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ORIGINAL PAPER

Drought response of two bedding plants

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Abstract Bedding plants are an important part of the urban public space and private gardens. However, they are not always properly watered and suffer from drought stress, especially when grown in containers. In this trial a response to water stress of two commonly used species, impatiens (*Impatiens walleriana* Hook) and geranium (*Pelargonium hortorum* L. H. Bailey) were compared. The former is highly herbaceous and prone to wilting whereas the latter has hairy leaves and is better adapted to drought. Plants were grown at three levels of soil water content (SWC): 80% (control), 60% (mild stress) and 30% (severe stress). Drought was maintained during three 10 day cycles, separated by 10 day periods of normal watering. In both species roots were significantly longer in plants grown at 30% SWC as compared to 80% SWC while plant height and flower number were reduced by drought only in impatiens. The initial relative water content (RWC) was lower in geranium and decreased less in response to drought than in impatiens. Ammonium content in leaves of both species increased significantly under stress but the ranges of increase were different in both species. There was a significant increase in the free amino acids content in leaves of impatiens as compared to geranium but this rise was more time than drought dependent. The reduction in the $a + b$ chlorophyll concentration in leaves of impatiens was significantly time and stress dependent while no reaction in geranium was observed. The above results show that changes in leaf RWC merit further attention as a possible indicator of plant response to drought stress in ornamental plants but additional studies are needed before this or other parameters can be used to evaluate new bedding plants for introduction into urban growing conditions, or as selection criteria in breeding for adaptation to demanding growing conditions.

Keywords *Impatiens walleriana* · *Pelargonium hortorum* · SWC · RWC · Chlorophyll · Ammonium · Free amino acids

List of abbreviations

DW	Dry weight
FW	Fresh weight
RWC	Relative water content
SWC	Soil water content

Introduction

Plants have evolved many physiological and morphological strategies to cope with drought stress that adversely affect plant growth, productivity and their attractiveness (Weng and Yang 2004). One of the first responses to the abiotic stress is a reduction in plant growth. As the drought period is getting longer, most of plants reduce their shoot growth, while the elongation of roots is usually stimulated, which permits deeper soil penetration in search for water (Yin et al. 2005). For ornamental plants, the most decorative element is usually flowers. Therefore, it is essential to keep plants blooming for an extended periods. Plants subjected to various stresses reduce flowering to save

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assimilates needed for survival (Augé et al. 2003). Abiotic stresses also lead to a series of physiological, biochemical and molecular changes (Wang et al. 2003). A decrease in the relative water content (RWC) in response to drought has been observed in a wide variety of crop plants (Lin and Kao 1998; Clavel et al. 2005), or in plants living in naturally dry environments (Loik and Harte 1997; Gratani and Varone 2004) but data on changes in the water status in seasonal ornamental plants are scarce (Augé et al. 2003). One of the processes occurring in stressed plants is proteolysis and ammonium accumulation (Singh and Usha 2003). Lin and Kao (1998) suggested that the accumulation of ammonium, which is toxic to plants, might be a factor that further aggravates the stress. Also, an increase in the amount of free amino acids in plant tissues may be a result of disturbances in the plant environment, especially in drought and salt stresses (Good and Zaplachinski 1994; Gzik 1996). Chlorophylls are essential pigments of the higher plant assimilatory tissues, responsible for proper functioning of the photosynthetic apparatus. According to Ueda et al. (2003) the chlorophyll $a + b$ content in the leaves can be indicative of stressful conditions, such as water or salt stress.

Numerous experiments dealt with the effects of abiotic stresses on agricultural plants such as wheat (Siddique et al. 2000), rice (Chen et al. 1997), sugar beet (Gzik 1996), groundnut (Clavel et al. 2005) and many others. Until now, ornamental plants have not been a frequent object of such studies even though these plants constitute a major part of the horticultural production and play an important role in everyday humane life. The aim of this study was to compare the response to drought of two species of ornamental bedding plants originating from different habitats, and commonly used in urban settings. Growth parameters such as the shoot height, roots length and the number of flowers per plant, as well as, RWC were measured. In order to identify reliable parameters indicating plant's response and adaptability to water stress the contents of chlorophyll $a + b$, ammonium and free amino acids were measured and compared after successive drought cycles.

Materials and methods

Plant material and growing conditions

Two ornamental plant species: impatiens (*Impatiens walleriana* Hook 'Deep Rose') and geranium (*Pelargonium hortorum* L. H. Bailey 'Deep Red') were used. Both are commonly cultivated as annual plants, often in pots or beds. *Impatiens walleriana* is native to the tropical East Africa. It is a succulent perennial with soft, fleshy stems and leaves, and pink flowers. *Pelargonium hortorum* is a hybrid of species indigenous to southeastern South Africa with dry, hot summers and mild, humid winters. This subshrub is a compact plant with woody stems and red flowers. Plants were grown in the greenhouses of the Faculty of Horticulture and Landscape Architecture, Warsaw Agricultural University in 2004 and 2005. Eight-week old seedlings, obtained from S&G Seeds Co., were transferred to pots (0.6 dm^{-3}) containing peat/bark substrate (1:1 v/v) with multimineral fertilizer Azofoska (2 kg m^{-3}). The experiment was started after 5 weeks of acclimatization. The average temperature in the greenhouse ranged from 21.0 ± 4.0°C (day) to 10.0 ± 4.0°C (night), and the relative humidity was 60 ± 10.0%. In both years, the experiment was carried out from May to July. Twice during the experiment plants were treated with a liquid multimineral fertilizer (Florovit 0.2%).

Three levels of SWC: 80% (control), 60% (mild drought stress) and 30% (severe drought stress) were imposed during the experiments. Drought stress was maintained during three 10-day cycles separated by 10-day period of standard watering, so the entire experiment lasted 50 days (Fig. 1). At the end of every stress period the growth parameters were measured and plant material was sampled for biochemical analysis.

There were 15 pots for every SWC level (five plants per each of three drought cycles) for the total of 45 plants of each species (+5 plants as material for initial measurements and analyses). Each plant was individually tagged and treated as a single replication.

Evaluation of drought stress

The soil water content in pots was measured by daily weighting (morning hours). Before the experiment, the substrate (in volume used in the trials) was weighted (FW), and oven dried at 105°C for 24 h to determine its dry weight (DW). The total soil water content (SWC) was calculated according to Turner (1981):

$$\mathrm{SWC} = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{DW}} \times 100\%$$

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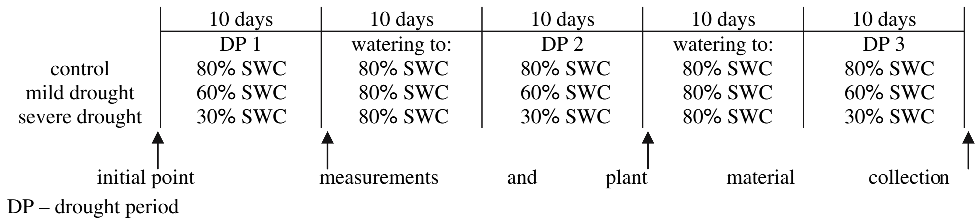
Growth parameters RWC and biochemical analysis

At the end of every drought cycle the total height in cm, root length (the longest root) in cm and the number of flowers per plant were recorded. The relative plant water content (RWC) was measured on five leaves collected from each of five plants at each sampling date. Leaves for RWC were weighed immediately (FW), then floated on distillated

Fig. 1 Experiment scheme with drought periods



water and weighed again when they reached a turgid weight (TW). Leaves were dried in the oven at 105°C for 24 h and their dry weight DW measured. The RWC was calculated using the formula of Barrs (1968):

$$RWC = \frac{FW - DW}{TW - DW} \times 100\%$$

For the analyses of the ammonium, free amino acid and chlorophyll $a + b$ contents and for DW determination, leaves from five plants were pooled, finely cut with a razor blade, mixed and then 0.5 g triplicate samples were prepared. Ammonium content was determined according to Lin and Kao (1996), free amino acids according to Rosen (1957) and chlorophyll $a + b$ according to Lichtenthaler and Wellburn (1983). The ammonium content was expressed in µmol g^{-1} DW. Free amino acids concentration was calculated from the previously plotted standard curve (Rosen 1957) and expressed in µmol leucine per one gram of DW.

Experimental design and statystical analysis

The data (means from 2 years observations and/or analyses) were subjected to ANOVA 3 (SWC × sampling date × species) using STATGRAPHICS® (version Plus 4.1) and differences between the means were compared with the Duncan test at the probability level $P = 95\%$.

Results

Plant growth and flowering

There was no significant difference in the height of geranium plants at various SWC levels and only the severe stress (30% SWC) slightly reduced plant height in impatiens (Table 1). In both species, roots were significantly longer in plants growing under 30% SWC relative to 80% SWC (on the average, by 35 and 15% in impatiens and geranium, respectively); mild stress did not affect root length. In impatiens, the number of flowers per plant was reduced in response to reduced levels of SWC (Table 1) and remained similar to the initial count during the 50 days of the experiment. Drought did not affect the flower numbers in geranium where they tripled over the initial during the experiment.

Relative water content

The initial relative water content in impatiens was 96% (Table 2) while in the more stress resistant geranium it was 82%. The former is more succulent plant; the latter is more drought resistant. In both species, plants grown under severe stress had significantly reduced RWC, to 56% in impatiens, i.e. by two-fifths as compared to the initial value, and to 66% in geranium, i.e. by one-fifth of the

Table 1 The effect of drought on mean height, root length and the number of flowers of *Impatiens walleriana* 'Deep Rose' and *Pelargonium hortorum* 'Deep Red'

Species	SWC level (%)	Height (cm)	Root lenght (cm)	Number of flowers
Impatiens walleriana	80	31.5 c	20.3 c	14.6 c
	60	29.3 b	20.2 c	12.4 b
	30	29.1 b	25.1 d	10.7 a
Pelargonium hortorum	80	23.3 a	13.5 a	23 d
	60	23.1 a	13.8 a	22 d
	30	23.3 a	16.3 b	22 d

DP drought period. Values followed by the same letter do not differ significantly at $P < 0.05$

Table 2 RWC (%) in plants of *Impatiens walleriana* 'Deep Rose' (initial level = 96.4%) and *Pelargonium hortorum* 'Deep Red' (initial level = 82.4%)

Species	SWC level	DP 1	DP 2	DP 3	Mean
Impatiens walleriana	80% SWC	97.1 c	95.8 c	96.3 c	96.4 c
	60% SWC	72.6 b	76.9 b	77.1 b	75.5 b
	30% SWC	56.2 a	57.1 a	55.1 a	56.1 a
Mean		75.3 a	75.6 a	76.2 a	
Pelargonium hortorum	80% SWC	81.3 c	80.1 c	79.2 c	80.2 b
	60% SWC	70.9 ab	73.1 b	73.2 b	72.4 ab
	30% SWC	65.7 a	67.1 a	66.0 a	66.3 a
Mean		72.6 a	73.4 a	72.8 a	

DP drought period. Values followed by the same letter do not differ significantly at $P < 0.05$

initial RWC. The mild stress (60% SWC) did not affect water content in geranium plants while it caused a drop in the RWC of impatiens by one fifth as compared to that at the beginning of the experiment. In neither species, successive drought cycles intensified plant response as expressed by RWC: plants had similar water contents after the first and third drought cycle.

Ammonium

The initial ammonium concentration in the impatiens leaves was more than twice as high as that of geranium (Table 3). In both species it increased significantly during the 50 days of the experiment, independently of the drought conditions imposed on plants, but the two taxa differed in the range of this increase. In geranium control plants (grown under 80% SWC), the ammonium content doubled during the experiment while in impatiens the increase in NH_3 content after 50 days was less than 60%. In plants of impatiens subjected to the severe drought stress (30% SWC) the ammonium content has already doubled after the first drought cycle while in geranium no significant change was observed on this sampling date. At the end of the experiment the impatiens plants grown under severe stress (30% SWC) had 73% more ammonium than those grown at 80% SWC. In geranium, after the third cycle of severe stress (30% SWC) the ammonium content increased only by 30% relative to the control, i.e. plants grown under 80% SWC. However, at the end of the experiment the heavily stressed plants of both species showed a similar range of ammonium accumulation: 273 and 290% of the initial level in impatiens and geranium, respectively, though the absolute values for impatiens were more than double of those for geranium.

Free amino acids

There was a significant increase in the free amino acids content in the leaves of impatiens under the increasing water stress and after every drought period (Table 4). However, it needs to be noted that during the 50 days, free amino acids were accumulating in leaves of impatiens irrespective of the SWC. They have increased by approx. 250% in control plants grown under 80% SWC relative to the initial value. Generally, the range of the increase in the free amino acids concentration was more time than drought dependent. The response of impatiens to water stress was the most pronounced after the first drought cycle: the free amino acid content in plants grown under 30% SWC was over 160% of that in control plants (80% SWC), whereas after the third drought cycle this increase relative to control plants was only 15%. In geranium, the increase in the free amino content during time course of the experiment was steady but negligible: approximately 10% in control geranium plants at the last sampling date. Neither was the drought effect as pronounced as in impatiens. The highest content of free amino acids was observed after the third drought cycle in plants grown under 30% SWC but it was only 40% over the initial value. Geranium plants responded in a similar fashion to the mild (60% SWC) and severe (30% SWC) water stresses.

Chlorophyll *a* + *b*

In impatiens, a reduction in the chlorophyll *a* + *b* content was more time- than drought dependent (Table 5). The pigment concentration dropped during the 50 days by approx. 50%, irrespective of the water regime applied; the changes being the most pronounced between the first and the second drought cycle. The global effect of water stress

Table 3 Effect of drought on ammonium content in leaves of *Impatiens walleriana* 'Deep Rose' (initial level = 7.32 μmol g^{-1} DW) and *Pelargonium hortorum* 'Deep Red' (initial level = 2.89 μmol g^{-1} DW)

Species	SWC level (%)	DP 1	DP 2	DP 3	Mean for SWC level	Mean for species
Impatiens walleriana	80	9.03	11.75	11.54	10.77 b	14.52 b
	60	11.92	16.19	14.30	14.15 c	
	30	16.37	19.60	19.95	18.64 d	
Mean for DP		12.44 a	15.85 b	15.26 b		
Pelargonium hortorum	80	2.92	6.14	6.36	5.14 a	5.75 a
	60	3.10	6.87	7.11	5.69 a	
	30	3.46	7.43	8.37	6.42 a	
Mean for DP		3.16 a	6.81 b	7.28 c	Mean for SWC level	
SWC level	80	5.98	8.95	8.95	7.96 a	
	60	7.51	11.53	10.71	9.92 b	
	30	9.92	13.52	14.16	12.53 c	
Mean for DP		7.80 a	13.33 b	11.27 ab		

DP drought period. Values followed by the same letter do not differ significantly at $P < 0.05$

Table 4 The effect of drought on the free amino acids accumulation in leaves of *Impatiens walleriana* 'Deep Rose' (initial level = 168.5 µmol g^{-1} DW) and *Impatiens walleriana* 'Deep Rose' (initial level = 150.9 µmol g^{-1} DW)

Species	SWC level	DP 1	DP 2	DP 3	Mean for SWC level	Mean for species
Impatiens walleriana	80	213.3	292.7	409.5	305.2 c	365.9 b
	60	255.4	413.1	456.5	357.0 d	
	30	348.9	430.8	472.6	417.4 e	
Mean for DP		272.5 a	378.9 b	446.2 c		
Pelargonium hortorum	80	158.7	168.6	163.2	163.5 a	191.3 a
	60	183.7	203.2	209.9	198.9 b	
	30	202.1	211.0	211.6	211.6 b	
Mean for DP		181.5 a	194.3 a	194.9 a	Mean for SWC level	
SWC level	80	186.0	230.7	286.4	234.4 a	
	60	219.6	308.2	333.3	287.0 ab	
	30	275.5	320.9	342.1	312.8 b	
Mean for DP		227.0 a	286.6 ab	320.6 b		

DP drought period. Values followed by the same letter do not differ significantly at $P < 0.05$

Table 5 Effect of drought on chlorophyll $a + b$ content in leaves of *Impatiens walleriana* 'Deep Rose' (initial level = 11.43 mg g^{-1} DW) and *Pelargonium hortorum* 'Deep Red' (initial level = 13.19 mg g^{-1} DW)

Species	SWC level (%)	DP 1	DP 2	DP 3	Mean for SWC level	Mean for species
Impatiens walleriana	80	9.52	5.83	6.31	7.22 b	7.01 a
	60	11.66	5.65	4.87	7.39 b	
	30	10.17	5.17	3.62	6.41 a	
Mean for DP		10.45 b	5.55 a	4.93 a		
Pelargonium hortorum	80	13.07	12.39	12.22	12.56 a	12.52 b
	60	13.21	11.31	12.08	12.20 a	
	30	13.51	12.67	12.23	12.81 a	
Mean for DP		13.26 a	12.12 a	12.18 a	Mean for SWC level	
SWC level	80	11.30	9.11	9.27	9.89 a	
	60	12.44	8.48	8.48	9.80 a	
	30	11.84	8.92	7.93	9.56 a	
Mean for DP		11.86 b	8.84 a	8.56 a		

DP drought period. Values followed by the same letter do not differ significantly at $P < 0.05$

was less significant but at the last sampling date in plants grown under 30% SWC the chlorophyll content dropped by more than two third as compared to the initial level and was over 40% lower than in controls grown under 80% SWC. Compared to impatiens, the leaves of geranium had a slightly higher initial concentration of chlorophyll $a + b$. None of the experimental factors affected pigment level: neither its concentration decreased insignificantly during the time course of the experiment, nor an effect of drought was found in geranium.

Discussion

Drought frequently affects bedding plants in urban areas causing adverse effects on plant growth and flowering, therefore on their aesthetic value. It is therefore essential to choose for planting, species that can cope with water shortage without losing their ornamental values. Drought tolerance tests based on the measurements of certain parameters related to the plant water status could help both in correct plant selection for urban settings as well as in breeding programs aiming to create new cultivars that would be better adapted to urban conditions. There is little information available on the use of selection criteria for selecting the appropriate ornamental plant species for urban green areas, or for the improvement of plant water stress tolerance through breeding. To assess the tolerance of plants to water stress their growth or survival is often measured. Indirect selection for drought tolerance using physiological or biochemical attributes as markers in breeding may be a simpler and a more effective approach.

However, it is difficult to identify one single attribute to be used as an effective selection criterion. A reliable indicator of drought tolerance should show a direct correlation between its numerical score and the degree of stress to which the plant is subjected, and it should be measured by a simple and standardized method. In a widely grown commodity crop such as wheat, the cell membrane stability was chosen to measure the drought and heat tolerance (Blum and Ebercon 1981). In rose breeding programs, parameters relating to water status and the properties of cell sap were found to be good indicators of the potential flower longevity (Gudin 2003). The lethal leaf water potential was chosen as a parameter allowing to evaluate ornamental plants for their foliar dehydratation tolerance (Augé et al. 2003). Proline accumulation was considered as a useful stress indicator in sugar beets (Gzik 1996). Numerous researchers have attempted to relate parameters indicating water status of leaves to the reflectance spectral properties (Yu et al. 2000). A range of physiological selection criteria for salt tolerance in plants such as osmotic adjustment, photosynthesis, water relations and ion relations has been recently discussed (Ashraf and Harris 2004). Here we report on an attempt to quantify several parameters of responses of two common bedding plants to an imposed water stress: morphological, physiological and biochemical. The aim was to test if any of these may serve as a drought stress indicator to estimate a priori the plant response to water shortage, and could be useful in breeding programs and in selection of plants for urban areas.

The results of our tests on impatiens and geranium confirm earlier observations that plant taxa differ in their responses to drought (Volaire 2003; Holopainen et al. 2006). Plants chosen for this study represent different habitats. In the regions from which the geraniums originated, drought periods are frequent. Therefore leaves become hairy and stems woody. On the other hand, impatiens which come from tropical forests are very succulent and their leaves lack any protection against water loss during a water stress. Indeed, impatiens plants grown under drought conditions in this study suffered more than geraniums: were shorter and flowered poorly. Evidently, due to an innate adaptation mechanism, geraniums grown under severe stress did not lose their aesthetic value as expressed by plant height and flowering intensity. According to Augé et al. (2003) both geranium and impatiens are the most dehydratation intolerant herbaceous plants among 30 taxa they have tested. However, although geranium symplasm is not tolerant of desiccation, plants have very sensitive stomata so their leaves can retain water and survive a long drought period; evidently this is not the case in impatiens.

One of the plant responses to drought is root elongation as shown by Volkmar (1997) on wheat or by Yin et al. (2005) in *Populus kangdigensis*. Both species of bedding plants tested here under severe drought (30% SWC) had longer roots at the end of the experiment, with a larger increase in impatiens than in geranium. However, this form of coping with the stress was not sufficient for impatiens, as the morphological parameters of the above soil parts in plants from severe stress (30% SWC) were significantly lower than in plants grown under 80% SWC, or in stressed geranium plants. However, plants growing in soil, i.e. in a flower bed, can favor root growth to a greater extent than shown in this trial when plants were constrained to limited soil volumes in pots.

The relative water content in plant tissues decreases due to the diminishing soil water content. RWC dropped in both species in response to drought, however the ranges of the responses were different in geranium and impatiens. Water loss occurred earlier in the succulent impatiens (already by 60% SWC) and was double that of what was observed in geranium. Gratani and Varone (2004) working on *Erica* sp. and *Rosmarinus officinalis* have shown that the low RWC values in these species during drought were indicative of a low recovery capacity. The same was true in the present trial in impatiens whose growth parameters were more negatively impacted by the imposed water stress than in geranium with higher RWC values under stress conditions.

One of the compounds which levels change under abiotic stress such drought is ammonium (Kronzuker et al. 2001). Ashraf and Harris (2004) showed that the ammonium contents in wheat and rice grown in normal (available water) and dry (water withheld) conditions were different. Lin and Kao (1998) reported that the NH^{+}_{4} accumulation was associated with senescence of water stressed, detached rice leaves. The same was true in this experiment: an increase of the ammonium content, especially in the leaves of impatiens to more than 173% of control level at 30% SWC. In geranium leaves, the ammonium accumulation was much lower at the same SWC level relative to plants grown at 80% SWC. However, in spite of the much lower initial ammonium content the accumulation of this compound in geranium progressed with time irrespective of a stress degree so its increase after 50 days of experiment was even larger than in impatiens. Therefore, changes in NH^{+}_{4} contents do not reflect differences between impatiens and geranium in their response to drought. Presently we have no data on the influence of water stress on nitrogen metabolism in the two species under study where the ammonium accumulation may have resulted from different metabolic pathways. Generally, an accumulation of free amino acids occurs in plants grown under stress conditions (Smolders et al. 1996; Starratt and Lazarovits 1996; Costa and Spitz 1997). An increase in the free amino acid pool is mostly explained by protein degradation. An increase in the concentration of free amino acids in stressed plants may play a significant role in drought sustenance as these compounds contribute to the osmoregulation in the leaves of plants under stress. Here, in impatiens the species more heavily affected by the imposed drought cycles than in geranium, the concentration of free amino acids increased after the third drought period to 280% of the initial level. In geranium, this parameter rose only by 40% in plants subjected to 30% SWC. However, in impatiens, the increase in the free amino acid concentration was much more time dependent. Therefore this attribute cannot be considered as a water stress indicator. Rather changes in the amino acids composition caused by drought might reveal differences between the species compared in this trial.

Contrary to the less drought affected geranium, the effects of the severe stress were manifested in the leaves of impatiens by a significant reduction in the chlorophyll $a + b$ content. These results are in line with the observations on various plant species by the authors like Parys et al. (1998), Ueda et al. (2003) or Gratani and Varone (2004). No effect of drought on pigment content was observed in geranium what might confirm better drought tolerance of this species.

The results of this study show that both species used for this study and representing different habitats differed in their response to drought almost in every aspect, and confirmed that geranium is better adapted to water stress. We consider the RWC and chlorophyll $a + b$ contents as parameters which merit further attention as possible indicators of plant responses to drought stress in ornamental plants but additional studies are needed before any of these parameters or other biochemical indicators could be used to evaluate new bedding plants before introducing them into urban conditions or as a selection criterion in breeding for adaptation to difficult growing conditions.

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