Morphological and Physiological Changes of *Brassica oleracea* Acephala Group Seedlings as Affected by Ion and Salt Stress

Gvozden DUMIČIĆ [1]
Juan CARLOS DÍAZ-PÉREZ [2]
Harwinder Singh SIDHU [2]
Branimir URLIĆ [1(✉)]
Smiljana GORETA BAN [3]
Dan MACLEAN [2]
Sarah WORKMAN [4]

Summary

The aim of this study was to determine the effect of salt stress on morphological and physiological changes of *Brassica oleracea* acephala group seedlings. Seedlings of kale cultivar Red Russian (RR) and collard Croatian population Konavle 2 (K2) were grown in a floating hydroponic system in Tifton, Georgia, USA. Seedlings were treated with seven different nutrient solutions (NS). The control NS (EC 2 dS m^{-1}) was concentrated to achieve EC 4, 6 or 8 dS m^{-1}. Three additional salt treatments included addition of NaCl solution to the control NS to get: EC 4 NaCl (2 NS + 2 NaCl), EC 6 NaCl (2 NS + 4 NaCl) and EC 8 NaCl (2 NS + 6 NaCl) dSm^{-1}. Leaf gas exchange parameters decreased with increased EC. Seedlings treated with EC 6 NaCl and 8 NaCl dS m^{-1} had the lowest leaf relative water content (less than 59%). Seedlings treated with 2 dS m^{-1} had the greatest (187 cm^2) leaf area (LA). Cultivar RR had greater LA (131 cm^2) than population K2 (84 cm^2). Increased percentage of shoot (14.1%) and root (10.4%) dry weight (DW) was recorded in seedlings treated with EC 8 dS m^{-1}, c. Population K2 had higher shoot (10.9%) and root (10.4%) DW percentage compared with cv. RR. In conclusion, the nutrient solution of EC 4 NaCl had negative effect on morphological characteristics, compared to the same solution without NaCl. Increased concentrations of NS significantly affected the leaf thickness (SLA) of *B. oleracea* acephala group seedlings. This can be used as production tool for seedlings hardening.

Key words

dry weight, leaf area, leaf gas exchange, nutrient solution, relative water content

[1] Institute for Adriatic Crops and Karst Reclamation, Split, Croatia
✉ e-mail: branimir@krs.hr
[2] University of Georgia, Department of Horticulture, Tifton, Georgia, USA
[3] Institute of Agriculture and Tourism, Poreč, Croatia,
Centre of Excellence for Biodiversity and Molecular Plant Breeding (CroP-BioDiv), Zagreb, Croatia
[4] University of Georgia, CAES Center for Agribusiness and Economic Development,
Athens, Georgia, USA

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Introduction

Salinity is one of the stress factors that affect the most vegetable production (Maiti et al., 2002). Osmotic and salt stress in the root zone, generally inhibit the growth of cultivated plants (Lin and Like, 2002). The same electrical conductivity (EC) value, achieved by different salt sources has different effects on plant height in melons (Nukaya et al., 1983). Cultivated plants, generally, had been selected for high productivity while their capacity for adaptation to growth changes is low (Parra et al., 2007). Also, the genotype may influence the different resistance to excessive salt concentration in the substrate. The plant growth depends on the degree of each of these factors, plant genotype and environmental conditions (Bayuelo-Jiménez et al., 2003). Leafy vegetables are sensitive to increased salinity in the soil. Shannon et al. (2000) found that yields of nine tested leafy vegetables were clearly reduced by increasing levels of salinity. The negative impact of salt stress on the plant growth can be used for growing lower and harder seedlings. Shannon and Grieve (1999) also indicated that the effect of increasing salt concentration is visible in the reduced growth of plants, which is manifested by smaller leaves, shorter stems, and sometimes less leaves. High levels of dissolved salts can damage the plant due to its deleterious effects on the function of roots and plant veins even when the substrate is adequately moistened (Granberry and Boyhan, 2003). The goal of this study was to determine the effect of salt stress on morphological and physiological changes of two genotypes of *B. oleracea* acephala group seedlings.

Material and methods

Seedlings of kale (*Brassica oleracea* var. *acephala* f. *sabellica*) cultivar Red Russian (RR) and collard (*Brassica oleracea* var. *acephala* f. viridis) Croatian population Konavle 2 (K2) were grown in a floating hydroponic system in a greenhouse in Tifton (31°28'N 83°31'E), Georgia, USA. During the experiment, average daily temperatures ranged from 27.8 to 32.7°C. The minimum daily temperature was between 21.7 to 25.6°C, while the maximum temperature values ranged from 35.3 to 43°C.

Seeds were sown on May 27 in polystyrene trays with 60 pots of 15 mL volume (30 pots per each genotype), which was 15, and that were filled with the peat-base medium (Pro-Mix, Quakertown, CA, USA). After sowing, trays were irrigated with tap water, and left in the shadow in environmental temperature conditions (day/night: 35/23°C) suitable for germination.

Seven days old seedlings, three centimeters high and with fully developed cotyledons, were transferred into tanks with 10 L of nutrient solution EC 2 dS m^{-1} (control NS, composition for kohlrabi, according to Sonneveld and Straver (1994) and pH 5.5). Additionally, six different nutrient solutions were made. Three NS were made by concentrated control NS to get EC 4, 6 or 8 dSm^{-1}, and three salt treatments included addition of NaCl solution (40 mmol NaCl solution without iodine) to the control NS to get EC 4 NaCl (2 NS + 2 NaCl), EC 6 NaCl (2 NS + 4 NaCl) and EC 8 NaCl (2 NS + 6 NaCl) dS m^{-1}. After trays were put in NS, during the four consecutive days concentration of nutrient solution was increased every 12 hours for EC 1 dS m^{-1} to achieve the required EC. Experiment was set up as randomized block design with three replications.

On 14th day after treatment started leaf gas exchange parameters were measured on first fully expanded leaf [stomatal conductance (g_{sw}), leaf intercellular CO_2 (C_i), photosynthetic rate (A), and transpiration (E)] using LI 6400 infrared gas analyzer (LI-COR, Inc., Lincoln, NE, USA). Relative water content (RWC) using method by Yamasaki and Dillenburg (1999), root, shoot and leaf dry weights (DW) after drying at 70°C to constant weigh were measured. Leaf area (LA) was determined with leaf area meter LI-3000 (LI-COR, Inc., Lincoln, NE, USA). Specific leaf area (SLA, cm^2 g^{-1}) was calculated by dividing the leaf area by leaf dry weight.

The data were analyzed by analysis of variance (ANOVA) using StatView statistical software (StatView for Windows; SAS Institute Inc. Copyright_ 1992-1998; Version 5.0). Following a significant F test, means were compared using the LSD test ($P \leq 0.05$).

Results and discussion

By increasing EC from EC 2 dS m^{-1} to EC 8 dS m^{-1} (regardless of the salt source) seedlings leaf gas exchange parameters were decreased (Table 1). High EC, especially the EC 8 NaCl dS m^{-1} had significantly lower C_i, as compared with values achieved with nutrient solution (Table 1). These results were in agreement with results of Morales-Garcia et al. (2008) who found that these physiological parameters decreased linearly as salinity increased. Intercellular CO_2 and transpiration were significantly higher in cv. Red Russian than in Konavle 2 population (Table 1). Dumičić et al. (2014) had opposite findings between these two genotypes grown under field condition. Probably cv. 'Red Russian' is less drought tolerant and high temperature conditions in field what is not case in hydroponic system. Sato et al. (2004) reported that photosynthetic rate was lower in stressed than in non-stressed cabbage seedlings. The EC level and genotypes had also interactive effect on photosynthetic rate and stomatal conductance. These parameters were more pronounced in Konavle 2 in combination with increased concentration of NS without NaCl and in cv. Red Russian with increased NS with NaCl (data not shown).

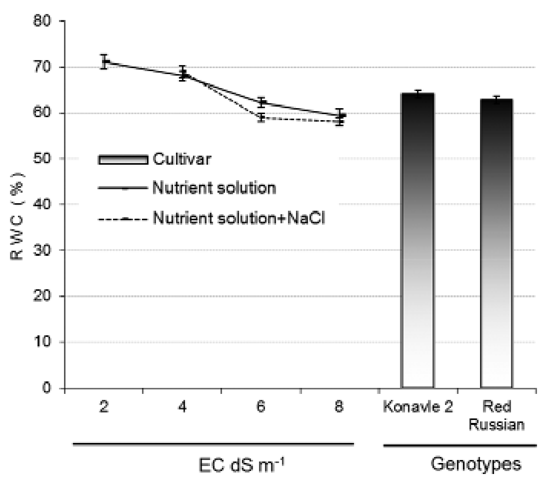
The RWC (EC level $P = <0.0001$; cultivar $P = 0.1569$) was between 58% and 71.1%, and decreased with increased EC level (Figure 1). The greater RWC values were recorded in seedlings treated with EC 2 and EC 4 dSm^{-1} (Figure 1), regardless of the salt source. Similar results were found by Salarizdah et al. (2012) on the leaves of canola plants treated with different salt levels and by Stepien and Klobus (2006) on cucumber seedlings.

By increasing solution EC from EC 2 to 8 dSm^{-1} (regardless of the salt source) seedling morphological parameters (root, shoot and leaf dry weight, leaves area and specific leaf area) were significantly decreased (Table 2). Salarizdah et al. (2012) also reported that fresh and dry weights of canola plants decreased with increased salinity. Seedlings treated with EC 4 NaCl and EC 6 NaCl dSm^{-1} had significantly less root weight than seedlings treated with same EC without NaCl (Table 2). Shoot DW of seedlings treated with EC 2 dSm^{-1} was not significantly different compared to seedlings treated with the EC 4 dSm^{-1}, while a significant difference was recorded for leaf DW (Table 2). The highest leaf DW and leaf area were recorded for seedlings treated with EC 2 dSm^{-1} (Table 2). A significant difference was recorded between the same EC treatments achieved with different sources of salt at EC 4 dSm^{-1} for both traits (Table 2). According to Colla et al. (2006) watermelon reduced leaf and stem DW when nutrient solution was of higher EC and NaCl addition. Results of our research were confirmed by Maggio et al. (2007) and Schwarz et al. (2002) who reported that regardless of the source of salinity the leaf area was decreased by increased EC

Table 1. Leaf gas exchange parameters: photosynthetic rate (A), stomatal conductance (g_{sw}), intercellular CO_2 (C_i) and transpiration (E) of two genotypes of *B. oleracea* acephala group grown with seven nutrient solutions (NS).

Treatment	Gas exchanges parameters			
	A (µmol $m^{-2}s^{-1}$)	g_{sw} (mol $m^{-2}s^{-1}$)	C_i (µmol mol^{-1})	E (mmol H_2O $m^{-2}s^{-1}$)
EC[a]				
2 NS[b]	29.5	0.85a[d]	311.2a	13.40ab
4 NS	30.6	0.91a	311.9a	14.70a
2+2 NaCl[c]	32.1	0.77ab	297.2ab	13.92ab
6 NS	30.7	0.65bcd	285.4b	13.13ab
2+4 NaCl	30.7	0.67bc	288.5b	13.72ab
8 NS	28.4	0.60cd	286.8b	12.28bc
2+6 NaCl	27.5	0.49d	262.4c	10.68c
Genotype (Gt.)				
'Konavle 2'	31.0	0.68b	285.5b	12.18b
'Red Russian'	28.9	0.72a	298.3a	14.05a
Significance				
EC	0.464	<0.0001	<0.0001	0.0006
Gt.	0.119	0.217	0.011	<0.0001
EC ×Gt.	0.0006	<0.0001	0.359	0.733

[a] EC 2-8 dS m^{-1}; concentrated nutrient solution or nutrient solution with NaCl; [b] NS; treatment achieved with standard nutrient solution; [c] NaCl; treatment achieved with EC 2 NS and NaCl; [d] Mean values (n = 3) followed by different letters in each column indicate significant differences according to *LSD* test ($P \leq 0.05$).



of nutrient solution in different vegetable species. The leaf thickness (SLA) was the lowest in both EC 8 treatments. Bayuelo-Jiménez et al. (2003) also stated that the SLA decreased in plants under salt stress. Cultivar Red Russian had greater root, shoot and leaf DW and leaf area than population Konavle2 (Tables 2). The interaction of genotype and different salt sources on the seedlings leaf area was recorded. Leaf area of Konavle 2 population was smaller compared to cv. Red Russian on all EC levels, except on EC 8 regardless of salinity source.

Figure 1. Relative water content (RWC; %) of two genotypes of *B. oleracea* acephala group treated with seven different nutrient solutions of EC from 2 to 8 dS m-1 (four achieved by concentrating the nutrient solution and three by adding NaCl in a standard nutrient solution). Vertical bar indicates mean ±1 SE.

Table 2. Growth characteristics: root, shoot and leaf dry weight (g), leaf area (cm^2) and specific leaf area (SLA; cm^2/g) of two genotypes of *B. oleracea* acephala group treated with seven nutrient solutions (NS).

Treatment	Dry weight			Leaf area	Specific leaf area
	Root	Shoot	Leaf		
EC[a]					
2 NS[b]	0.15a[d]	0.90a	0.74a	186.7a	256.6a
4 NS	0.14ab	0.79a	0.66b	150.8b	240.3a
2+2 NaCl[c]	0.10c	0.62b	0.51c	118.9c	233.8a
6 NS	0.12bc	0.65b	0.54c	121.1c	236.7a
2+4 NaCl	0.08d	0.55b	0.47c	103.8c	232.6a
8 NS	0.06e	0.34c	0.28d	48.1d	175.2b
2+6 NaCl	0.07de	0.31c	0.26d	46.7d	180.5b
Genotype					
Konavle 2	0.09b	0.49b	0.38b	83.5b	221.6
Red Russian	0.11a	0.66a	0.59a	130.6a	219.5
Significant					
EC	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cv.	0.0286	<0.0001	<0.0001	<0.0001	0.68
EC × Cv.	0.458	0.316	0.109	0.011	0.47

[a] EC 2-8 dS m^{-1}; concentrated nutrient solution or nutrient solution with NaCl; [b] NS; treatment achieved with standard nutrient solution; [c] NaCl; treatment achieved with EC 2 NS and NaCl; [d] Mean values (n = 3) followed by different letters in each column indicate significant differences according to *LSD* test ($P \leq 0.05$).

In conclusion, increased concentration of nutrient solution from EC 2 to EC 8, regardless of the salt source, negatively influenced the morphological and physiological changes of *B. oleracea* acephala group seedlings. The nutrient solution of EC with NaCl had higher negative effect on morphological characteristics compared to EC achieved without NaCl.

Increased concentrations of NS significantly affected the leaf thickness (SLA) of *B. oleracea* acephala group seedlings. This can be used as production tool for seedlings hardening.

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