



The effect on photosynthesis and osmotic regulation in *Beta vulgaris* L. var. *Flavescens* DC. by salt stress

Deok-Gyun Choi¹, Jeong-Sook Hwang¹, Sung-Chul Choi¹, Sung-Hwan Lim¹, Jong-Guk Kim² and Yeon-Sik Choo^{1,*}

¹Department of Biology, Kyungpook National University, Daegu 41944, Korea

²Department of Life Science and Biotechnology, Kyungpook National University, Daegu 41944, Korea

Abstract

This study was to investigate the effect of salt stress on physiological characteristics such as plant growth, photosynthesis, solutes related to osmoregulation of *Beta vulgaris*. A significant increase of dry weight was observed in 50 mM and 100 mM NaCl. The contents of Chl a, b and carotenoid were lower in NaCl treatments than the control. On 14 day after NaCl treatment, photosynthetic rate (P_N), the transpiration rate (E) and stomatal conductance of CO_2 (g_s) were reduced by NaCl treatment. On 28 day after NaCl treatment, the significant reduction in g_s and E was shown in NaCl 200 mM. However, P_N and water use efficiency (WUE) in all NaCl treatments showed higher value than that of control. Total ion contents (TIC) and osmolality were higher than the control. On 14 day after treatment, the contents of proline (Pro) increased significantly in 200 mM and 300 mM NaCl concentration compared with control, whereas on 28 day in all treatments it was lower than that of the control. The contents of glycine betaine (GB) increased with the increase of NaCl concentration. The contents of Na^+ , Cl^- , GB, osmolality and TIC increased with the increase of NaCl concentrations. These results suggested that under severe NaCl stress conditions, NaCl treatment did not induce photochemical inhibition on fluorescence in the leaves of *B. vulgaris*, but the reduction of chlorophyll contents was related in a decrease in leaf production. Furthermore, increased GB as well as Na^+ and Cl^- contents resulted in a increase of osmolality, which can help to overcome NaCl stress.

Key words: chlorophyll fluorescence, osmotic regulation, photosynthesis, proline, salt stress

INTRODUCTION

Many parts of the world are effected by salt (Munns and Tester 2008), which is known as one of the main environmental constraints that are affecting the productivity of crops and plants (Munns 2002). Among various environmental stresses, excessive salinity in soil and groundwater suppresses plant growth, causes decrease in net photosynthesis, reduces vitality of enzymes that control metabolism, and accelerates production of harmful active oxygen species in plant cells (Cheeseman 1988, Bor-

sani et al. 2001, Gill and Tuteja 2010). Salt constrains plant growth largely in three ways. The first is water deficiency caused by reduced water potential in soil around roots. The second is toxic effect of ions caused by excessive absorption of Na^+ and Cl^- . The last is ionic imbalance due to constraints in transportation and absorption of ions (Marschner 1995). Adaptive response of plants to salt can be divided broadly into the exclusion or accumulation of salts. Salt exclusion can be observed mainly in monocot

<http://dx.doi.org/10.5141/ecoenv.2016.009>



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Received 24 August 2015, Accepted 23 November 2015

*Corresponding Author

E-mail: yschoo@knu.ac.kr

Tel: +82-53-950-5346

www.kci.go.kr

plants while salt accumulation can be observed normally in dicotyledonous halophytes. Most halophytes survive by developing resistance to salt stress through two methods. One method is to remove accumulated salt from their bodies by forming salt glands as well as trichomes, special epidermal structure. The other is to dilute absorbed salt ion by increasing water content through the shape and structural changes of cell, and by accumulating absorbed salt in vacuole (Reimold and Queen 1974, Jefferies et al. 1979, Reimann and Breckle 1988, Breckle 1990). Species resistant to saline conditions increase concentration of compatible solutes in cytoplasm to carry out a process called osmoregulation, which plays a crucial role in the adaptation of the plants under saline environment. Representative compatible solutes are amino acids (esp. proline, Pro), betaines, low-molecular-weight carbohydrates, and sugar alcohols (Stumpf 1984, Smirnov 1993). Under salt or water stress, many Chenopodiaceae plants are known to accumulate Pro and glycine betaine (GB) used as osmolytes for osmoregulation (Storey and Jones 1979). Salt accumulation in irrigated soil especially in arid and semiarid region is known as the primary factor of reduced crop productivity (Serrano 1996, Volkmar et al. 1998). In general, decreased crop productivity due to various environmental stress including salt is related to decrease in photosynthetic performance (Ziska et al. 1990). Salt reduces the photosynthetic ability of plant, by affecting one or more processes in connection with photosynthesis. Environmental stresses decrease light energy that plants use (Singh and Dubey 1995), reduce the amount of chlorophyll or destroy the structure of chloroplast, damage photosystem II (Downton et al. 1985), reduce stomatal conductance by closing stomatal pore and eventually reduce the amount of fixed CO₂ (Seemann and Sharkey 1986, Singh and Dubey 1995).

This research was performed to investigate the physiological changes of leaf beet (*Beta vulgaris*) through growth response, solute pattern and photosynthetic activity as a part of adaption under saline conditions.

MATERIAL AND METHODS

Plant material and growth conditions

Seeds of *B. vulgaris* L. var. *flavescens* DC. were purchased from Danong Co. in Korea. After washing 5-6 times the seeds of *B. vulgaris* in distilled water, they were allowed to germinate for 5 days at 26°C incubator. Germinated plants were transferred to a plastic pot (diameter

10 cm x height 15 cm) filled with vermiculite. This experiments were conducted in greenhouse where mean temperature was maintained about day (14 h, 25°C), night (10 h, 18°C). Plants were irrigated 200 ml with Hoagland's solution and four different NaCl concentrations (0, 50, 100, 200 and 300 mM) every day for 45 days.

Measurement of plant water content and inorganic ions

After measuring the fresh weight (FW), leaf samples were dried in an oven at 70°C for 3 days to determine dry weight (DW). Plant water (PW) content was determined as the difference between the FW and DW. The dried plant material was ground to a homogeneous powder and extracted with 95°C distilled water for 1 h, then the sample was filtered with a GF/A filter (4.7 cm, Whatman, UK). Inorganic cations (Na⁺, K⁺, Ca²⁺ and Mg²⁺) were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Optima7300DV; Perkin Elmer, Waltham, MA, USA). The chloride content was measured using a chloride titrator (Titrators DL 50; Mettler Toledo Inc., Columbus, OH, USA).

Measurement of total ions

Total ion contents (calculated as NaCl equivalents) were determined using a electrical conductivity instrument (Mettler Check Mate 90; Mettler Toledo Inc.).

Measurement of water-soluble carbohydrates and osmolality

Total soluble carbohydrates of the plants were assayed using the phenol-sulfuric acid method (Chaplin 1994). 20 µL of plant extract was mixed with 580 µL of distilled water, 400 µL of 5% phenol, and 400 µL of sulfuric acid, and the solution was allowed to stand for 10 min before being shaken vigorously. Total carbohydrates were quantified by determining the absorbance at 490 nm by using a UV mini 1240 spectrophotometer (Shimadzu, Kyoto, Japan) after 30 more minutes. Glucose (2–40 µg in 200 µL) was used as a standard solution.

Osmolality was measured by cryoscopy using an Micro-Osmometer 3MO (Advanced Instruments, Needham Heights, MA, USA).

Measurement of Pro

Dried leaves (0.5g) were extracted in 3% sulphosala-

icylic acid and the homogenate filtered through a 0.45 μm pore size CF/C (Whatman). Filtrate of 2 ml was reacted with 2 ml of acid ninhydrin reagent and and glacial acetic acid of 2 ml in a test tube for 1 h at 100°C and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml of toluene and mixed vigorously with a vortex mixture for 15-20s. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance measured at 520 nm using toluene as blank. Pro concentration was calculated from a standard curve using L-Pro (Bates et al. 1973).

Measurement of GB

The extract was diluted with 2N H_2SO_4 (1 : 1 / v) and 0.5 mL of the acidified extract was cooled in ice water for 1 h. Later, 0.2 mL of cold potassium tri-iodide solution was added and mixed by vortex, and the tubes were stored at 4°C for 15 min and centrifuged at 10,000 rpm for 15 min. The supernatant was aspirated with a fine-tipped glass tube. The periodide crystals were dissolved in 9 mL of 1,2-dichloroethane with vigorous shaking. After 2.5 h, the absorbance was determined at 365 nm in a spectrophotometer. GB (200 μg in 1 N H_2SO_4 1000 mL) was used as a standard solution (Grieve and Grattan 1983).

Leaf gas-exchange measurements

Photosynthesis of the plant leaves was determined using a LCi Portable Photosynthesis System (ADC BioScientific LTD, Hertfordshire, UK), and then was stabilized for five minutes to one-minute interval was measured five times.

Chlorophyll fluorescence

Before measuring chlorophyll fluorescence, leaves were put in dark-adapted state for 20 min using light exclusion clips and then minimal fluorescence in dark-adapted leaves (F_0), maximal fluorescence (F_m), maximum photochemical quantum yield of photosystem II (F_v/F_m), effective photochemical quantum yield of photosystem II ($Y(II)$), quantum yield of light-induced non photochemical fluorescence quenching ($Y(NPQ)$) and quantum yield of non-regulated heat dissipation and fluorescence emission ($Y(NO)$) were measured using a Portable Chlorophyll Fluorometer PAM-2500 (Heinz Walz GmbH, Effeltrich, Germany).

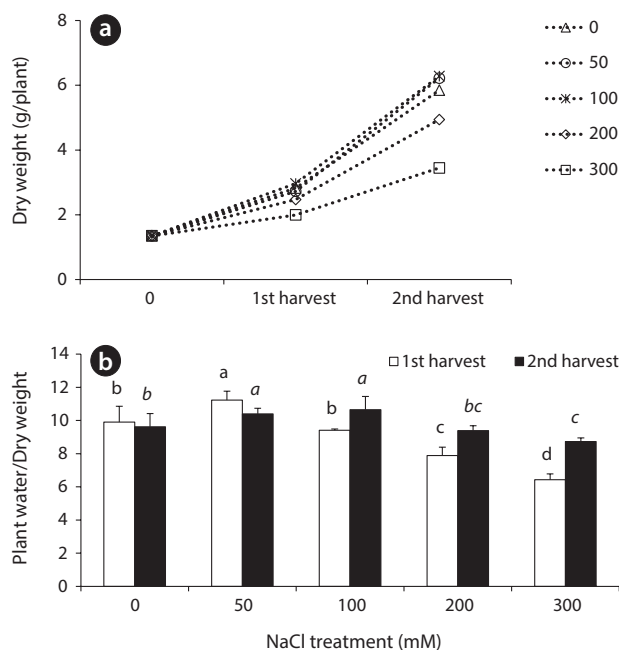


Fig. 1. Effect of NaCl treatments (0, 50, 100, 200 and 300 mM) on (a) leaf dry weight and (b) the ratio of plant water to dry weight in leaves of *Beta vulgaris*. Data represent mean value of five replicates with standard deviation. The different letters indicate significant differences among treatments tested with one-way ANOVA and Duncan's multiple range test ($P < 0.05$).

Measurement of chlorophyll contents

The leaf samples for chlorophyll and carotenoid were extracted by dimethyl sulfoxide (DMSO) at 60°C for 24 h. The contents of chlorophyll a, chlorophyll b and carotenoid were estimated from absorbance at 645 nm, 663 nm and 480 nm with UV mini-1240 spectrophotometer (Shimadzu).

Statistical analyses

Data were analyzed by analysis of variance (ANOVA) using SPSS ver. 19.0 (SPSS Inc., Chicago, IL, USA). Graphs show means with standard deviation (SD). A Duncan's multiple range test was carried out to determine significant differences ($P < 0.05$, $N = 5$) among the groups.

RESULTS AND DISCUSSION

Growth and plant water content

DW change and the ratio of plant water content to DW in beet leaves after salt treatments are shown in Fig. 1. A great difference could not be observed in dry weight be-

tween the control and treatment groups until after the first 14 days salt treatments. However, DW showed a significant difference at 28 days after salt treatment. The DW of the two treatments of 50 mM and 100 mM NaCl were more than that of the control. When treated with 200 mM NaCl, however, DW was slightly lower than the control, and plant growth was significantly inhibited at 300 mM NaCl treatment (Fig. 1). Until 14 days after treatment, the ratio of plant water content to DW in beet leaves was increased at 50 mM NaCl and then gradually decreased, and showed the lowest value at the 300 mM treatment. Over 100 mM NaCl treatments, the ratio of plant water content to DW in leaves of the 28 day treatment increased due to continued salt stress, compared to those given the 14 day treatment (Fig. 1).

Compared with most crops which generally exhibit low salt tolerance, many chenopodiaceous plants possess a somewhat higher salt tolerance (Choi et al. 2014). For example, *Suaeda japonica* and *Salicornia herbacea* belonging to the Chenopodiaceous plants that grow on salt marshes displayed high salt resistance even at 400 mM NaCl treatments, showing an increase of DW (Kim et al. 2002). In the case of salt sensitive plants, a rapid decrease in the DW and fresh weight of roots and leaves was observed at 200 mM NaCl (Greenway and Munns 1980). Sugar beets, which belong to the same genus such as beets showed sensitive growth response with the increase of salinity during germination and initial stages of growth. However, it is known to possess somewhat salt resistance, showing high ability of osmotic adjustment though the accumulation of solutes such as inorganic ions, Pro and GB (Gzik 1996, Ghoulam and Fares 2001). The beets used in this research also displayed a growth response similar to the control group even under 200 mM NaCl treatments.

It is considered that beets overcome salt stress without a definite inhibition to growth up to 200 mM.

Chlorophyll fluorescence

In the NaCl treated group, a large difference was not observed in F_0 , F_m and F_v/F_m , which represent quantum yield of photosystem II's photochemical reaction to maximum photosynthesis (Table 1). Chlorophyll fluorescence and electron transport in leaves of beets were hardly affected by salt treatments, displaying a high F_v/F_m value of over 0.81 in all experimental groups treated with NaCl.

F_v/F_m is used not only to measure photosynthetic electron transport ability, but also as an indicator of environmental stresses (Schansker and van Rensen 1999). The F_v/F_m ratio of plants in stress-free environments is generally known to be 0.75-0.85 (Peterson et al. 1988). Typically plants tend to show an increase of F_0 and decrease of F_m when they are stressed. However, stressed plants possess a mechanism that relaxes the energy of excited electrons derived from chlorophyll by releasing the energy as a form of heat and light in order to suppress the generation of active oxygen species, which brings it back to the ground state. In this way, the photosynthetic apparatus in leaves is protected. This is the photoprotection mechanism of plants, called non-photochemical quenching (NPQ) (Niyog 1999). The results of this experiment did not show any significant difference between the control and experimental groups in terms of the values of F_0 , F_m , F_v/F_m , $Y(NO)$, $Y(II)$ and $Y(NPQ)$. Judging from the results, NaCl treated beet plants perform photosynthesis effectively without significant salt effect. It is considered that beets under salt stress is not damaged to photosystem.

Table 1. Characteristics of chlorophyll fluorescence (F_0 , F_m , F_v/F_m , $Y(II)$, $Y(NPQ)$ and $Y(NO)$) under NaCl stress.

NaCl (mM)	Harvest	F_0	F_m	F_v/F_m	$Y(II)$	$Y(NPQ)$	$Y(NO)$
0	1st	0.22 ± 0.00	1.23 ± 0.05	0.82 ± 0.01	0.53 ± 0.04	0.16 ± 0.05	0.31 ± 0.03
	2nd	0.21 ± 0.02	1.15 ± 0.13	0.82 ± 0.01	0.52 ± 0.10	0.18 ± 0.05	0.31 ± 0.06
50	1st	0.21 ± 0.01	1.18 ± 0.02	0.82 ± 0.00	0.45 ± 0.06	0.28 ± 0.08	0.27 ± 0.03
	2nd	0.22 ± 0.01	1.22 ± 0.02	0.82 ± 0.01	0.47 ± 0.03	0.24 ± 0.04	0.29 ± 0.02
100	1st	0.21 ± 0.02	1.16 ± 0.07	0.82 ± 0.01	0.49 ± 0.09	0.24 ± 0.08	0.28 ± 0.02
	2nd	0.21 ± 0.01	1.17 ± 0.04	0.82 ± 0.01	0.43 ± 0.03	0.28 ± 0.02	0.28 ± 0.02
200	1st	0.20 ± 0.02	1.10 ± 0.10	0.82 ± 0.01	0.54 ± 0.06	0.19 ± 0.08	0.27 ± 0.02
	2nd	0.22 ± 0.02	1.18 ± 0.05	0.82 ± 0.01	0.47 ± 0.05	0.27 ± 0.05	0.26 ± 0.01
300	1st	0.20 ± 0.01	1.10 ± 0.07	0.82 ± 0.00	0.53 ± 0.07	0.22 ± 0.07	0.25 ± 0.01
	2nd	0.20 ± 0.01	1.05 ± 0.09	0.81 ± 0.01	0.59 ± 0.04	0.17 ± 0.04	0.25 ± 0.01

F_0 , minimal fluorescence in dark-adapted leaves; F_m , maximal fluorescence in dark-adapted leaves; F_v/F_m , maximum photochemical quantum yield of PS II; $Y(II)$, effective photochemical quantum yield of PS II; $Y(NPQ)$, quantum yield of light-induced non-photochemical fluorescence quenching; $Y(NO)$, quantum yield of non-regulated heat dissipation and fluorescence emission.

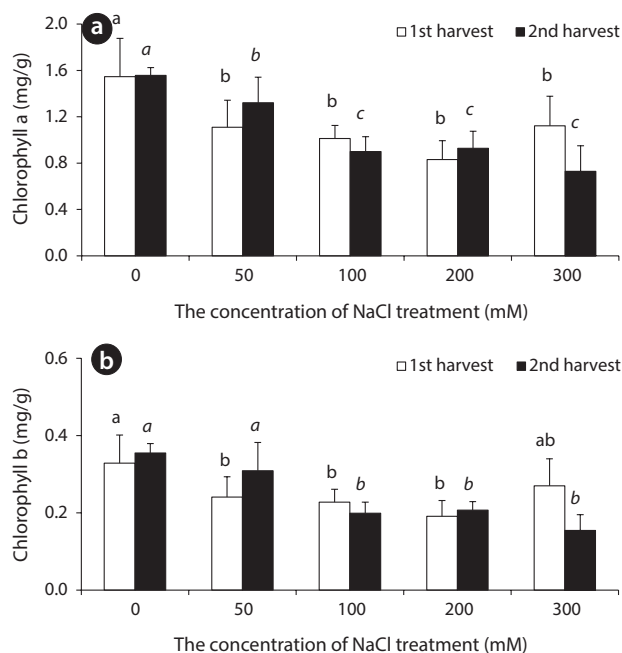


Fig. 2. Effect of NaCl treatments (0, 50, 100, 200 and 300 mM) on (a) chlorophyll a and (b) chlorophyll b contents in leaves of *Beta vulgaris*. Data represent mean value of five replicates with standard deviation. The different letters indicate significant differences among treatments tested with one-way ANOVA and Duncan's multiple range test ($P < 0.05$).

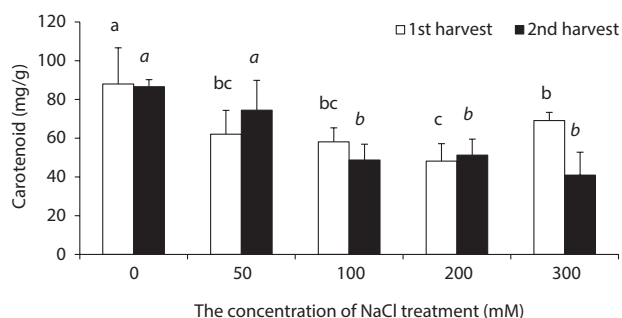


Fig. 3. Effect of NaCl treatments (0, 50, 100, 200 and 300 mM) on carotenoid contents in leaves of *Beta vulgaris*. Data represent mean value of five replicates with standard deviation. The different letters indicate significant differences among treatments tested with one-way ANOVA and Duncan's multiple range test ($P < 0.05$).

Chlorophyll a, b and carotenoid content

Chlorophyll a (Chl a) content of beets displayed a tendency to decrease in accordance with the increase of salinity. With lengthened treatment time, a difference in content was not observed up to 200 mM, but a decrease in Chl a content was observed at 300 mM. Up until the 14th day, Chl b content decreased somewhat, but did not display a great change. However, a decrease was observed

at concentrations over 100 mM on the 28th day (Fig. 2).

Carotenoid is known to play two roles in photosynthesis: light harvesting and photoprotection (Hopkins and Hüner 2004). With respect to concentration level of NaCl treatment, carotenoid content was lower in all experimental groups than in control groups (Fig. 3). In general, chlorophyll content decreased in leaves of NaCl-stressed plants (Meloni et al. 2003). Decreased Chl a, Chl b and carotenoid contents in leaves of beets treated with NaCl perhaps indicate that NaCl hinders the synthesis of chlorophyll and carotenoid in beet.

Photosynthesis

With the increase of NaCl concentration, both stomatal transpiration rate and stomatal conductance showed a tendency to decrease significantly, while water use efficiency displayed a tendency to increase (Fig. 4). Transpiration usually occurs through stomata, so that the degree of stomatal opening and closing is of absolute importance in water retention. Stomatal conductance, which is diffusion of water into the atmosphere, is influenced by many environmental factors such as light intensity, vapor pressure deficit, concentration of carbon dioxide, temperature and relative humidity (Sim and Han 2003). With decreased stomatal conductance, stomatal transpiration rate decreases and photosynthetic performance is affected. Photosynthesis in higher plants became especially affected when plant was provided with less than available water. Thus, abscisic acid (ABA) content in plants is increased. With the change in ABA content, stomata begins to close up, which affects the absorption of carbon dioxide (Salisbury and Rose 1992, Yu and Bae 2004).

Fourteen days after NaCl treatment, an obvious difference in transpiration rate and stomatal conductance between control group and experimental groups was observed. The difference of the 1st harvest was more significant than the 28th day. With NaCl treatment, a decrease in transpiration rate and stomatal conductance appeared before photosynthesis. As a result, the beets decreased stomatal conductance and transpiration rate in order to reduce water loss within the plant body under NaCl stress. Therefore, appropriate water-carbon dioxide exchange could not take place, hindering photosynthetic performance.

Osmotic concentration, total ion and inorganic cation content

Ion uptake and compartmentalization are crucial not

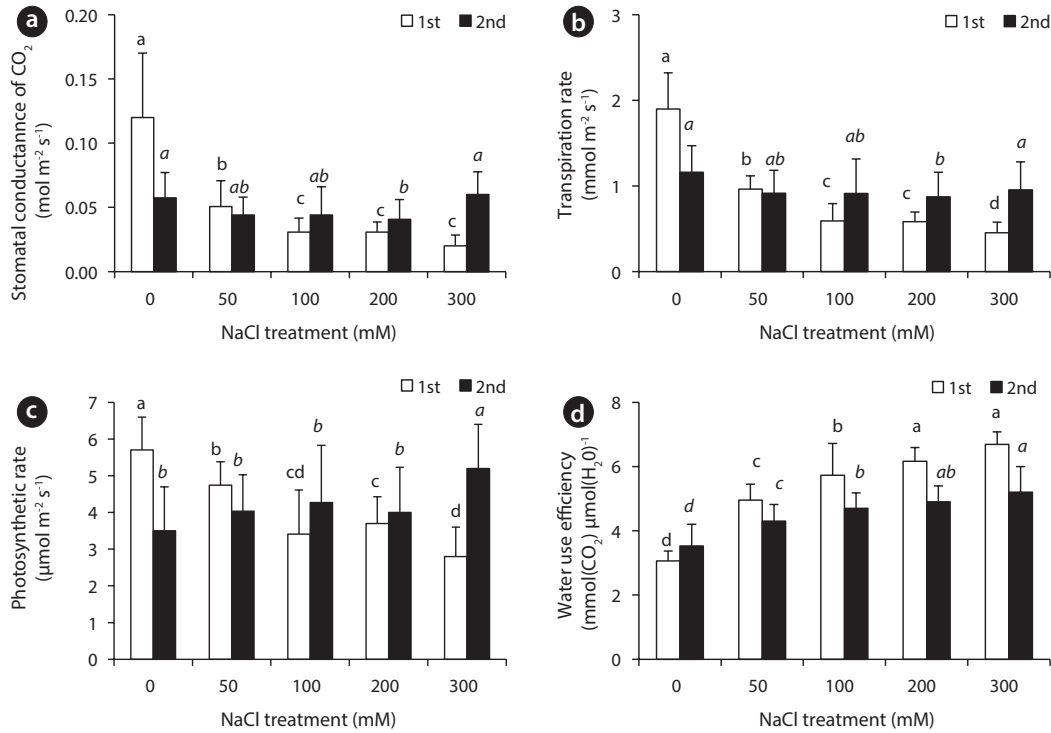


Fig. 4. Effect of NaCl treatments (0, 50, 100, 200 and 300 mM) on (a) stomatal conductance of CO₂ (gs), (b) transpiration rate (E), (c) photosynthetic rate (A) and (d) water use efficiency (WUE) in leaves of *Beta vulgaris*. Data represent mean value of five replicates with standard deviation. The different letters indicate significant differences among treatments tested with one-way ANOVA and Duncan's multiple range test ($P < 0.05$).

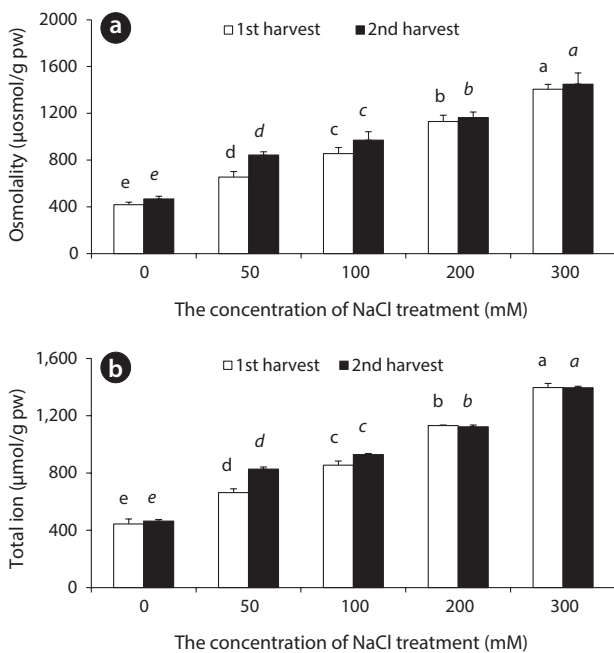


Fig. 5. Effect of NaCl treatments (0, 50, 100, 200 and 300 mM) on (a) osmolality(μosmol/g plant water) and (b) total ions concentrations (μmol/g plant water) in leaves of *Beta vulgaris*. Data represent mean value of five replicates with standard deviation. The different letters indicate significant differences among treatments tested with one-way ANOVA and Duncan's multiple range test ($P < 0.05$).

only for normal growth but also for growth under saline condition (Adams et al. 1992) because the stress disturbs ion homeostasis. The total osmotic concentration and ion content accumulated in beet leaves increased with an increase in salinity (Fig. 5). This had a connection to an increased influx of Na⁺ and Cl⁻ ions. In order to maintain water potential of leaves under saline conditions, the beet increases osmotic concentration in the body. The study shows that in the beginning of salt stress, inorganic ions constituted the majority of osmotic concentration, and then, organic solutes such as GB were accumulated in the plant body. *S. asparagoides* and *S. maritime*, which are also Chenopodiaceous plants that grow in salt marshes, are known to use mainly inorganic ions such as Na⁺ and Cl⁻ in order to appropriately maintain osmotic concentration of leaves in accordance with the environment they inhabit (Choi et al. 2012). Beets, which belong to the Chenopodiaceous plant family, are also thought to accumulate mainly Na⁺ and Cl⁻ to use in osmotic adjustment as a response to NaCl. Inorganic cation content of beet leaves treated with NaCl is shown in Fig. 6. The control group accumulated mainly K⁺ ion among inorganic cations. With an increase in NaCl concentration and treatment time, cation content in beet leaves increased. Mainly, Na⁺ ions accumulated in

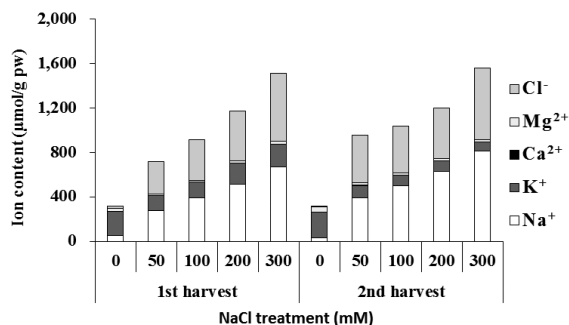


Fig. 6. Effect of NaCl treatments (0, 50, 100, 200 and 300 mM) on the inorganic cation concentrations ($\mu\text{mol/g}$ plant water) in leaves of *Beta vulgaris*. Data represent mean values.

the plant body. 14 days after saline treatment, Na^+ ion content in the plant body tended to increase, however, a great change was not observed in inorganic cation content including that of K^+ ions. 28 days after NaCl treatment, a remarkable increase in Na^+ ion content was observed. However, a great difference was not observed in content between experimental groups. Due to noticeable increase in Na^+ ions in experimental groups treated with 300 mM NaCl, K^+ ion content tended to display a relative decrease.

High salt (NaCl) uptake competes with the uptake of other nutrient ions, especially K^+ , leading to K^+ deficiency. Increased treatment of NaCl induces increase in Na^+ and Cl^- and decrease in Ca^{2+} , K^+ , and Mg^{2+} levels in a number of plants (Khan et al. 1999, Aziz and Khan 2001). In plants that cannot remove salt out of the body, absorption of K^+ and Ca^{2+} ions is hindered under environments with high concentrations of NaCl. At the same time, growth is hindered by the imbalance in inorganic nutrients and ion toxicity caused by excessive influx of Na^+ ion (Ashraf et al. 1994). In some plants, excessive Ca^{2+} ion content in the plant body can have a harmful effect, so a low Ca^{2+} concentration is usually maintained in the cytoplasm. Chenopodiaceous plants are especially known to accumulate only a small amount of Ca^{2+} ions in the plant body (Choo and Song 1998). Plants adapted to saline environments possess various physiological control mechanisms: In order to avoid NaCl hinderance, plants dilute salt accumulated in cells by increasing water content (succulents), emit it through salt glands, accumulate in trichome, accumulate to the roots or old leaves, and store it in vacuoles (Shabala and Mackay 2011). Sugar beets, a Chenopodiaceous plant, accumulates a great amount of K^+ and Na^+ to use for osmotic adjustment during early stages of its growth. As it grows, it gradually dilutes accumulated ion concentration by increasing water content within the plant body (Kenter

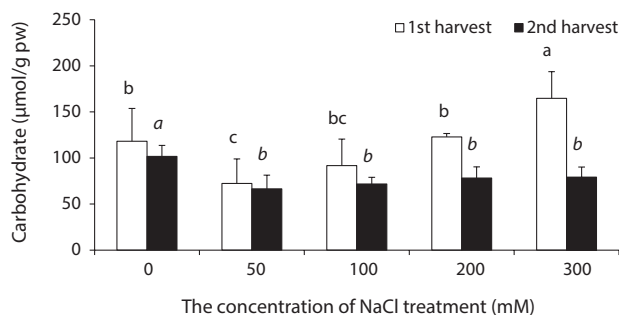


Fig. 7. Effect of NaCl treatments (0, 50, 100, 200 and 300 mM) on carbohydrate concentrations ($\mu\text{mol/g}$ plant water) in leaves of *Beta vulgaris*. Data represent mean value of five replicates with standard deviation. The different letters indicate significant differences among treatments tested with one-way ANOVA and Duncan's multiple range test ($P < 0.05$).

and Hoffmann 2006). As a response to NaCl stress, beets overcome ion toxicity by appropriate compartmentalization of Na^+ ions accumulated in the leaf. At the same time, beets increase water content and maintain a balance between Na^+ ions and K^+ ions in leaves.

Soluble carbohydrate content

Beets contained a small amount of soluble carbohydrates. The smallest change in soluble carbohydrate content was observed in experimental groups treated with 50 mM NaCl, while the greatest change was observed in experimental groups treated with 300 mM NaCl (Fig. 7). As a response to drought or NaCl stress, most plants accumulate soluble carbohydrates such as sucrose, Gramineous crops and Cyperaceous plants are especially known to contain a great amount of sugar (Albert and Popp 1978, Stewart et al. 1979, Huetterer and Albert 1993, Choo and Albert 1999). A physiological characteristic of *Salsola collina*, a Chenopodiaceous plant that grows in coastal sand dunes, is that it holds more soluble carbohydrates compared to other Chenopodiaceous plants (Popp and Smirnoff 1995, Choo and Albert 1999, Choi et al. 2004). Sugar beet accumulated a great amount of sugar in its roots in early stages of growth; and as it grows, the amount continuously increased (Kenter and Hoffmann 2006). In this study, the sugar concentration of beet increased somewhat under NaCl environments, but a small amount of soluble carbohydrates was observed in general. The reason why a great change in sugar content was not observed with NaCl treatment is because carbohydrates are not considered to be the beet's main osmolytes.

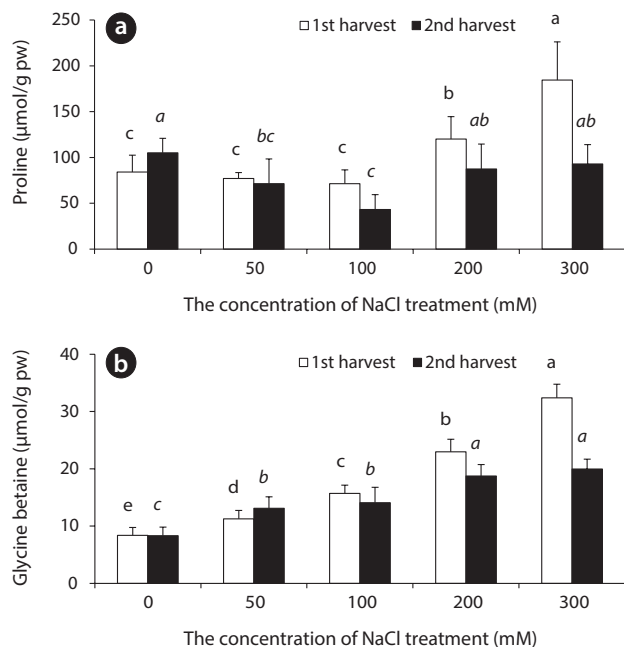


Fig. 8. Effect of NaCl treatments (0, 100, 200 and 300 mM) on (a) proline and (b) glycine betaine concentrations ($\mu\text{mol/g}$ plant water) in leaves of *Beta vulgaris*. Data represent mean value of five replicates with standard deviation. The different letters indicate significant differences among treatments tested with one-way ANOVA and Duncan's multiple range test ($P < 0.05$).

Pro and GB content

In NaCl treated beets, changes in Pro content did not show in the control group, nor did they appear in experimental groups treated with up to 50–100 mM NaCl, but beets showed an increase in content at NaCl concentration of 200 mM and 300 mM on the 14th day. On the 28th day, a great difference in Pro content between all experimental groups, and the control group was not observed. In comparison, GB content following saline treatment showed an increase in accordance with an increase in salinity (Fig. 8).

Pro, which is known as one of the compatible solutes, is accumulated as a response to water deficiency as well as salt and low temperature stress in higher plants, and this accumulation plays an important role in osmotic adjustment. Also, Pro assists in protecting cells from many negative effects caused by drought (Binzel et al. 1987, Voetberg and Sharp 1991, Rhodes and Hanson 1993). GB, which is known as a cytoplasmic osmolyte for osmotic adjustment, is accumulated in the cytoplasm as a response to stress such as salt, cold, heat and drought in many plants. Even though a great amount of GB may accumulate in the cytoplasm under osmotic stress, it does

not hinder biochemical responses. Rather, it is used to maintain cell membrane, to stabilize cell structure, and to maintain photosynthetic efficiency (Wang and Showalter 2004). The accumulation of GB not only has a connection to NaCl resistance, but is known to increase NaCl resistance in plants that do not form GB when the supply of GB from the outside (Harinasut et al. 1996). In sugar beets and spinach, which are Chenopodiaceous plants, formation of GB in chlorophyll is catalyzed by choline mono-oxygenase (CMO) and betaine aldehyde dehydrogenase (BADH) enzymes as a response to various stress factors such as drought and NaCl, and is accumulated in the cytoplasm (Sakamoto and Murata 2002). In beets, GB is accumulated for osmotic adjustment with accumulation of inorganic ions such as Na^+ and Cl^- in the plant body. Thus, GB is thought to be the main cytoplasmic osmolyte for osmotic adjustment in regards to NaCl stress in beets.

These results suggested that under severe NaCl stress conditions, NaCl treatment did not induce photochemical inhibition on fluorescence in the leaves of *B. vulgaris*, but the reduction of chlorophyll contents was related in a decrease in leaf production. Furthermore, increased GB as well as Na^+ and Cl^- contents resulted in an increase of osmolality, which can help to overcome NaCl stress. In conclusion, beet overcomes salt stress without a definite hindrance to growth in NaCl environments with up to 200mM NaCl by accumulating GB and with effective accumulation of inorganic ions.

By understanding the physiological characteristic of beet under salt stress, it may give a clue to solve salt accumulation problem in agricultural land.

ACKNOWLEDGEMENTS

This work was supported by the Eco-Innovation project of the Ministry of Environment, Korea.

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