Volume 67 | 41 | Number 2, 2019

PLANTS RECOVERY PERFORMANCE FROM WATER STRESS

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To link to this article: https://doi.org/10.11118/actaun201967020453
Received: 15. 10. 2018, Accepted: 14. 1. 2019

To cite this article: ŠAJBIDOROVÁ VIERA, HILLOVÁ DAGMAR, ŽIVČÁK MAREK, LICHTNEROVÁ HELENA. 2019. Plants Recovery Performance from Water Stress. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 67(2): 453–462.

Abstract

Recovery of plants after water stress events represents their high suitability for the urban condition areas. Reaction to drought and recovery of woody plants *Cornus mas* L., *Lonicera caerulea* L. and perennials *Alchemilla mollis* (Buser) Rothm., *Geranium maculatum* L., *Geranium x magnificum* Hyl. 'Rosemoor' and *Geranium* 'Philippe Vapelle' were evaluate. Within a pot experiment the non-destructive methods of monitoring: measurement of chlorophyll content using chlorophyll meter CL-01, leaf stomatal conductance using Delta T Leaf porometer AP4 and modulated chlorophyll fluorescence using Hansatech FMS 1 were chosen. Based upon our results we may demonstrate different protective mechanisms of plants in water stress conditions. An increase in the chlorophyll concentration in stress-exposed tissues and recovery of stomatal conductance in *Cornus mas* L. and in *Alchemilla mollis* (Buser) Rothm. were observed. In *Cornus mas* L. and *Lonicera caerulea* L. the early recovery of parameter ϕPSII (after 2 days of re-watering) and the delayed recovery (after 6 days of re-watering) in *Geranium* plants and *Alchemilla mollis* (Buser) Rothm.) were shown.

Keywords: water stress, recovery performance, drought tolerant woody plants, drought tolerant perennials

INTRODUCTION

Plants stress recovery caused from drought is crucial for their survival in the urban environment in course of the climate change. Especially crops production is being put at risk from drought events during the season. Better understanding of plant post-drought recovery performance could improve our predictions on the ecosystem productivity in a rapidly changing climate (Yin, 2017). Ornamental plants in public areas are also threatened by water stress and it is important to select trees, shrubs and herbs species with high tolerance to drought events.

Plants stress recovery is predetermined by geographical variation and biological adaptation characteristics of plants. In order to understand the influences of geographic variation and plant physiology/morphology on post-drought recovery performance across many taxonomic groups, it would be helpful to compare the recovery performance among plant groups with similar plant biological characteristics or those which are adapted to similar habitats (Yin, 2017). Herb species have been found to have greater and faster post-drought photosynthetic recovery than shrubs because herbs must reach their carbon balance before ending their life cycle in the late spring (Galmés *et al.* 2007). Yet we do not have sufficient knowledge of how extreme events shape individuals, communities and ecosystems. Extreme events (climatic extremes, physical disturbances, insect outbreaks and invasions by exotic species) play a disproportionate role in shaping the physiology, ecology and evolution of organisms, with the emphasis on terrestrial plants (Gutschick, 2003).

Diverse drought-survival strategies and leaf hydraulic features among multiple plant functional types may imply equally diverse plant strategies also exists in response to re-watering (Yin, 2017).

One of the crucial factors for a plant growth is the accessibility of water in the soil. Plants have a set of adaptive mechanisms that help them to tolerate adverse environmental conditions. There has been quite a lot of research about the drought response of crops but also more testing of drought tolerant ornamental plant species is needed in the field of landscape architecture. Based upon the aforementioned the aim of this experiment has been set – comparing the three perennial species's drought stress reactions with similar demands on the environment. In order to point out to possible differences, we have decided to add species with different demands on the environment or those from another plants group (perennials and shrubs).

Woody plants species with different demands on the environment with the aim to compare the reaction to water stress and recovery in this experiment were selected. *Cornus mas* L. is very suitable for urban habitats after the expected climate change because of drought tolerance and hardiness (Roloff *et al.* (2009) and has a high plasticity, growing in all kinds of soils (Da Ronch *et al.* 2016).

Lonicera caerulea L. naturally grows near water and is not drought-tolerant species (Lauritzen *et al.* 2015).

Herbaceous perennials were selected according to growth attributes, habitat classification defined by Hansen and Stahl (1993) and categories of herbaceous plants according to physiological traits (Hillová, 2016). Four low widely spreading herbaceous plants for sun and semi-shade on the woodland edge (*Geranium maculatum* L., *Geranium x magnificum* Hyl. 'Rosemoor', *Geranium* 'Philippe Vapelle') and loosely bound to the woodland edge (*Alchemilla mollis* (Buser) Rothm.) were chosen for experiment. *Geranium* taxa were selected as a resistant to dehydration stress, while *Alchemilla mollis* as the most 'susceptible' to dehydration.

The aim of the article is to evaluate the reaction of plants in the condition of water stress (without any water supply) and the plants recovery (regeneration after water supply) based on selected characteristics – chlorophyll content, stomatal conductance and the effective quantum yield of PSII.

Chlorophyll is one of the major chloroplast components for photosynthesis. Relative chlorophyll content has a positive relationship with a photosynthetic rate. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation (Anjum *et al.* 2011).

The stomatal conductance (g_s) of the leaves was used to monitor the reactions of stomata through water deficit impact. This is a very important defense mechanism against a loss of water (Tardieu and Davis, 1993; Živčák, 2006). Impact of water deficit results in closure of stomata and a decrease in photosynthesis. Therefore, a maintenance of higher g_s values due to osmotic adjustment in long-lasting drought may represent important part of drought tolerance (Živčák *et al.* 2009).

The chlorophyll fluorescence signal is very sensitive to environmental effects and well reflects the changes in photosynthesis (Kalaji *et al.*, 2014). The effective quantum yield of PSII (ΦPSII) is a real yield of active PSII reaction centers in the processing of absorbed light energy. ΦPSII represents the fraction of the light energy used in photochemistry (Genty *et al.*, 1989; Schreiber, 2004). The decrease of photosynthetic assimilation can be well detected by a decrease of ΦPSII, as it was documented also in woody plants (Peguero-Pina *et al.*, 2008, Gallé *et al.*, 2007, Šajbidorová *et al.* 2015).

MATERIAL AND METHODS

Two shrubs species (*Cornus mas* L., *Lonicera caerulea* L.) and four perennials taxa (*Alchemilla mollis* (Buser) Rothm., *Geranium maculatum* L., *Geranium x magnificum* Hyl. 'Rosemoor' and *Geranium* 'Philippe Vapelle') were chosen for the experiment of plants recovery performance after water stress.

The five plants form each taxa were cultivated into the substrate based on the white peat, enriched with the clay (20 kg/m^3), pH 5.5–6.5, with NPK fertilizer 14:10:18 (Klasmann TS3, Klasmann-Deilmann GmbH, Germany), in the 3L pot size, under the polypropylene cover with 40 % of shading.

Within a pot experiment physiological responses of plants in relation to water scarcity and water stress recovery were monitored. The non-destructive methods of monitoring the impact of a lack of water in the soil to plants, measurement of chlorophyll content, leaf stomatal conductance and modulated chlorophyll fluorescence were chosen.

At the beginning of the experiment, plants were hydrated in 60 % of the soil water supply for one month to root the plants, The soil water supply at 60 % represents optimal condition (control condition) of substrate water supply based on our previous experiments (Šajbidorová *et al.,* 2015). After rooting of plants they were exposed to water stress – plants were not hydrated anymore. After 7, 14 days and (after 21 days only in species *Cornus mas* L.) measurements were made on two leaves per plant from each taxa. After 14 days (after 21 days only in species *Cornus mas* L.) plants were re-irrigated in 60 % of the soil water supply with the goal to observe the regeneration of plants after water scarcity. The experiment started in April 2015 (with the one month of rooting phase), measurement started at the beginning of June and finished at the end of June, respectively at the beginning of July 2015 (according to experimental taxa).

Except for *Cornus mas* L. all taxa persisted without water supply 14 days, only *Cornus mas* L. resisted for 21 days, without of wilting syptoms the wilting of plants was the signal of the drought stress limit and the beginnig of re-irrigating of plants and their regeneration.

Determination of chlorophyll content was performed using portable chlorophyll meter CL-01 *(PP Systems, USA).* The portable chlorophyll meter determines the relative chlorophyll content of the leaves by measuring the absorbance at wavelength (620 and 940 nm). The instrument measures the absorbance of both wavelengths and expressed the values in CCI (Chlorophyll Content Index) units.

When measuring leaf stomatal conductance Delta T leaf porometer AP4 (Delta-T Devices Ltd, United Kingdom) was used. Measurement of a loss of water vapor through the stomata took place before midday. The leaf stomatal conductance was determined in mm. s^{-1}, together with the recording the current time, light intensity in μmol. m^{-2}. s^{-1} and the current temperature in degrees Celsius.

Chlorophyll fluorescence was measured by the chlorophyll fluorometer Hansatech FMS 1 (Hansatech Instruments Ltd, United Kingdom) in the morning hours. The following characteristics of measurement protocol of the chlorophyll fluorescence were used: one second light pulses of red light with an intensity of 895 μmol. m^{-2}. s^{-1}, the intensity of actinic light 34 μmol. m^{-2}. s^{-1} and the saturation light pulse 10 000 μmol. m^{-2}. s^{-1}.

For the mathematical and statistical analysis of the data one-way Anova and LSD test, $P < 0.05$ were used. The statistical assessment of the data was conducted using software Statgraphics Centurion XVII (StatPoint Technologies, USA, XV, license number: 7805000000722).

RESULTS AND DISCUSSION

We have observed if there is any difference in chlorophyll content values over 14 days of drought treatment and 2 respectively 6 days after re-irrigating of plants originally exposed to 14 days of drought treatment.

The chlorophyll content reached in *Lonicera caerulea* L. statistically significant lower values compared to *Cornus mas* L. and did not changed recognizably after re-irrigating of plants (Tab. I.). The decrease in the water content in the substrate may lead to an increase in the chlorophyll concentration, obvious for example in *Cornus mas* L. (after 7 days), which does not reflects any real increase in the chlorophyll synthesis, but the concentration increases due to lower water content in stress-exposed tissues. The next drought (after 14 days) may cause some decrease in chlorophyll, which may be interpreted as a protective response of some species to prevent excessive photoinhibitory damage in drought stress conditions (Munné-Bosch and Alegre, 2000). The changes to chlorophyll content may be associated also with the drought-induced leaf senescence. That means avoiding of large losses through transpiration, thus contributing to the maintenance of the favourable water balance of the whole plant. Drought - leaf senescence is considered to be the adaptive mechanisms of the survival because it reduces the water demand on the whole-plant level (Munné-Bosch and Alegre, 2004).

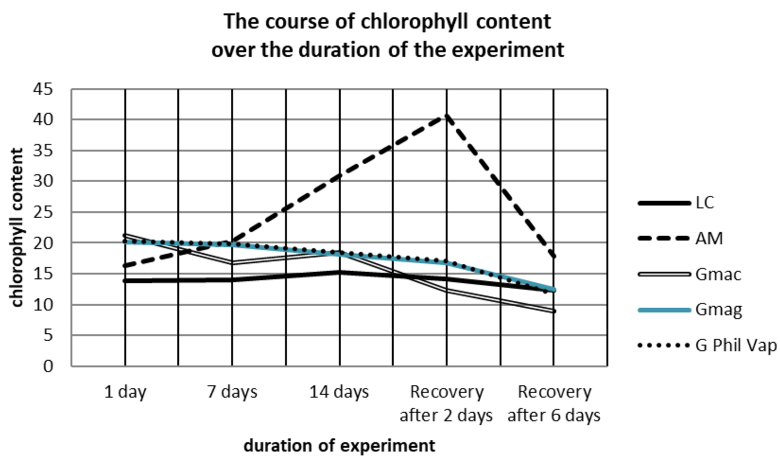
The decrease in the chlorophyll content in *Geranium maculatum* L. was not statistically significant after 14 days of drought treatment (by 13 %). After re-irrigating of plants we have observed statistically significant decrease of values by 42 % (recovery after 2 days) and 58 % (recovery after 6 days), compared to first day of drought treatment.

Also in *Geranium x magnificum* Hyl. 'Rosemoor' was not statistically significant decrease in the chlorophyll content after 14 days of drought treatment (by 10 %). Re-irrigating leaded to decrease in the values by 17 % (recovery after 2 days) and statistically significant 38 % (recovery after 6 days), compared to first day of drought treatment.

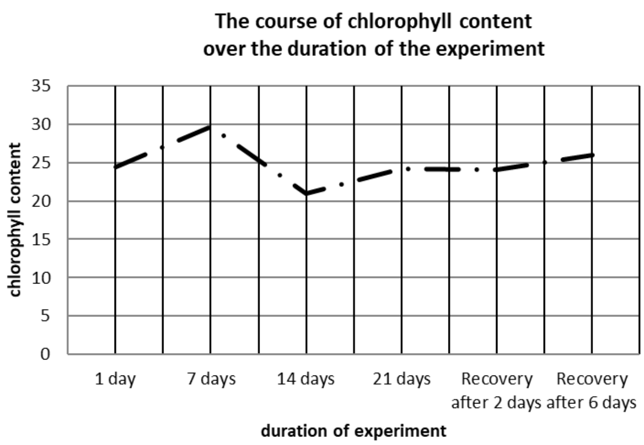
The decrease in the chlorophyll content in *Geranium* 'Philippe Vapelle' was not statistically significant after 14 days of drought treatment (by 9 %). Re-irrigating leaded to decrease of values by 16 % (recovery after 2 days) and statistically significant 42 % (recovery after 6 days), compared to first day of drought treatment.

In the *Geranium* – plants were observed the decrease in the chlorophyll content values during drought treatment and also after re-irrigating of plants. In comparison to the experimental perennial taxa (*Alchemilla mollis* (Buser) Rothm.) the changes in this parameter were not so obvious (Fig. 1) which might have been due to a different adaptation ability to water stress condition.

However, we observed a significantly different response in drought-exposed plants of *Alchemilla mollis* (Buser) Rothm., in which the chlorophyll content increased by 90 % after 14 days of drought treatment (Fig. 1). It was shown that water stress probably may change the structure of leaves (Ennajeh *et al.*, 2010). In this respect, the changes to the chlorophyll content may be associated with adjustment of leaf anatomy in *Alchemilla mollis* that we can interpret it as an adaptation mechanism. The fact that no decrease in the chlorophyll content was observed two days



1: *The course of chlorophyll content over the duration of the experiment*
Explanation: LC - *Lonicera caerulea* L., AM - *Alchemilla mollis* (Buser) Rothm., Gmac - *Geranium maculatum* L., Gmag - *Geranium x magnificum* Hyl. 'Rosemoor', G Phil Vap - *Geranium* 'Philippe Vapelle '



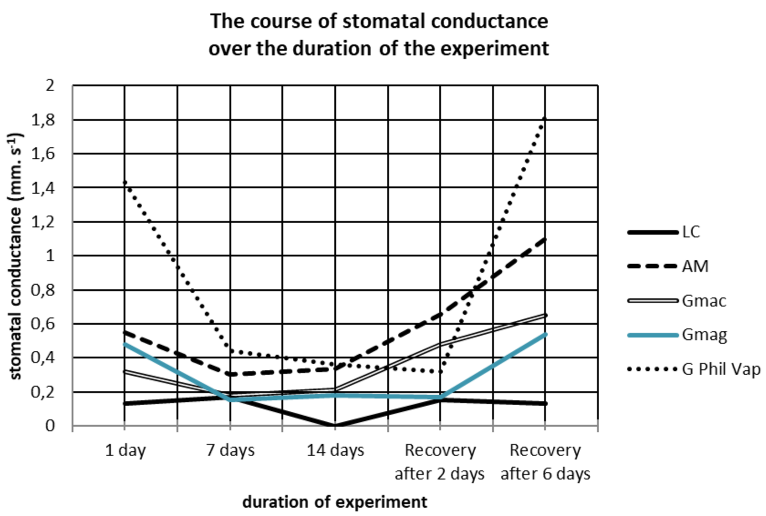
2: *The course of chlorophyll content over the duration of the experiment in Cornus mas L.*

after re-irrigating of plants in this species confirms that the increase in chlorophyll was not caused by the loss of water, but probably by structural changes in leaf tissues. However, the next growth in well-watered conditions led to significant decrease in the chlorophyll content and *Alchemilla* plants reached the initial values of chlorophyll. These reactions indicate very strong ability of this species to modify photosynthetic apparatus for actual conditions, which is typical for herbaceous plants, whereas the chlorophyll content of woody plants use to be more stable (Evans and Poorter, 2001). The results indicate the importance of anatomical analysis of leaves in our future experimental tasks.

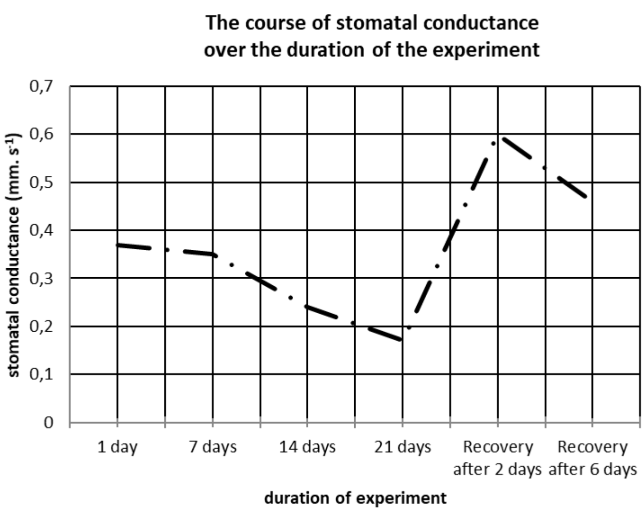
As the photosynthetic productivity in drought is limited mostly due to stomatal closure, we have measured stomatal conductance to test if there is any difference in stomatal conductance values over 14 days of drought treatment and 2 respectively 6 days after re-irrigating of plants originally exposed to 14 days of drought treatment.

In the woody plant *Lonicera caerulea* L. after 14 days of water stress no leaf stomatal conductance can be measured. Water stress lead to closure of stomata. After re-irrigating of plants the values of g_s reached the initial values (Tab. I.).

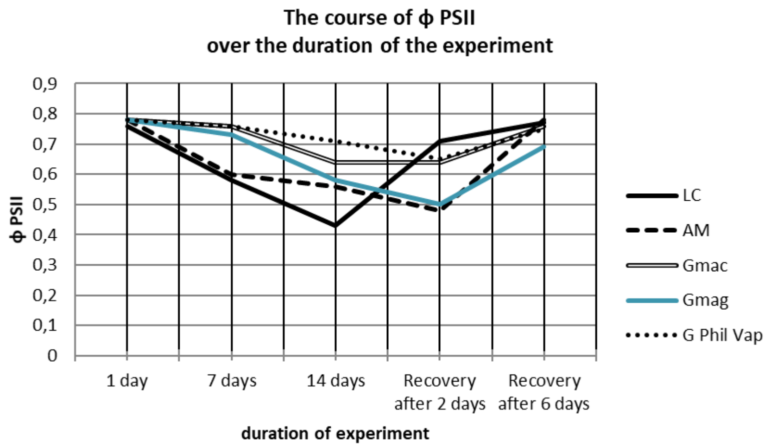
In *Cornus mas* L. after 21 days we have observed not statistically significant decrease of g_s values (Fig. 4). Re-irrigating of plants led to the statistically significant increase in g_s values by 60 % in contrast to the initials values. This species is able to regenerate from water scarcity that we



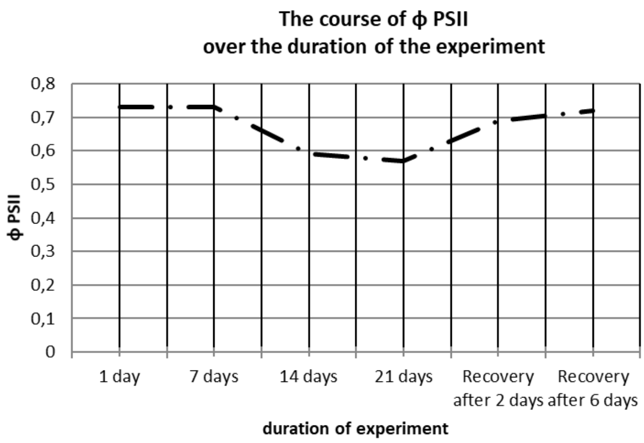
3: *The course of stomatal conductance over the duration of the experiment*
Explanation: LC - *Lonicera caerulea* L., AM - *Alchemilla mollis* (Buser) Rothm., *Gmac* - *Geranium maculatum* L., Gmag - *Geranium x magnificum* Hyl. 'Rosemoor', G Phil Vap - *Geranium* 'Philippe Vapelle'



4: *The course of stomatal conductance over the duration of the experiment in Cornus mas L.*



5: *The course of ϕ PSII over the duration of the experiment*
Explanation: LC - *Lonicera caerulea* L., AM - *Alchemilla mollis* (Buser) Rothm., *Gmac* - *Geranium maculatum* L., Gmag - *Geranium x magnificum* Hyl. 'Rosemoor', G Phil Vap - *Geranium* 'Philippe Vapelle'



6: *The course of ϕ PSII over the duration of the experiment in Cornus mas L.*

have assumed because their drought tolerance (Roloff *et al.* 2009).

In *Alchemilla mollis* (Buser) Rothm. and *Geranium maculatum* L. the reaction of stomata in condition of water stress and recovery was very similar. In *Geranium maculatum* L. The gs values after 14 days of drought was lower but not significant (by 34 %). Two days after re-irrigating of plants the gs values increased by 50 % compared to the initial values. Six days after re-irrigating of plants the gs values statistically and significantly increased by 103 % compared to the 1 day of drought stress. The slow recovery of stomata conductance may be associated with accumulation of abscisic acid or other inhibitory stress compounds, which prevent stomata opening despite the water content was recovered (Miyashita *et al.*, 2005).

In *Alchemilla mollis* (Buser) Rothm. The decrease of gs values after 14 days of water stress was not significant (by 39 %). Two days after re-irrigating of plants (plants water stress recovery) increased the g_s values by 20 % compared to the initial values. Six days after re-irrigating of plants the g_s values statistically significant increased by 102 % compared to the 1 day of drought stress (Fig. 3). The fact that the stomatal conductance on the 6th day of the recovery increased reduces the likelihood that the decrease in the chlorophyll content was caused by the senescence, but supports the structural changes towards a non-stressed phenotype.

In *Geranium x magnificum* Hyl. 'Rosemoor' and *Geranium* 'Philippe Vapelle' water stress lead to bigger differences between the initial values of g_s and values after 14 days of drought treatment (increase of g_s values *Geranium x magnificum* Hyl. 'Rosemoor' by 63 % and in *Geranium* 'Philippe Vapelle' by 75 %). These plants were not able to regenerate in this parameter to the initial

I: *The mean values of the analyzed parameters and 95 % LSD test for the studied taxa. Values with the same letter are not significantly different.*

Duration of experiment	chl		g_s		ϕ PSII	
Lonicera caerulea						
1 day	13.91	ab	0.13	b	0.76	c
7 days	13.97	ab	0.17	bc	0.58	ab
14 days	15.17	b	0.00	a	0.43	a
Recovery after 2 d	14.11	ab	0.15	bc	0.71	bc
Recovery after 6 d	12.32	a	0.13	bc	0.77	c
Cornus mas						
1 day	24.42	ab	0.37	bc	0.73	b
7 days	29.66	c	0.35	bc	0.73	b
14 days	20.91	a	0.24	ab	0.59	a
21 days	24.22	ab	0.17	ab	0.57	a
Recovery after 2 d	24.12	ab	0.60	d	0.69	b
Recovery after 6 d	26.03	bc	0.46	c	0.72	b
Alchemilla mollis						
1 day	16.26	a	0.55	ab	0.78	b
7 days	20.25	ab	0.30	a	0.60	a
14 days	30.92	bc	0.34	ab	0.56	a
Recovery after 2 d	40.70	c	0.65	b	0.48	a
Recovery after 6 d	17.84	ab	1.10	c	0.78	b
Geranium maculatum						
1 day	21.15	c	0.32	ab	0.78	b
7 days	16.74	abc	0.18	a	0.76	b
14 days	18.47	bc	0.21	a	0.64	a
Recovery after 2 d	12.26	ab	0.48	bc	0.64	a
Recovery after 6 d	8.97	a	0.65	c	0.76	b
Geranium magnificum ’Rosemoor’						
1 day	20.22	a	0.48	b	0.78	d
7 days	19.74	a	0.15	a	0.73	cd
14 days	18.13	a	0.18	ab	0.58	b
Recovery after 2 d	16.80	a	0.17	a	0.50	a
Recovery after 6 d	12.44	b	0.54	b	0.69	c
Geranium ‘Philippe Vapelle’						
1 day	20.34	b	1.43	bc	0.78	c
7 days	19.83	b	0.44	a	0.76	c
14 days	18.43	b	0.36	a	0.71	b
Recovery after 2 d	17.10	b	0.32	ab	0.65	a
Recovery after 6 d	11.78	a	1.82	c	0.75	bc

values 2 days after re-irrigating, but after 6 days of re-irrigating the values reached the initial values, even in the *Geranium* 'Philippe Vapelle' was the recovery values after 6 days about 27 % higher than initial values, which may reflect the structural changes to the leaves caused by drought.

Stomata are the entrance of water loss and CO_2 absorbability and stomatal closure is one of the first responses to drought stress which results in lower rate of photosynthesis. Stomata close progressively with increased drought stress (Anjum *et al.* 2011) that we consider as well (decrese of g_s is more evident 7 days of water stress duration).

Drought stress progressively decreases CO_2 assimilation rates due to reduced stomatal conductance, it disrupts photosynthetic pigments and reduces the gas exchange leading to a reduction in plant growth and productivity (Anjum *et al.* 2011).

To analyze the photosynthetic responses, the chlorophyll fluorescence measurements were driven. We have observed if there is no difference in ϕ PSII values over 14 days of drought treatment and 2 respectively 6 days after re-irrigating of plants originally exposed to 14 days of drought treatment.

In all taxa we have observed statistically significant decrease in ϕ PSII values during 14 days of drought stress treatment (Tab. I.) with the biggest decrease to have been reported in *Lonicera caerulea* L. (by 43 %), the smallest decrease was in *Geranium* 'Philippe Vapelle' (by 9 %). In another species the decrease accounted for appr. 20–25 % .

After re-watering of plants we observed the recovery of values ϕ PSII nearly to the initial values in *Lonicera caerulea* L. and *Cornus mas* L. (Tab. I., Fig. 5, Fig. 6).

Reaction in *Alchemilla mollis* (Buser) Rothm. 2 days after re-irrigation the 38-percent decrease in the values was reported and 6 days after re-irrigation the values were the same as during the 1st day (absolute regeneration).

In *Geranium maculatum* L. after re-watering was monitored the decrease by 18 % from initial values. Six days after re-irrigation the values was nearly the same than in the 1st day.

In *Geranium x magnificum* Hyl. 'Rosemoor' after re-watering was monitored the decrease by 36 % from initial values. Six days after re-irrigation the values was nearly the same than in the 1st day.

In *Geranium* 'Philippe Vapelle' after re-watering was monitored the decrease by 17 % from initial values. Six days after re-irrigation the values was nearly the same than in the 1st day.

In general, we observed that in the drought stress ϕ PSII values decreased in all species; however, the level of the decrease varied in different species. It was fully in accordance with the expectations, as the PSII quantum yield is a very good indicator of the decrease in the photosynthetic rate in drought (Maxwell and Johnson 2000). The most significant differences were observed in drought recovery, in which we reported both early recovery (*Cornus, Lonicera*) or delayed recovery (*Geranium, Alchemilla*). These responses mostly correspond to responses of stomatal conductance. The only species, which did not recover the values of PSII quantum yield to the initial values was *Geranium maculatum* L., which may indicate some irreversible damage caused by drought, which was not present in other species.

CONCLUSION

Based upon this experiment results different plants reaction to drought may be defined.

Short lasting water stress increased chlorophyll concentration in stress-exposed tissues and long lasting water stress leaded to the decrease in the chlorophyll content as a protective response in *Cornus mas* L. Probably the leaf anatomy adjustment represents a very strong ability to modify photosynthetic apparatus in *Alchemilla mollis* (Buser) Rothm. according to stomatal conductance reaction and chlorophyll content.

No significant changes in chlorophyll content in *Lonicera caerulea* L. and *Geranium* plants were identified.

Stomata responded very intensively in *Lonicera caerulea* L. because of their closure after 14 days of drought, but the re-irrigation led to absolute regeneration of stomatal conductance. Stomatal conductance values in *Cornus mas* L. after re-irrigation were even higher than initial values. We may assume that both woody plants were able to regenerate after drought period, but the course of the reaction was different.

Very interesting reactions in parameter ϕPSII in drought recovery were shown, in woody plants *Cornus mas* L. and *Lonicera caerulea* L. the early recovery were observed (after 2 days of re-watering) and the delayed recovery (after 6 days of re-watering) in *Geranium* plants and *Alchemilla mollis* (Buser) Rothm.).

The following experiment with another perennials and woody plants species are crucial for better understanding of plants drought reactions.

These findings may be applied in the rules of designing and maintenance management of ornamental plants to be planted in extreme condition.

The information on plant reactions to drought is important in decision-making: about the plant selection (according to their changes to morphological characteristics, which are related to some physiological parameters) and about the spacing of plants in flower beds. Dense spacing of plants in drought condition is needed and the species with lower drought resistance should be combined with those with higher drought stress tolerance, which can help them to resist and overcome the drought.

Acknowledgements

This paper was created within the project KEGA *Interactive experimental garden.* Project registration number 035SPU-4/2016.

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