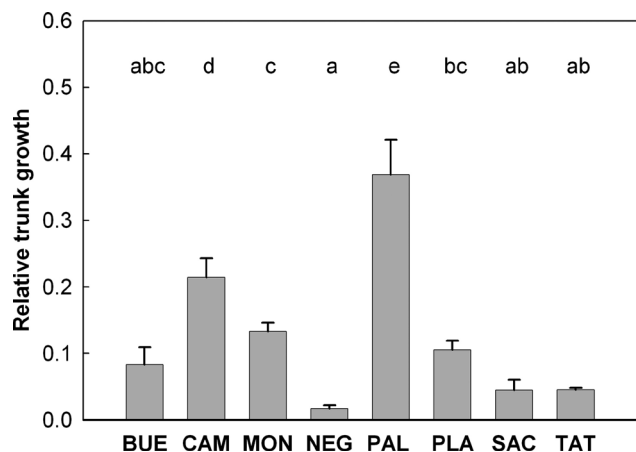
Table 2. Pearson's correlation coefficients (r) calculated for relations between physiological parameters differences derived from data recorded before and after the dry summer period in 2019 or after resuming of the vegetative growth in 2020 (RTG)

Species	Pearson's correlation coefficient (r)						
	RWC-PRO	RWC-SI	RWC-SLA	RWC-A	RWC-RTG	PRO-SLA	PRO-RTG
BUE	0.807*	0.056	−0.083	0.827*	0.506	−0.538	0.856*
CAM	−0.758*	0.478	−0.746*	–	−0.952**	0.666	0.742*
MON	0.727*	−0.580	0.880*	–	−0.650	0.578	0.014
NEG	0.055	0.473	0.063	−0.281	0.754*	0.467	0.136
PAL	−0.124	−0.352	0.733	–	0.648	0.243	−0.722*
PLA	−0.559	0.314	−0.410	−0.102	0.397	0.190	0.310
SAC	0.475	0.277	−0.757*	–	−0.092	0.113	0.343
TAT	0.913**	−0.866*	−0.443	0.224	0.010	−0.420	0.417

Abbreviations: BUE – *Acer buergerianum* Miq., CAM – *A. campestre* L., MON – *A. monspessulanum* L., NEG – *A. negundo* L., PAL – *A. palmatum* Thunb., PLA – *A. platanoides* L., SAC – *A. saccharinum* L., TAT – *A. tataricum* L., RWC – relative water content, PRO – leaf free proline concentration, SLA – specific leaf area, RTG – relative trunk growth at 1 m height in 2020, * – $0.7 \leq r < 0.9$ – strong correlation, ** – $0.9 \leq r < 1.0$ – very strong correlation.

and TAT. On the other hand, NEG reached the lowest values (0.017±0.006).

Calculation of the Pearson's correlation coefficients (Table 2) revealed strong relation of RWC difference between the sampling terms (before and after the dry summer period) with PRO difference in BUE, CAM and MON, with SI difference only in TAT, with SLA difference in CAM, MON and SAC, with A difference only in BUE, and with RTG in NEG. Difference in PRO was strongly related only to RTG (in BUE, CAM and PAL). However, two very strong relationships were found, as well – between RWC and PRO difference in TAT (r = 0.913) and between RWC difference and RTG in CAM (r = −0.952).

Discussion

Summer drought period in 2019, approximately one month (July) without larger precipitation, with maximal daily air temperatures often exceeding 35 °C and the average daily vapour pressure deficit attacking 1 kPa (Fig. 1), was one of the typical dry periods of the last decade in Vieska nad Žitavou, Slovakia. Since the leaf relative water content in most of the studied species decreased only of about 5–6%, in *A. saccharinum* ca. 2%, in *A. platanoides* almost 8% and *A. campestre* a little more than 10%, we can state that no from them suffered from drought stress (Banks et al., 2019; Raček et al., 2020). On the other hand, activation of protective mechanisms in some species, namely water/resource translocation (leaf shedding) in *A. negundo* and *A. tataricum* or osmotic adjustment (free proline increase) in *A. buergerianum*, *A. palmatum* and *A. saccharinum* means that species sensed concrete levels of stress, which triggered these morphological/metabolic changes. However, whereas leaf shedding is one of the earliest acclimatory response, osmotic adjustment turns on under deeper water deficit (Fitter & Hay, 2002). Since elevated stomatal

index (*A. buergerianum*, *A. negundo*, *A. platanoides* and *A. saccharinum*) and reduced specific leaf area (*A. buergerianum*, *A. monspessulanum*) were associated only with leaves developed during the summer, their effect on the plant water status was limited (Table 2). Thus, we could suppose that three of the studied maple species, which did not activate the leaf area reduction or osmotic adjustment (*A. monspessulanum*, *A. platanoides* and *A. campestre*), avoided stress probably by reaching water reserves in deeper soil layers. However, little is known about the root growth stimulation under drought in maples. Root-to-shoot ratio of *A. buergerianum* seedlings grown under 15% field water capacity rose very slightly (by 0.15) in comparison to those cultivated in soil of 75% field capacity (Guo et al., 2013), what points to larger role of another protection mechanisms, including osmotic adjustment.

Second, plant memory of past drought stress as well as cross stress tolerance need to be taken into account in this experiment (Walter et al., 2013; Li & Liu, 2016). Before our measurement were carried out, plants could sense water shortage (despite of summer watering in the first three years of cultivation) as well as temperature extremes. Concretely, July 2015 and August 2017 were found as extremely dry (20–30% of the long-term normal) and July and August 2015 along with June 2019 as extremely hot months (4 °C higher average temperatures compared to the normal values).

Two different reactions to the water shortage were observed in stem radial growth of coniferous species after drought pre-treatment. For instance, *Abies concolor* (Gordon) Lindl. ex Hildebr. and *Pinus ponderosa* Douglas ex C. Lawson showed low resistance and slow recovery of this parameter, but an opposite answer was found in *Juniperus* species (Peltier et al., 2016). Similar results were obtained for *Pinus sylvestris* L. and *Larix decidua* Mill. on one side and *Pinus nigra* L. and *Pseudotsuga menziesii* (Mirb.) Franco

on the other one (Eilmann & Rigling, 2012). Examination of growth patterns in deciduous black locust (*Robinia pseudoacacia* L.) after resprouting under different cycles of drought or regular watering revealed higher drought tolerance in drought pre-treated individuals (Mantovani et al., 2014). Two grape cultivars (Sangiovese and Montepulciano) exposed to severe drought for 4 years, compared to those kept at 90% field capacity, showed a shift to less conservative strategy towards water loss and decreased water use efficiency (Tombesi et al., 2018). Furthermore, drought primed saplings of mulberry (*Morus multicaulis* (Perr.) Loudon) sustained comparable or slightly higher biomass accumulation under second drought than not-primed ones. They also exhibited lower osmolyte accumulation and oxidative damage in roots and leaves (Liu et al., 2022).

Altogether, some of tree species can benefit from early experiences when they cope with a drought stress episode, but some of them cannot. Calculation of the relative trunk growth (RTG) in our collection of maple species revealed the largest drought sensivity in the fast growing *A. negundo*, *A. saccharinum* and in *A. tataricum* (Fig. 5). On the other hand, the slowliest growing *A. palmatum* showed the largest growth stability. We found a strong negatively relationship between RTG and species stem diameter at 1.0 m height (r = −0.845), indirectly describing the plant growth rate. It was strongly correlated with RWC in *A. negundo* and free proline in *A. buergerianum* and *A. campestre* (Table 2), showing clear association of RTG with the plant water status improvement ability.

Thus, taking into account the decisive protective mechanisms onset and the trunk growth stability (RTG), species in our experiment can be ordered in respect of drought resistance as follows: 1. *A. campestre*, 2. *A. monspessulanum*, 3. *A. platanoides*, 4. *A. tataricum*, 5. *A. negundo*, 6. *A. saccharinum*, 7. *A. palmatum* and 8. *A. buergerianum*. This ranking nicely corresponds with the natural conditions (lack of precipitation and temperature elevation in summer) they come from (Krüssmann, 1960; Bertová, 1984; Dostál & Červenka, 1991; Slavík, 2010), except for *A. tataricum*, which occupies similar habitats as *A. campestre*.

However, what is their ranking in other works carried out mostly in adult individuals? Niinemets & Valladares (2006) indicated the highest drought tolerance index for *A. monspessulanum* (4.31 out of 5), then 3.37 for *A. tataricum* and 2.73–3.03 for the rest species (except for *A. buergerianum* and *A. palmatum*). Sjöman et al. (2015) ordered these species according to summer leaf water potential at turgor loss (Ψ_{P0}) from the least to the most drought tolerant, as follows: *A. negundo* with Ψ_{P0} of ca. −2.5 MPa, *A. campestre* and *A. platanoides* – ca. −3.0 MPa, *A. saccharinum* and *A. tataricum* – about 3.5 MPa and *A. monspessulanum* with values around −4.5 MPa. As reported by

Tissier et al. (2004), maple species preferring moist stands (*A. campestre*, *A. platanoides* and *A. negundo*) lost xylem conductivity at almost −3 MPa, but *A. mospessulanum* from drier stands at about −4.5 MPa. Lens et al. (2011) studied mean cavitation pressure at zero stem hydraulic conductivity in some species from our collection: *A. negundo* – approx. −2.5 MPa, *A. saccharinum* – about 3.0 MPa and *A. platanoides* – ca. −5.5 MPa. Work of Mao et al. (2016), based on testing antioxidant defense in maple seedlings against dessication caused by polyethylene glycol (PEG 6000), showed *A. palmatum* as more sensitive than *A. negundo*. The only one paper on trident maple (*A. buergerianum*) acclimation to water stress revealed excellent water acquisition ability of this species (Guo et al., 2013).

Nevertheless, drought stress resistance can substantially change with the tree age. As referred by Lucas-Borja et al. (2021), effect of water inbalance (precipitation minus evapotranspiration) on basal area increment was lower in younger age classes (1–39 years old) of black pine (*Pinus nigra* Arn. ssp. *salzmannii*) forest than in older ones. In opposite, older Qinghai spruce (*Picea crassifolia* Kom.) stands exhibited higher resistance to drought than younger stands, but the post-drought recovery showed opposite pattern. These age-related discrepancies in drought resilience were significantly smaller going from wetter region to drier one (Wang et al., 2022). Dominant tree species in an old-grown tropical forest in Thailand were more vulnerable to embolism and hence more sensitive to water stress than those from younger sites (Unawong et al., 2022). However, pressure-volume curve parameters, namely RWC and water potential at turgor pressure loss and osmotic potential at water saturated state, decreased markedly with age in *Robinia pseudoacacia* L. and *Populus simonii* Carrière, suggesting rise in the drought tolerance (Li et al., 2012). Aging of a drought-tolerant shrub *Cistus clusii* Dunal led to enhanced oxidative stress (increased lipid peroxidation, reduced photosystem II efficiency), and rise in abscisic acid concentration at similar water and nitrogen level in leaves during stressful spring and summer period (Munné-Bosch & Lalueza, 2007).

The tree age-related drought resistance development can differ among species and this needs to be considered when new long-term cultivated plantations should be established. Comprehension of above listed literature rankings of maple species based on adult individuals evaluation showed approximately following order: *A. monspessulanum*, *A. tataricum*, *A. platanoides*, *A. campestre*, *A. saccharinum* and *A. negundo*. However, considering this species order, not only age, but also genotypic and environmental differences from our experiment need to be taken into account in estimation of the shift in drought resistance.

Conclusions

Knowledge on drought resistance of ornamental tree species is crutial for their application in plantations in adverse urban environment. This work brought information on physiological and morphological reactions of juvenile individials of attractive native as well as foreign maple species to summer drought in southern Slovakia along with consideration of stress memory effect and age-related changes in drought resistance. Finally, the species drought resistance order was formed (with the mostly drought resistant *A. campestre*), as a practical output.

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