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Research Article

PLANT GROWTH OF CURLY KALE UNDER SALINITY STRESS

Piotr Salachna[1], Rafał Piechocki[1], Andżelika Byczyńska[1]

[1] Department of Horticulture, West Pomeranian University of Technology in Szczecin, Papieża Pawła VI 3 Str., 71-459 Szczecin, Poland, e-mail: piotr.salachna@zut.edu.pl, rafal.piechocki@zut.edu.pl, andzelika.woskowiak@zut.edu.pl

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ABSTRACT
Ornamental plants growing in urban areas are exposed to soil salinity that negatively affects their quality. Identifying species that retain high ornamental value despite salt stress is therefore of high practical importance. Curly kale (*Brassica oleracea* L. var. *sabellica* L.) is an attractive plant with leaves of both edible and ornamental character. The aim of the study was to evaluate a response of ornamental curly kale to different concentrations of NaCl. The study material was 'Scarlet' cultivar. The plants were grown in pots in a plastic tunnel. They were irrigated with NaCl solution at the following concentrations: 50, 100, 200, 400, and 800 mM mmol·dm^{-3}. NaCl treatment resulted in a significant increase in pH and electrical conductivity (EC) of the substrate. Salt stress significantly affected plant growth and number, width and length of leaves, and the effects depended on NaCl concentration. Fifteen days after the salt supply ceased, relative chlorophyll content in leaves (SPAD) decreased due to NaCl treatment in a concentration-dependent manner. Treatments with 200, 400, and 800 mmol·dm^{-3} NaCl reduced stomatal conductance, and the changes were greater on the 5th day following the stress cessation than on the 15th day. Irrigation with a 200, 400, and 800 mmol·dm^{-3} NaCl solution negatively affected plant bonitation score. The plants treated with 50 i 100 mmol·dm^{-3} NaCl were not significantly different visual score from the control plants.

Keywords: sodium chloride, ornamental plants, stomatal conductance, SPAD

INTRODUCTION

Ornamental plants growing in urban areas should be resistant to air pollution and soil salinity [Pląskowska, 2010]. Soil salinity along city transportation routes is due to human activity, such as using sodium chloride to remove snow from roads, streets, and pavements [Bach et al., 2009]. Salt concentrations that exceed plant tolerance level cause growth inhibition [Villarino and Matsson, 2011], reduce flower quality [Sonneveld et al., 1999], trigger leaf browning and drying [Cassaniti et al., 2012], and finally result in plant organ death [Niu and Cabrera, 2010; Parihar et al., 2015]. Salinity has a negative impact on photosynthesis [Vetach-Blohm et al., 2013], water management [Ma et al., 2012] and enzymatic activity [Zhang et al., 2014]. It also disturbs nutrient absorption and ion balance [Valdez-Aguilar et al., 2009], induces osmotic stress [Munns and Tester, 2008] and dysfunction of plasma membranes [Mansour et al., 2015]. Plant responses to salinity are variable and depend on plant genotype, growth phase and soil moisture content [Niu and Cabrera, 2010; Cassaniti et al., 2013]. Recent years witnessed numerous research studies on the sensitivity of different species of ornamental plants to salinity that were aimed at identifying the plants tolerant to excess soil salt and estimating the degree of this tolerance [Niu et al. 2007; Niu and Cabrera 2010; Breś et al., 2014; Sun et al., 2015; Wu et al., 2016]. Learning more about salt tolerant plants is of great practical importance in the selection of species and cultivars grown in urban green areas [Niu et al., 2010; Cassaniti et al., 2013; Escalona et al., 2013; Salachna and Piechocki, 2016].

Ornamental cultivars of vegetables planted in flowerbeds are currently highly valued [Gibson and Whipker, 2001; Zhao and Li, 2011; Haghighi et al., 2016]. Curly kale (*Brassica oleracea* L. var. *sabellica* L.), cultivated already in ancient Greece and Rome for ornamental and food purposes, is a good example of such a species [Murray et al. 2005]. Its plants produce thick stems with curly leaves arranged in a characteristic rosette. Kale leaves are edible and decorative, and depending on the cultivar they can be less or more curly, dark green to purple brown [Lewandowska 2000]. Their colors turn very bright after the first light freezes. The plants grow rapidly and have low soil requirements. Curly kale can be successfully cultivated in flowerbeds and pots [Hirvela 2014]. Due to its high resistance to low temperature curly kale is also highly decorative in autumn and winter [Watson, 1996].

Most studies on the response to salt stress conducted in ornamental plants of Brassicaceae family concerned ornamental cabbage and kale (*Brassica oleracea* var. *acephala*). Chen et al. [2011] proved that germination of ornamental cabbage seeds depended on the cabbage cultivar and NaCl concentration. In 'Nagoya series' cv., salt tolerance was the highest (lethal concentration 213.65 mmol/L NaCl), while in 'Pigeons series' it was the lowest (lethal concentration 87.32 mmol/L). Zheng et al. [2010] reported that rising doses of NaCl first increased and then decreased the content of soluble protein and CAT and POD activity in ornamental kale seedlings. They claimed that the content of soluble protein and CAT activity were the highest when NaCl concentration was 600 mmol/L, while POD activity reached its highest level at 800 mmol/L NaCl. According to Haghighi et al. [2016], ornamental kale is recommended for phytoremediation of saline soils with 10 and 16 $mg \cdot kg^{-1}$ lead and cadmium contents, respectively.

So far, scientific literature lacks papers on the effects of salinity in ornamental curly kale cultivars. Therefore, the aim of this study was to evaluate the effects of moderate soil salinity caused by sodium chloride on morphological features, stomatal conductance, greenness index of leaves and ornamental value of curly kale.

MATERIAL AND METHODS

Curly kale (*Brassica oleracea* L. var. *sabellica* L.) plants of 'Scarlet' cv. (purple leaves) were derived from seedlings produced in a heated greenhouse. The seeds obtained from Breeding and Seed Company W. Legutko (Poland) were sown on 30th March 2015, and after 2 weeks the seedlings were transferred into pots 8 cm in diameter, filled with TS1 plant substrate (Klasmann-Deilmann, Poland). On 6th May 2015, single seedlings were transferred into black PCV pots of 17 cm diameter and 2 dm^3 capacity. The substrate was deacidified peat with pH 6.5 mixed with Yara Mila Complex fertilizer (Yara International ASA, Norway) containing 12% N, 11% P_2O_5, 18% K_2O, 2.7% MgO, 8% S, 0.015% B, 0.2% Fe, 0.02% Mn and 0.02% Zn, used at 3 $g \cdot dm^{-3}$. The pots were placed on 60 cm high tables in an unheated tunnel covered with double layer of plastic located in the area of West Pomeranian University of Technology in Szczecin (53°25' N, 14°32' E; 25 m a.s.l.). The temperature inside the tunnel was monitored by vents that opened automatically when the temperature exceeded 20°C. The plants were irrigated with a solution of pure p.a. 99.9% sodium chloride (NaCl) (Chempur, Poland). The irrigation started on 27th May 2015 and the following NaCl concentrations were used: 50, 100, 200, 400, and 800 $mmol \cdot dm^{-3}$. Salt treatment was repeated four times, every 5 days, using 300 ml of the solution per plant. Control plants were irrigated with tap water with EC 0.27 $mS \cdot cm^{-1}$. Five and fifteen days after the last treatment, two physiological parameters, i.e. greenness index and stomatal conductance were assessed. The greenness index (relative chlorophyll content) was measured in SPAD units (Soil Plant Analysis Development) with an optical device Chlorophyll Meter SPAD-502 (Minolta, Japan). The measurements included five leaves located in the central section of the plant and three readings were taken per each leaf. Stomatal conductance (g_s) was assessed with SC1 porometer (Dekagon Devices, USA). The measurements were conducted from 10:00 a.m. to 12:00 p.m. and included three leaves from each plant. PAR radiation during measurement was 910–1033 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (as per Radiometer-Fotometr RF-100, Snopan, Poland). The plants were grown under natural photoperiod till 15th July 2015. On the last day of the experiment, the following parameters were determined: plant height, number of leaves per plant, length and width of the largest leaf from the central section of the plant. The visual score of the plants was assessed by three people according to the bonitation scale from 1 to 5 points, where 5 meant the plants of the highest quality. In each variant of the experiment, three replications of the substrate sample were taken in order to determine pH and electrical conductivity (EC). The measurements were performed with microcomputer pH-METER CP–315 M (Elmetron, Poland) and conductivity meter CC-411 (Elmetron, Poland), in aqueous extract (substrate : distilled water 1 : 2).

Each experimental variant included 20 plants, 5 plants per repetition. The results were statistically evaluated by the analysis of variance (ANOVA) for univariate experiments. Statistical software ANALWAR-4.3, (based on Microsoft Excel) developed by Franciszek Rudnicki was used. Mean values were compared using Tuckey test for a significance level α = 0.05.

RESULTS AND DISCUSSION

Analysis of the substrate after the experiment completion revealed a significant increase in pH and electrical conductivity (EC) as a result of NaCl administration (Table 1). The increased pH may be due to alkalizing properties of NaCl, mainly sodium, which trigger the following series of reactions: NaCl → $NaHCO_3$ → Na_2CO_3 → NaOH [Breś, 2008]. High pH disturbs absorption of phosphorus and most of the micronutrients [Marschner, 1995], which could induce discoloration and necrosis of leaves in the plants treated with the highest concentration of NaCl, i.e. 400 and 800 mM (Fig. 1). Plant injury could result from increasing ion concentrations that cause chlorosis and necrosis on the leaf margins and premature leaf fall [Cassaniti et al., 2012].

Table 1. The pH and electrical conductivity (EC) of growing medium solution in the end of experiment

NaCl (mmol·dm^{-3})	pH	EC (µS·cm^{-1})
0	6.72 ± 0.03	47.0 ± 4.90
50	7.07 ± 0.08	243 ± 6.55
100	7.29 ± 0.03	327 ± 6.60
200	7.38 ± 0.07	385 ± 4.50
400	7.41 ± 0.02	565 ± 4.50
800	7.45 ± 0.03	621 ± 7.75
$LSD_{0.05}$	0.135	15.598

Table 2 presents the effect of NaCl on stomatal conductance (g_s) in the leaves of 'Scarlet' cv. In plants treated with low concentration of NaCl, i.e. 50 and 100 mmol·dm^{-3}, this parameter was not significantly different from the control plants five and fifteen days after the stress cessation. Significant reduction in stomatal conductance was observed in the plants treated with 200, 400, and 800 mmol·dm^{-3} NaCl, particularly five days after the last NaCl dose. Significant reduction in this physiological parameter due to salt stress was observed in previous studies in *Chrysanthemum ×morifolium* 'Yellow Blush' [Lee and van Iersel, 2008], *Narcissus* 'Dutch Master' and 'Tete-e-Tete' [Vetach-Blohm et al., 2013], and *Plectranthus ciliatus* E.Mey. ex Benth. [Salachna et al., 2015]. Stomatal conductance is an important indicator of stomatal control of water loss and CO_2 assimilation. Lowered stomatal conductance in plants exposed to salt stress may be due to a reduction in water potential or disturbances in photosynthesis and respiration [Parihar et al., 2015].

Changes in relative chlorophyll content in the leaves of 'Scarlet' cv. exposed to salt stress are presented in Table 3. Five days after the last salt application, the plants treated with the highest NaCl concentrations (400 and 800 mmol·dm^{-3})

Figure 1. Effect of salinity on growth curly kale
(left to right: nontreated control, 50, 100, 200, 400, and 800 mmol·dm^{-3} NaCl)

Table 2. Effect of NaCl at concentrations of 0–800 mmol·dm^{-3} on stomatal conductance (g_s) and stomatal conductance rate to control (%) in curly kale. Values are presented as the means ± standard deviation

NaCl (mmol·dm^{-3})	5 days after the stress cessation		15 days after the stress cessation	
	g_s (mmol m s)	g_s rate to control (%)	g_s (mmol m s)	g_s rate to control (%)
0	30.9 ± 1.47	0.00	43.0 ± 1.32	0.00
50	28.7 ± 0.90	-7.12	35.0 ± 2.65	-18.6
100	26.8 ± 2.76	-13.3	35.0 ± 2.00	-18.6
200	18.2 ± 2.03	-41.1	33.6 ± 4.71	-21.9
400	16.3 ± 1.30	-47.2	31.0 ± 4.36	-27.9
800	12.5 ± 2.72	-59.5	28.2 ± 0.93	-34.4
$LSD_{0.05}$	5.465	--	8.291	--

Table 3. Effect of NaCl at concentrations of 0–800 mmol·dm^{-3} on relative chlorophyll content (SPAD) and relative chlorophyll content rate to control (%) in curly kale. Values are presented as the means ± standard deviation

NaCl (mmol·dm^{-3})	5 days after the stress cessation		15 days after the stress cessation	
	SPAD	SPAD rate to control (%)	SPAD	SPAD rate to control (%)
0	43.2 ± 1.11	0.00	43.5 ± 1.10	0.00
50	44.6 ± 0.71	3.24	37.8 ± 2.01	-13.1
100	43.4 ± 1.25	0.46	34.0 ± 3.27	-21.8
200	43.0 ± 1.23	-0.46	30.8 ± 1.42	-29.2
400	37.5 ± 1.14	-13.2	24.8 ± 1.50	-42.9
800	31.2 ± 2.00	-27.8	18.5 ± 0.64	-57.5
$LSD_{0.05}$	3.562	--	5.086	--

had significantly lower leaf greenness index as compared with control, by 5.7 and 12 SPAD, respectively. After the next 10 days, chlorophyll content in curly kale leaves was reduced in all NaCl variants, and the changes were concentration dependent. Similar findings were published by Zheng et al. [2010], who investigated the seedlings of *Brassica oleracea* L. var. *acephalea* DC. and reported chlorophyll loss that increased with growing NaCl concentrations. Leaf greenness index markedly decreased in other salt treated ornamental plants, such as *Calendula officinalis* L., *Calceolaria ×herbeohybrida* Voss [Fornes et al., 2007], *Glandularia canadensis* (L.) Nutt., *Lantana montevidensis* (Spreng.) Briq. [Niu et al., 2007], or *Chrysanthemum ×morifolium* 'Yellow Blush' [Lee and van Iersel, 2008]. According to the literature (Nandy et al., 2007; Jaleel et al., 2008), excessive amounts of salt may accumulate in chloroplasts and exert a direct toxic effect on photosynthesis through destabilization and destruction of protein complexes of photosynthetic pigments. Differences in chlorophyll content five and fifteen days after the last treatment can be explained by the fact that the time after which salt-related damage is visible depends on the rate of Na^+ and Cl^- ion accumulation and efficiency of their sequestration in cells and tissues [Munns, 2002].

Irrigation of 'Scarlet' curly kale with NaCl solutions significantly affected plant height in the concentration-dependent manner (Table 4). A substantial growth reduction due to salinity was observed in plants treated with 200, 400, and 800 mmol NaCl·dm^{-3}. They were lower than the control plants by 21.9%, 27.9%, and 34.4%, respectively. Salt stress related growth inhibition was confirmed in several studies [Ibrahim et al., 1991; Valdez-Aguilar et al., 2009; Krzymińska and Ulczycka-Walorska, 2015; García-Caparrós et al., 2016; Salachna et al., 2016]. Salinity was reported to inhibit mitoses and elongation growth of cells and consequently reduce fresh and dry weight, especially of the above ground parts [Cassaniti et al., 2012].

In this study, 'Scarlet' curly kale grown under salt stress conditions had fewer leaves as compared with the control plants (Table 4). This relationship depended on NaCl concentration. The plants treated with the highest concentration of NaCl, i.e. 800 mmol NaCl·dm^{-3} produced the lowest number of leaves. Reduced number of leaves in plants exposed to excessive salinity was also observed in such bedding plants as *Coleus blumei* 'Xenia Field' and *Salvia splendens* 'Flare Path' [Ibrahim et al. 1991].

Table 4. Effect of NaCl at concentrations of 0–800 mmol·dm^{-3} on plant characteristics in curly kale. Values are presented as the means ± standard deviation

NaCl (mmol·dm^{-3})	Plant height (cm)	Number of leaves	Leaf width (cm)	Leaf length (cm)	Visual score
0	43.0 ± 1.32	18.7 ± 1.53	26.7 ± 2.08	10.2 ± 0.46	5.00 ± 0.00
50	35.0 ± 2.65	16.7 ± 0.58	26.3 ± 0.58	9.03 ± 0.50	4.67 ± 0.29
100	35.0 ± 2.00	15.0 ± 1.00	25.0 ± 1.00	8.27 ± 0.25	4.50 ± 0.50
200	33.6 ± 4.71	15.0 ± 1.73	24.3 ± 1.16	8.60 ± 0.36	3.83 ± 0.29
400	31.0 ± 4.36	15.3 ± 0.58	25.0 ± 1.00	8.27 ± 0.25	3.33 ± 0.29
800	28.2 ± 0.93	14.0 ± 0.00	23.0 ± 1.00	7.83 ± 0.29	2.50 ± 0.50
$LSD_{0.05}$	8.291	2.963	3.359	1.006	0.970

The plants treated with 800 mmol NaCl·dm^{-3} produced the shortest leaves with the narrowest blades, as compared with other and non-treated control plants (Table 4). Adverse effects of strong salt stress on leaf surface were also reported in chrysanthemum [Lee and van Iersel, 2008] and *Grewia tenax* (Forssk.) Fiori [Saied et al., 2010]. Reduction in the leaf number and size in plants exposed to salinity causes a decrease in photosynthesis rate and depletion of energy resources necessary for plant growth and development [Kłosowska, 2010].

The study demonstrated that the visual score of the plants treated with 50 and 100 mmol NaCl·dm^{-3} was not significantly different from that of the control plants (Table 4). A clear reduction of plant quality was observed after the application of 200, 400, or 800 mmol NaCl·dm^{-3}, and the bonitation score decreased along with increasing NaCl concentration. The fact that 'Scarlet' curly kale growing in the presence of 50 and 100 mmol NaCl·dm^{-3} retained its decorative properties suggests that this cultivar is moderately tolerant to soil salinity. However, this assumption requires further study. Plant response to salinity under controlled condition may be different from that in the field, and the differences are due to an interaction of many factors, such as climatic conditions, nutrient status or soil properties that modify plant tolerance to salinity [Wu et al., 1995; Munns and Termaat, 2002; Niu et al., 2007].

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