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EFFECTS OF DROUGHT TREATMENT ON THREE MATRIX PLANTING PERENNIALS

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Abstract. The goal of research was to evaluate the above-ground physiological and overall plant responses of chosen herbaceous matrix plants to varying water soil content in confined root zone. *Stachys macrantha* (C. Koch) Jalas, *Brunnera macrophylla* (Adams) IM Johnston. and *Geranium macrorrhizum* L. were subjected to drought. We measured a) stomatal conductance, b) leaf area, c) dry matter content, d) leaf pigments content, e) visual quality rating. Drought stress led to considerable decline in stomatal conductance (75% *Stachys*, 60% *Geranium*, and 42% *Brunnera*) and in leaf area (*Stachys* 59%, *Geranium* 53%, *Brunnera* 45%). There were observed opposite trend: increased in leaf dry matter content (33% *Stachys*, 14% *Brunnera*), root dry matter content (22% *Stachys*, 14% *Brunnera*) and ratio carotenoid/total chlorophyll content (4% *Brunnera*). Two from investigated plants (*Brunnera* and *Geranium*) survived in drought condition at acceptable morphological damage, however, *Stachys* did not perform well in a confined root zone. These results lead to the conclusion that *Brunnera* and *Geranium* are well adapted to dry conditions and would be suitable for use in low water use landscape, however, *Stachys* is partially recommended.

Key words: stomatal conductance, SLA, chlorophyll, carotenoids, visual quality

INTRODUCTION

The access to professional herbaceous planting design has changed dramatically in recent years. Herbaceous perennial plants are increasingly being recognized as a vital part of urban and garden environments [Oudolf and Kingsbury 2013], with multifunc-

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tional importance, not just a luxury or an unnecessary. The interest of perennial plantings has focused to large-scale planting project and long-term performances concept plantings with auto-regulation in maintenance. The earlier ordered group (block) plantings has gradually substituted by spontaneous types: matrix, randomized, mixed plantings, where are mingling currents, inspired by nature. One of those, matrix evokes the situation in many natural habitats where a small number of species form the vast majority of the biomass, studded with a larger number of species present in much smaller numbers but which are a visually important element [Oudolf and Kingsbury 2013]. The matrix proposal overlaps two approaches: planned placing of plants with inevitable randomness [Kircher et al. 2011]. The main function of matrix plants are to be a ground cover and to visually highlight or to create the framework for impressing a dominant plants. The ground cover ability of matrix plants has increased resistance to weeds infiltration [Dunnett and Hitchmough 2007] and consequently has reduced the labor and financial costs of planting maintenance. Trends in spontaneity, longevity and low maintenance in herbaceous plantings should emphasize to thoroughly thought out selection of plants based on skills, experience, knowledge and experiments [Hansen and Stahl 1993, Scarfone 2007, Oudolf and Kingsbury 2013]. This is also related to responsible and sophisticated approach in consideration of principles of creating plant compositions for those plantings.

Urban herbaceous plantings are damaged due to detrimental effects of various biotic and abiotic stresses, therefore minimizing these losses is a major area of consideration in herbaceous plantings design process. Drought is the most important environmental stress, severely impairs plant growth and development [Anjum et al. 2011], limits herbaceous plant aesthetics and their performance, more than any other environmental factors. Evidently, ornamental plants are very much undervalued in targeting research on drought resistance (compared with crop plants). Thus, there is a strong need to increase systematic work on selection of herbaceous perennials adapted to dry urban conditions. Contemporary perspectives of research in landscape planting design should be targeted to identify responses of newly selected plants for limited water growing conditions in urban environment and definition new selection criteria target directly toward herbaceous perennials growth, survival and sufficiently aesthetic appearance.

The objective of this study was evaluate the above-ground physiological and overall plant responses of 3 herbaceous matrix plants to varying water soil content in confined root zone by experimental set up. The evaluated plants were selected from uniform garden habitats: loosely bound to the woodland edge, according to Hansen and Stahl [1993].

MATERIALS AND METHODS

The study was conducted at the Experimental Station of Horticulture and Landscape Engineering Faculty in Nitra, over a 2-year period (2013–2014). The experiment was arranged as a split plot complete block design, with species and variants of experiment subplot. Each main plot consisted of 1.5 l containers with saucers arranged in 2 rows with 15 replications. The experiment was based on retaining stable drought level of soil water content: 30 and 60% soil water content. We retained stable drought level by adequate watering 3 time per week, without rainfall affect, because experiment was located in sheltered conditions (Clingfilm tunnel). The determination of weight of the substrate at different soil water content at the beginning of the experiment was key to set up the watering amount. Each pot was regularly weighted out before watering and adequate water content was replenished to the required level (30 and 60% soil water content). Differentiated water regime was applied for 45 days. Plugs of *Stachys macrantha* (C. Koch) Jalas, *Brunnera macrophylla* (Adams) IM Johnston. and *Geranium macrorrhizum* L. from commercial nursery were potted into 1.5 l nursery containers. Individual pots were filled with trade peat-clay medium Klassman TS-3. The weight of each planted pots and dry soil was determined for possibilities of calculation desired soil moisture content of trial pots. All plants were well watered to saturate the growing media during the first month. All plants were fertilized with slow release stick fertilizer NPL 10-5-6 (Agro CS a.s.): two sticks per pot in setting up the experiment (April 2013 and 2014). Treatments of 30 and 60% soil water content (gravimetric measured as grams of water per gram of oven-dried soil) in trial pots were applied for 45 days in May and June 2013 and 2014 (*Geranium macrorrhizum* only 2014 year). Necessary irrigation water was supplied to each trial pots according to foreordination, different in individual pots.

Plant visual quality was assessed on a scale from 0 to 5, as described by Zollinger et al. [2006], in mid-May and June 2013 and 2014 (*Geranium macrorrhizum* only 2014 year). Plants were rated every other week, during 7 week treatment period for the degree of wilt and leaf burn. Stomatal conductance, as an index of plant stress and indicator of photosynthetic activity, was measured with an AP4 Leaf Porometer. Measurements were taken on two fully expanded leaves of one plant with 10 replications per treatment between 07.00 and 15.00 hours. Plants were harvested in end-June. Leaves and stems were separated. All leaves per plant were detached to determine their relative water content (RWC). After cuttings, the petiole was immediately immersed in distilled water inside of a glass tube, which was immediately sealed. The tubes were then taken to the laboratory where the increased weight of the tubes was used to determine leaf fresh weight (FW). After 4 h, the leaves were weighed to obtain the turgid weight (TW). The dry weight (DW) was then measured after oven drying at 80°C for 48 h, and RWC was calculated follows: RWC = 100 (FW – DW) / TW – DW. Leaf area was measured for green leaf tissue using the scanner, and then calculated by free software Image J. Specific leaf area (SLA) was calculated as the plant leaf area divided by the dry mass of leaves. Leaf dry matter content (LDMC) was expressed as percentage of the ratio of leaf dry mass to saturated leaf fresh mass and calculated as follows: LDMC = (DW / FW) × 100%. Similarly was determined Root dry matter content (RDMC) as the ratio of root dry mass to saturated root fresh mass. Photosynthetic pigments like leaf chlorophylls and carotenoids were extracted from fresh leaves with 85% acetone and estimated spectrophotometrically as describe by Šesták and Čadský [1966]. Statistical analyses of experimental data were performed using Statgraphics Plus 4.0® (Statistical Grafics Corp., Herndon, Va. U.S.A.). Analysis of variance (ANOVA) was performed to estimate statistically significant differences between their mean values at a confidence level of 95% (P-value < 0.05). A multiple range test of least significant difference test (LSD tests) was used to analyze the existence of homogenous samples. The Kruskal-Wallis test was used to test the difference medians between plant visual qualities in the drought treatments.

RESULTS AND DISCUSSION

Ornamental plants serve an aesthetic as well as functional role, and should be able to withstand drought and still be visually appealing in landscapes [Zollinger et al. 2006]. The advantages of doing visual evaluations are that they can be taken quickly in the field and provide valuable information for evaluation and selection of plant materials that may not be otherwise obtained due to time or cost constraints [Wolfe et al. 1998]. The overall aesthetic value of plant under water stress, would be an ideal criterion but it is a subjective indicator. Authors were rated decorative value as observed symptoms of wilting and leaf burning [Zollinger et al. 2006, Mårtensson et al. 2014], or their inflorescences´ height [Chylinski and Lukaszewska 2010], or plant density and canopy dieback [Scheiber et al. 2008]. The lowest values in visual quality rating were observed for *Stachys*. These plants had foliage injury with symptoms of necrosis and browning and eventually died during drought treatment (tab. 1). Leaves injury of *Stachys* occurred the earliest among evaluated species (tab. 2). Short-term drought stress (during 7 days under 30% soil water content) decreased the visual quality scores to the value 1. There was no substantial difference in visual quality scores among *Geranium* and *Brunnera*. The visual quality score for these species was only mildly reduced at different treatment. Mid-term drought stress (during 30 days under 30% soil water content) decreased visual quality scores only to the value 3. The better visual quality of *Geranium* and *Brunnera* in drought conditions may by partly related to its ability to regulate water loss by closing stomata [Zollinger et al. 2006] or leaf trichomes and hairs, which may improved leaf water status by entrapping and retaining surface water, thus assisting in its final absorption into the mesophyll [Grammatikopoulos and Manetas 1994]. Plants that are able to minimize water loss via stomatal closure, formation of a cuticle and wax layer, sclerophyll, leaf trichomes and hair [Grammatikopoulos and Manetas 1994], essential oil production [Machovec and Jakábová 2006], changes in leaf shape and orientation [Zollinger et al. 2006] or higher allocation to roots are likely to have higher ornamental value when exposed to drought stress. Stomatal or morphological acclimation would be more desirable ornamentally than plants that acclimate by reducing total transpiration via drastically eliminating leaf area, or wilting and leaf burning.

Table 1. Visual quality rating for leaf burn and wilting of leaves across species and drought treatments

Species	Burn					Wilt				
	rating		N	SD	p	rating		N	SD	p
	DT 30%	DT 60%				DT 30%	DT 60%			
GM	4.0 (3.6)a	4.0 (4.2)b	40	0.775821	0.0004	3.0a	4.0b	40	0.798634	0.0000
SM	1.0a	3.0b	80	1.06333	0.0000	1.0a	3.0b	80	1.14093	0.0000
BM	4.0a	4.0a	80	0.621642	0.0000	3.0a	4.0b	80	0.812418	0.0000

Abbreviations: GM – *Geranium macrorrhizum*, SM – *Stachys macrantha*, BM – *Brunnera macrophylla*, DT – drought treatment, SD – standard deviation. Different letters indicate significant differences in rating visual quality (median) of leaves, between drought treatments for each species, tested by Kruskal-Wallis at P = 0.05. Mean values are given in brackets

Table 2. Visual quality rating for leaf burn and wilting of leaves in different drought stress duration

Species	N	Burn						Wilt					
		rating/duration of stress (days)				SD	p	rating/duration of stress (days)				SD	p
		7	15	30	45			7	15	30	45		
GM	40	5.0c	4.0b	3.0a	3.0a	0.837	0.00	4.0b	4.0b	3.0 (2.9)a	2.5 (a)	0.833	0.00
SM	80	3.0b	1.0a	1.0a	1.0a	0.973	0.00	3.0b	1.0a	1.0a	1.0 a	0.932	0.00
BM	80	4.0c	4.0c	3.0 (3.4)b	3.0 (2.6)a	0.745	0.00	4.0c	4.0c	3.0b	2.0 a	0.885	0.00

Abbreviations: SD – standard deviation. Different letters indicate significant differences in rating visual quality (median) of leaves, between duration of drought stress for each species, tested by Kruskal-Wallis at P = 0.05. Mean values are given in brackets

Table 3. Stomatal conductance (g_s) across species and drought treatments

Species	Stomatal conductance (g_s)		N	SD	F-ratio	p
	DT 30%	DT 60%				
GM	0.65 a	1.63 b	160	1.03751	45.35	0.0000
SM	0.74 a	2.98 b	176	2.35424	51.19	0.0000
BM	0.85 a	1.46 b	180	1.19579	12.53	0.0005

Abbreviations: DT – drought treatment, SD – standard deviation. Different letters indicate significant differences in stomatal conductance (g_s), between drought treatments for each species, tested by ANOVA at *P* = 0.05

Table 4. Effect of drought stress duration on stomatal conductance (g_s) of individual plants

Species	Stomatal conductance (g_s)/duration of stress (days)			N	SD	F-ratio	p
	15	30	45				
GM	0.48 a	0.90 b	0.60 a	60	0.473921	4.59	0.0142
SM	0.55 a	0.87 a	0.81 a	85	0.827352	1.26	0.2885
BM	0.90 a	0.88 a	0.76 a	90	0.770462	0.28	0.7542

Abbreviations: SD – standard deviation. Different letters indicate significant differences in stomatal conductance (g_s), between duration of drought stress for each species, tested by ANOVA at *P* = 0.05

Table 5. Leaf area, Specific leaf area, Leaf dry matter content (LDMC) and Root dry matter content (RDMC) across species and drought treatments

Species	Drought treatment (% soil water content)	Leaf area (cm^2 $plant^{-1}$)	SLA (cm^2 g^{-1})	LDMC (%)	RDMC (%)
GM	30	354.6 ±35.9 a	335.4 ±17.7 b	22.6 ±0.8 a	32.0 ±2.0 a
	60	752.1 ±115.5 b	308.0 ±20.2 a	23.0 ±1.3 a	30.3 ±2.5 a
SM	30	211.7 ±209.5 a	250.9 ±38.5 a	35.0 ±8.0 b	39.0 ±9.4 b
	60	511.5 ±270.2 b	254.8 ±31.6 a	23.5 ±1.9 a	30.4 ±5.5 a
BM	30	305.1 ±122.9 a	226.8 ±40.7 a	25.6 ±4.0 b	31.2 ±2.8 b
	60	557.0 ±191.5 b	247.3 ±48.9 a	21.9 ± 1.4a	26.9 ±2.9 a

Different letters indicate significant differences in leaf area, specific leaf area (SLA), Leaf dry matter content (LDMC) and Root dry matter content (RDMC), between drought treatments for each species, tested by ANOVA at *P* = 0.05

Stomatal control is another way in which plants deal with drought stress [Levitt 1980]. The plant must balance the benefit of stomatal closure conserving water against limiting CO_2 uptake and decreased evaporative cooling of the leaf tissue [Lambers et al. 1998]. Increased stomatal sensitivity is a functional mechanism that allows plants to maintain high water status during drought periods [Pessarakli 2002]. Stomata close progressively with increased drought stress. It is well known that leaf water status always interacts with stomatal conductance [Anjum et al. 2011]. Drought stress led to considerable decline in stomatal conductance (75% *Stachys*, 60% *Geranium*, and 42% *Brunnera*) (tab. 3). We studied the effect of drought stress duration (during 15, 30 and 45 days under 30% soil water content) on stomatal conductance (g_s). There was significant difference only for *Geranium*, when 30 days duration of drought stress led to 47% increase in stomatal conductance. Gradually prolonged stress (45 days) led to 33% decrease in stomatal conductance. Similar results were observed for *Brunnera* only in 2014, when mid-term drought stress led to 34% increase in stomatal conductance, and extension stress at 45 days led to 28% gradually decrease.

Table 6. Effect of drought stress duration on leaf area, specific leaf area and relative water content (RWC) of individual plants

Species	Duration stress (day)	Leaf area (cm^2 $plant^{-1}$)	SLA (cm^2 g^{-1})	RWC (%)
GM	7	293.4 ±30.6 b	296.9 ±18.7 a	86.6 ±0.7 a
	45	166.6 ±47.4 a	327.8 ±43.8 a	85.8 ±2.5 a
SM	7	366.5 ±130.9 b	291.2 ±27.8 b	81.7 ±4.7 b
	45	106.5 ±42.1 a	258.4 ±35.8 a	76.4 ±9.3 a
BM	7	448.2 ±177.3 b	322.9 ±35 b	86.2 ±3.5 b
	45	262.8 ±122.5 a	225.4 ±20.3 a	74.6 ±8.9 a

Different letters indicate significant differences in leaf area, specific leaf area (SLA) and relative water content (RWC) between duration of drought stress for each species, tested by ANOVA at $P = 0.05$

Drought led to substantial impairment of growth in terms of plant height, leaf area, number of leaves/plants, shoot fresh and dry weight/plants [Anjum et al. 2011]. Significant decreases were observed in leaf area in every individual plants (*Stachys* 59%, *Geranium* 53%, *Brunnera* 45%) (tab. 5). Similar result has also been noted in other ornamental herbaceous perennials [Garland et al. 2012, Zollinger et al. 2006]. Drought-induced reduction in leaf area is ascribed to suppression of leaf expansion through reduction in photosynthesis [Anjum et al. 2011]. Specific leaf area (SLA), an indictor of leaf thickness, has often been observed to be reduced under drought condition [Marcelis et al. 1998]. Low SLA is preferable as it indicates higher drought resistance [Painawadee et al. 2009]. A decrease in SLA may also occurs in response to drought in herbaceous leaves as a result on an increased investment in structural tissues, allowing increased resistance to unfavorable environmental conditions [Maroco et al. 2000]. It could by hypothesized that all investigated species with low SLA have more photosynthetic machinery per unit leaf area and hence potential for greater assimilation under drought stress because thicker leaves usually have a greater photosynthetic capacity compared with thinner leaves [Painawadee et al. 2009]. Only *Geranium* plants show significant increased SLA, when drought treatment led to 8% increased in SLA and to non significant increased with the duration of stress, when SLA increased by 9% (tabs 5, 6). In the studies of Susiluoto and Berninger [2007] on *Eucalyptus microtheca* reported similar results. *Stachys* and *Brunnera* behaved the opposite way, there were drought induced decrease with the duration of stress in SLA (11% *Stachys* and 30% *Brunnera*). A common adverse effect of water stress on plants is the reduction in fresh and dry biomass production [Shao et al. 2008]. Investigated plants behaved the opposite way, there were drought induced increase of leaf and root dry matter content (*Stachys*: 33% LDMC and 22% RDMC, *Brunnera*: 14% LDMC and RDMC, too) (tab. 5). The higher dry matter may be an indication of such an increase in non-structural carbohydrate content [Pilon-Smith et al. 1995]. RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures [Anjum et al. 2011]. Leaf RWC is of the best growth / biochemical indices revealing the stress intensity. The rate of RWC in plant with high resistance against drought is higher than others [Arjenaki et al. 2012]. Extended drought treatment led to significant decline in RWC (6% *Stachys*, 13% *Brunnera*) (tab. 6).

Table 7. Effect of drought stress duration on total chlorophyll and total carotenoids of *Stachys macrantha*, *Brunnera macrophylla* and *Geranium macrorrhizum*

Species	Duration stress (days)	Total chlorophyll (mg m^{-2} leaf area)	Total carotenoids (mg m^{-2} leaf area)
GM	7	393.06 ±35.4b	89.38 ±4.7a
	45	350.11 ±21.5a	91.31 ±4.7a
SM	7	367.15 ±65.3b	84.97 ±13.1b
	45	286.94 ±65.8a	68.28 ±13.4a
BM	7	461.53 ±55.1b	101.38 ±11.9b
	45	354.06 ±74.7a	85.34 ±16.8a

Different letters indicate significant differences in total chlorophyll and total carotenoids, between duration of drought stress for each species, tested by ANOVA at $P = 0.05$

Table 8. Total chlorophyll and total carotenoids across species and drought treatments

Species	Drought treatment (% soil water content)	Total chlorophyll (mg m^{-2} leaf area)	Total carotenoids (mg m^{-2} leaf area)	Ratio carotenoid/total chlorophyll
GM	30	396.63 ±19 b	91.21 ±3.7 b	0.23 ±0.007a
	60	355.26 ±32.97 a	81.13 ±6.4 a	0.23 ±0.004a
SM	30	329.66 ±98.8 a	76.47 ±19.6 a	0.24 ±0.02a
	60	373.11 ±85.2 a	82.17 ±16.8 a	0.23 ±0.06a
BM	30	409.74 ±111.8 a	96.55 ±24.0 b	0.24 ±0.02b
	60	379.01 ±119.0 a	85.13 ±25.3 a	0.23 ±0.02a

Different letters indicate significant differences in total chlorophyll and total carotenoids, between drought treatments for each species, tested by ANOVA at $P = 0.05$

Chlorophyll content has a positive relationship with photosynthetic rate [Anjum et al. 2011] and is also indicator of photosynthetic capability of plant tissues [Arjenaki et al. 2012]. The decrease in chlorophyll content under stress has been considered as typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation. Loss of chlorophyll content under water stress is considered as main cause of inactivation of photosynthesis [Anjum et al. 2011]. Arjenaki et al. [2012] found that resistant genotypes of wheat had the highest chlorophyll content in drought stress – damage to leaf pigments as a result of water deficit. Generally, stress conditions did not affected leaf chlorophyll content (tab. 8) at investigated plants, only in *Geranium* drought condition led to opposite result – increased leaf chlorophyll content by 10% (tab. 8). These minor changes in chlorophyll content allow the conclusion that the pigment apparatus is comparatively resistant to dehydration [Nikolaeva et al. 2010]. Unchanged level of chlorophyll content under drought condition were indicated in *Sedum spectabile* [Chylinski and Lukaszewska 2010]. Effect of duration of drought stress was significant on leaf chlorophyll content at every investigated plants (decreased by 11% *Geranium*, 22% *Stachys*, 23% *Brunnera*) (tab. 7). Under drought condition two of the tested species (*Geranium* and *Brunnera*) had higher level of carotenoid pigments (tab. 8), but duration of stress condition significantly decreased level of carotenoid pigments (*Stachys* by 20%, and *Brunnera* by 16%) (tab. 7). Karimi et al. [2013] revealed higher concentration of carotenoids in the leaves of drought tolerant genotypes. Liu et al. [2011] conclude in their study that drought stress decreased pigments content but increased the ratio of carotenoid and chlorophyll content. We observed the same results, carotenoid and chlorophyll content ratio increased, but significantly only in plants *Geranium macrorrhizum* (tab. 8).

CONCLUSIONS

We have shown that the evaluated plants *Brunnera macrophylla* and *Geranium macrorrhizum* are well adapted to dry conditions and would be suitable for use in low water use landscape. Generally, investigated plants displayed the smallest changes in analyzed parameters (stomatal conductance plasticity, degradation leaf area and dry matter content, and ratio or content leaf pigments). Investigated plants survived with acceptable morphological damage under drought condition. *Stachys macrantha*, however, is partially recommended for low-water-use landscape, only provided thickened planting. Although the investigated plants are included in the same garden habitats, the design principles must be distinguishing according their morphological and physiological reflection under stress conditions. It is necessary to find new effective screening techniques and innovate herbaceous sorting to garden habitats, generally used in herbaceous perennials planting design.

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WPŁYW SUSZY NA TRZY GATUNKI BYLIN OKRYWOWYCH

Streszczenie. Celem badań była ocena fizjologicznej reakcji wybranych roślin zielnych na różną zawartości wody w glebie w zamkniętej strefie korzeniowej. *Stachys macrantha* (C. Koch) Jalas, *Brunnera macrophylla* (Adams) IM Johnston oraz *Geranium macrorrhizum* L. poddano działaniu niedoboru wody. Dokonano pomiaru: a) przewodnictwa szparkowego, b) powierzchni liści, c) zawartość suchej masy, d) zawartości pigmentów liści oraz e) wizualnej oceny jakości. Stres wywołany niedoborem wody doprowadził do znacznego spadku przewodnictwa szparkowego (75% *Stachys*, 60% *Geranium*, 42% *Brunnera*) oraz powierzchni liści (59% *Stachys*, 53% *Geranium*, 45% *Brunnera*). Zaobserwowano również tendencję odwrotną: wzrost zawartości suchej masy w liściach (33%

Stachys, 14% *Brunnera*), zawartości suchej masy w korzeniach (22% *Stachys*, 14% *Brunnera*) oraz stosunku zawartości karotenoidów do całkowitej zawartości chlorofilu (4% *Brunnera*). Dwie z badanych roślin (*Brunnera i Geranium)* przetrwały w warunkach suszy z akceptowalnym poziomem uszkodzenia morfologicznego, ale *Stachys* nie radził sobie w strefie korzeniowej. Na podstawie wyników wnioskuje się, że *Brunnera* i *Geranium* są dobrze przystosowane do warunków niedoboru wody i byłyby odpowiednie dla obszarów o niskim poziomie wody, gdzie nie zaleca się raczej wykorzystywania *Stachys*.

Słowa kluczowe: przewodność szparkowa, SLA, chlorofil, karotenoidy, jakość wizualna

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