

Response of *Hyacinthus orientalis* L. to salinity caused by increased concentrations of sodium chloride in the soil

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Abstract

Hyacinths are used in flowerbed in cities. Plants are exposed to difficult urban conditions prevailing in winter, including notably to soil salinity resulting mainly from the use of sodium chloride. The aim of the study was analysed the response of *Hyacinthus orientalis* 'Delft Blue' to increased concentrations of sodium chloride in the soil. Pots with bulbs were each treated with 100 cm³ of NaCl aqueous solution at a concentration of either 10 g dm⁻³ (dose level 1) or 20 g dm⁻³ (dose level 2). Pots were irrigated with the NaCl aqueous solution in September or in January of the following year. After keeping the bulbs at a cool temperature for 12 weeks, the plants were grown in a greenhouse where they were assessed in terms of growth parameters and free proline content. The obtained results showed that the growth and the ornamental qualities of *Hyacinthus orientalis* 'Delft Blue' after treatment with sodium chloride were sufficient for recommending this plant for growing in flower beds exposed to salinity of 6.1-8.6 g dm⁻³. The leaf greenness index and proline content (in the second season) were greater and the length of forcing period was longer (in the second season), when the sodium chloride was applied to the soil on the day of planting bulbs.

Keywords: bulbous plants; bulbs; flower bed; NaCl; proline

Introduction

Hyacinthus orientalis is a bulbous plant frequently used as a component of floral arrangements growing in spring flower beds in green spaces. *Hyacinthus orientalis* flowers in spring on stems growing from underground bulbs planted earlier in autumn. This makes it particularly exposed to difficult urban conditions prevailing in winter, including notably to soil salinity resulting mainly from the use of sodium chloride (NaCl) to remove ice from roads and pavements.

Salt stress induces various physiological disorders in plants (Parida and Das, 2005). Basically, salinity exerts a twofold negative influence on plants. The increased concentration of ions in the soil solution decreases

soil water potential thus triggering osmotic stress, which hinders water intake from the soil. This results in lower water content in cells, cell turgor loss, and stunted plant growth (Liang *et al.*, 2018). This process also involves stomata closure, which reduces carbon dioxide diffusion inside leaves and inhibits photosynthesis (Parihar *et al.*, 2015). The above changes slow down plant growth, limit biomass accumulation and disturb the plant development process (Gharsallah *et al.*, 2016).

Another harmful effect resulting from increased NaCl concentrations in the soil is an increased uptake of Na⁺ and Cl⁻, which disrupts ion metabolism and causes ionic stress (Teh *et al.*, 2016; Mansour and Ali, 2017). The consequence is disturbance in the absorption of potassium, phosphorus, calcium and nitrogen ions leading to insufficient levels of those elements in the plant (Chinnusamy *et al.*, 2006; Teh *et al.*, 2016; Mansour and Ali, 2017). The aforementioned changes as well as the toxic effect of sodium and chlorine ions on the activity of various enzymes disrupt many metabolic pathways and damage cellular structures (Chinnusamy *et al.*, 2006). The negative influence of the increased concentration of sodium and chlorine ions manifests as lower chlorophyll content in leaves and, finally, in chlorosis (Tavakkoli *et al.*, 2010). The above described disturbances cause changes in plant morphology, delayed flowering, accelerated aging and an earlier loss of the plant's ornamental value (Ramoliya *et al.*, 2004). The toxicity of those ions is also apparent through premature defoliation, and in extreme cases, even plant death (Läuchli and Grattan, 2007).

A plant may use two strategies to develop resistance to stress factors, including salinity; namely, it may either avoid or tolerate stress (Chinnusamy *et al.*, 2006; Parihar *et al.*, 2015; Singh *et al.*, 2017). Avoiding salt stress involves the formation of protective barriers to prevent harmful ions from invading the cytoplasm and the maintenance of the osmotic balance to prevent cell dehydration. Salt tolerance is a response to salinity by triggering mechanisms that limit damage and enable damage repair. Such mechanisms are linked to the synthesis of specific proteins and compatible substances that enable maintaining the osmotic balance in cells and protect cell structures from the consequences of dehydration and ionic toxicity (Mansour and Ali, 2017). One of such substances is an amino acid called proline, the level of which increases in many plant species exposed to salinity (Hayat *et al.*, 2012; Gharsallah *et al.*, 2016). It was shown that both the accumulation and exogenous application of proline under salt stress conditions improved plant resistance to this adverse environmental factor (Hayat *et al.*, 2012; Teh *et al.*, 2016; Mansour and Ali, 2017). Proline increases tolerance to salinity in that it reduces the cell water potential and thus prevents dehydration. In addition, this amino acid may protect cell structures and enzymes from damage caused by dehydration and the toxicity of ions (Hayat *et al.*, 2012; Mansour and Ali, 2017). Proline may also scavenge reactive oxygen species, which are produced during times of stress, disturb metabolic processes (Hayat *et al.*, 2012).

The aim of this study was to investigate the response of *Hyacinthus orientalis* 'Delft Blue', an ornamental plant with a magnificent and fragrant inflorescence, to salinity caused by increased NaCl concentrations in the soil. The study analysed the influence of two dose levels of sodium chloride applied on two different dates on the plant's resistance to salinity and the content of free proline in leaves. Resistance to salinity was assessed based on growth parameters (stem length, dry matter of the aerial part) and ornamental qualities (flowering start date, leaf greenness index (SPAD)).

Materials and Methods

The experiment was conducted in the greenhouse of the Department of Ornamental Plants at the Poznań University of Life Sciences during two seasons (2014/15 and 2015/16). Bulbs of *Hyacinthus orientalis* 'Delft Blue' were planted in calendar week 34 in 0.8 dm³ flower pots filled with a peat substrate of pH 6.2 (measured after the addition of minerals). The potting medium was enriched with the following mineral components of the soil: 146 mg dm⁻³ N-NO₃, 47 mg dm⁻³ N-NH₄, 65 mg dm⁻³ P, 321 mg dm⁻³ K, 1840 mg dm⁻³

³ Ca, 145 mg dm⁻³ Mg, 64 mg dm⁻³ Na, 36 mg dm⁻³ Cl. Soil salinity in terms of NaCl content was 1.9 gdm⁻³. The soil pH and salinity were measured using conductometer CPC-511 (Elmetron).

There were 140 pots in the experiment, each containing one bulb. The pots were divided into five treatments, 28 pots per treatment, as follows: (1) control, (2) a 10 g dm⁻³ aqueous solution of NaCl (dose level 1) applied on the bulb planting date, (3) a 10 g dm⁻³ aqueous solution of NaCl (dose level 1) applied in the first week of January, (4) a 20 g dm⁻³ aqueous solution (dose level 2) of NaCl applied on the bulb planting date, (5) a 20 g dm⁻³ aqueous solution of NaCl (dose level 2) applied in the first week of January.

On the planting date, the soil and the bulbs in 84 pots were watered with 100 cm³ of tap water. The remaining pots were each irrigated with 100 g dm⁻³ of NaCl aqueous solution at a concentration of 10 gdm⁻³ (28 pots) and 20 g dm⁻³ (28 pots). The pots with the bulbs were placed for 12 weeks in a room with an initial temperature of +9 °C to let them grow roots and prepare them for cultivation. In December the temperature was reduced to +5 °C, and in January to +2 °C so as to avoid elongated plant growth. In the first week of January, 56 pots earlier watered with tap water were selected of which 28 pots were irrigated with 10 gdm⁻³ NaCl aqueous solution and another 28 pots with 20 g dm⁻³ NaCl aqueous solution. The remaining pots were watered with 100 cm³ of tap water.

Soil salinity

After applying 10 g dm⁻³ NaCl aqueous solution, the soil salinity level was 6.1 g dm⁻³, and the content of Na and Cl in 1 dm⁻³ of the soil was, respectively, 134 mg and 2192 mg. Irrigation with 20 g dm⁻³ NaCl aqueous solution resulted in a soil salinity level of 8.6 g dm⁻³, with Na and Cl concentrations of 233 mg dm⁻³ and 3646 mg dm⁻³, respectively. In both cases, the pH value was 6.3.

Determination of proline content

Seven days before the planned date of moving the plants to the greenhouse, four pots with plants were selected from each treatment to determine proline content in leaves. Proline content was measured in 4 replications, and each replication was conducted on a different plant material sample. Proline content was measured according to Bates *et al.* (1973).

Greenhouse growing, observations and measurements

After the 12-week cooling period, the plants were moved to the greenhouse. The experiment involved 5 treatments, each including 24 pots. The number of pots per treatment was reduced by 4 pots which were earlier selected for the proline content measurement. One pot with bulb was one replication.

Water loss from pots placed in the greenhouse was replenished using tap water irrigation. In this process, pots with plants were weighed and supplemented with water until all pots had the same weight.

In both seasons, plants were grown in the greenhouse from 10 January to 5 April, i.e. till the end of flowering. During that time, the number of growing days until the beginning of flowering was determined. On the flowering start date, the stem length was measured from the base to the place of growth of the bottom-most flower. After the flowering, the dry matter of the aerial part was weighed.

The chlorophyll content was measured using the leaf greenness index (Test Soil Plant Analysis System - SPAD) (Pacewicz and Gregorczyk, 2009). At the beginning and after flowering, SPAD values were determined using a Yara N-tester. Based on the results, the percentage difference between the initial and final value was calculated. The measurement was conducted on the apex of the external leaf of each plant in a treatment.

Statistical analysis

The results were subjected to a two-way analysis of variance using Statistica software, while means were grouped using the Duncan test at the significance level $\alpha=0.05$. For percentage values, the normality of distribution of the studied features was checked using the Shapiro-Wilk normality test. A Bliss transformation ($y = \arcsin(x/100)^{-1/2}$, where y -value of a characteristic after transformation, x -observed value of the trait before

transformation) was performed in the case of abnormal feature distribution. In the remaining cases, the data were subjected to the standard Duncan test. The data were compared in each season separately.

Results and Discussion

The number of days that *Hyacinthus orientalis* 'Delft Blue' needed to start flowering in both seasons depended on the dose level of NaCl applied in the experiment (Table 1). The control plants were the first to start flowering. The length of exposure to the stress factor was significant only in the second season. The application of sodium chloride on the bulb planting date delayed flowering. Such response to saline conditions has been observed in many plant species, e.g. rice (Läuchli and Grattan, 2007), China aster (Küçükahmetler, 2002) and narcissus (Veatch-Blohm *et al.*, 2014).

Introducing the stress factor resulted in weaker plant growth in the saline soil (Table 2). The length of the stem on the flowering start date depended only on the soil salinity level. The longest stems were found in the control group and the shortest in the group subjected to dose level 2 of NaCl. It can be concluded that, NaCl inhibited growth, as was the case in *Gaillardia aristata* and *Salvia coccinea* (Niu and Rodriguez, 2006). Nevertheless, the inflorescence was fully developed and showed no signs of damage. *Hyacinthus* 'Delft Blue' is suitable for planting in soils with salinity of up to 8.6 g dm⁻³ of NaCl.

In the first season, the percent change in the SPAD value depended solely on the applied NaCl dose level (Table 3). The percent change in the SPAD value increased in the control treatment and after dose level 1 of NaCl, whereas it decreased after dose level 2 of NaCl. In the second season, the SPAD value percent change was positive and depended on both the NaCl dose level and the date of its application. The highest growth in the SPAD value was noted in the control treatment, and the lowest growth was observed after dose level 2 of NaCl. Irrigating the soil with the NaCl solution on the date of bulb planting led to a stronger increase in the percent change in SPAD value relative to the NaCl treatment in the first week of January. These results suggest that 'Delft Blue' is less resistant to high NaCl doses when applied later in the season, similar to *Calceolaria hybrida*, *Calendula officinalis* (Fornes *et al.*, 2007), *Vicia faba* (Tavakkoli *et al.*, 2010) and in research of Türkoglu *et al.* (2011). Rajesh *et al.* (1998) showed that the chlorophyll content of salinity resistant plants increased under the influence of NaCl.

The weight of the dry matter of the aerial part collected after flowering depended only on the applied NaCl dose level in both seasons (Table 4). In the first season the weight of the dry matter obtained from the control treatment was higher than that from plants treated with dose levels 1 and 2 of NaCl. In the second season, the dry matter of control plants and plants treated with dose level 1 NaCl had a higher weight compared to that of plants treated with dose level 2 of NaCl. This might indicate that 'Delft Blue' adapted to difficult environmental conditions or that sodium chloride might stimulate its growth. The speculation is confirmed by the study conducted by Niu and Rodriguez (2006). Different results were obtained by Rajesh *et al.* (1998). The fresh and dry matter of *Ceriops roxburghiana* was found to increase under salinity stress conditions.

The free proline content in young leaves was equal in both seasons and depended on both the NaCl dose level and the date of its application (Table 5). The plants treated with NaCl on the bulb planting date accumulated more free proline than the plants irrigated with the NaCl solution in the first week of January. The highest proline concentration was observed after dose level 2 of NaCl and the lowest – after dose level 1 of NaCl. Also, the proline content in young leaves increased with the length of exposure to the stress factor. Similar observations were made in the studies on *Atriplex halimus* (Martinez *et al.*, 2005), *Trachyspermum ammi* (Ashraf and Orooj, 2006), *Iris lactea* var. *chinensis* (Bai *et al.*, 2008), *Melisa officinalis* (Khalid and Cai, 2011), *Echium amoenum* (Ramezani *et al.*, 2011) and *Crocus sativus* (Rajaei *et al.*, 2009; Renau-Morata *et al.*, 2012). The results of this study seem to confirm that 'Delft Blue', like some other plant species, builds up resistance to NaCl by accumulating proline (Hayat *et al.*, 2012; Teh *et al.*, 2016; Mansour and Ali, 2017).

Table 1. The number of days of pre-flowering period of *Hyacinthus orientalis* 'Delft Blue' in greenhouse by timing of exposure to NaCl and NaCl dose (n= 24)

Term of NaCl application	Season							
	The first				The second			
	Dose level of NaCl				Dose level of NaCl			
	0	1	2	Mean	0	1	2	Mean
The day of planting bulbs	18.4 ^a	19.7 ^b	20.0 ^b	19.3 ^a	17.9 ^a	20.4 ^{bc}	21.4 ^c	19.9 ^b
The 1 st week of Jan.	18.4 ^a	19.6 ^b	19.8 ^b	19.3 ^a	17.9 ^a	18.3 ^a	20.0 ^b	18.7 ^a
Mean	18.4 ^a	19.7 ^b	19.9 ^b		17.9 ^a	19.3 ^b	20.7 ^c	

Dose level 1 - 10 g dm⁻³ aqueous solution of NaCl, dose level 2 - 20 g dm⁻³ aqueous solution.
The means followed by the same letter do not differ significantly at $\alpha=0.05$.

Table 2. The length of *Hyacinthus orientalis* 'Delft Blue' stem in the beginning of flowering by timing of exposure to NaCl and NaCl dose (n= 24) (cm)

Term of NaCl application	Season							
	The first				The second			
	Dose level of NaCl				Dose level of NaCl			
	0	1	2	Mean	0	1	2	Mean
The day of planting bulbs	17.0 ^c	15.3 ^b	13.0 ^a	15.1 ^a	13.3 ^b	10.7 ^{ab}	8.3 ^a	10.8 ^a
The 1 st week of Jan.	17.0 ^c	15.3 ^b	13.0 ^a	15.1 ^a	13.3 ^b	12.0 ^b	9.0 ^a	11.4 ^a
Mean	17.0 ^c	15.3 ^b	13.0 ^a		13.3 ^c	11.3 ^b	8.7 ^a	

Dose level 1 - 10 g dm⁻³ aqueous solution of NaCl, dose level 2 - 20 g dm⁻³ aqueous solution.
The means followed by the same letter do not differ significantly at $\alpha=0.05$.

Table 3. The change of leaf greenness index (SPAD value) of *Hyacinthus orientalis* 'Delft Blue' by timing of exposure to NaCl and NaCl dose (n= 24) (%)

Term of NaCl application	Season							
	The first				The second			
	Dose level of NaCl				Dose level of NaCl			
	0	1	2	Mean	0	1	2	Mean
The day of planting bulbs	1.5 ^c	2.4 ^c	-3.7 ^{ab}	0.1 ^a	11.2 ^b	10.4 ^b	3.7 ^a	8.4 ^b
The 1 st week of Jan.	1.5 ^c	-0.7 ^{bc}	-4.7 ^a	-1.3 ^a	11.2 ^b	3.3 ^a	4.2 ^a	6.2 ^a
Mean	1.5 ^b	0.8 ^b	-4.2 ^a		11.2 ^c	6.8 ^b	3.9 ^a	

Dose level 1 - 10 g dm⁻³ aqueous solution of NaCl, dose level 2 - 20 g dm⁻³ aqueous solution.
The means followed by the same letter do not differ significantly at $\alpha=0.05$.

Table 4. The dry matter of the aerial part of *Hyacinthus orientalis* 'Delft Blue' after the end of flowering by timing of exposure to NaCl and NaCl dose (n= 24) (g)

Term of NaCl application	Season							
	The first				The second			
	Dose level of NaCl				Dose level of NaCl			
	0	1	2	Mean	0	1	2	Mean
The day of planting bulbs	6.0 ^b	4.8 ^a	5.0 ^a	5.3 ^a	4.8 ^b	4.3 ^{ab}	3.8 ^a	4.3 ^a
The 1 st week of Jan.	6.0 ^b	5.5 ^{ab}	5.5 ^{ab}	5.7 ^a	4.8 ^b	4.3 ^{ab}	3.5 ^a	4.2 ^a
Mean	6.0 ^b	5.2 ^a	5.3 ^a		4.8 ^b	4.3 ^b	3.7 ^a	

Dose level 1 - 10 g dm⁻³ aqueous solution of NaCl, dose level 2 - 20 g dm⁻³ aqueous solution.

The means followed by the same letter do not differ significantly at $\alpha = 0.05$.

Table 5. The proline content in young leaves of *Hyacinthus orientalis* 'Delft Blue' by timing of exposure to NaCl and NaCl dose (n= 4) (mg g⁻¹ FM)

Term of NaCl application	Season							
	The first				The second			
	Dose level of NaCl				Dose level of NaCl			
	0	1	2	Mean	0	1	2	Mean
The day of planting bulbs	0.52 ^{bc}	0.42 ^a	0.96 ^d	0.63 ^b	0.52 ^{bc}	0.42 ^a	0.96 ^d	0.63 ^b
The 1 st week of Jan.	0.52 ^{bc}	0.56 ^c	0.49 ^b	0.52 ^a	0.52 ^{bc}	0.56 ^c	0.49 ^b	0.52 ^a
Mean	0.52 ^b	0.49 ^a	0.73 ^c		0.52 ^b	0.49 ^a	0.73 ^c	

Dose level 1 - 10 g dm⁻³ aqueous solution of NaCl, dose level 2 - 20 g dm⁻³ aqueous solution.

The means followed by the same letter do not differ significantly at $\alpha = 0.05$.

Conclusions

Plants used in urban flower beds should be able to flower for a long period and look decorative even under unfavourable soil and climatic conditions. A proper selection of plant species and cultivars is particularly important when it comes to designing spring floral arrangements including bulb plants. The experiment described in this study showed that the growth and the ornamental qualities of *Hyacinthus orientalis* 'Delft Blue' after treatment with sodium chloride were sufficient for recommending this plant for growing in flower beds exposed to salinity of 6.1-8.6 g dm⁻³. The leaf greenness index and proline content (in the second season) were bigger and the length of forcing period was longer (in the second season), when the sodium chloride was applied to the soil on the day of planting bulbs.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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