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Effect of osmotic stress on growth and osmolytes accumulation in sugar beet (*Beta vulgaris* L.) plants

G.-Q. Wu, R.-J. Feng, Q.-Z. Shui

School of Life Science and Engineering, Lanzhou University of Technology, Lanzhou, P.R. China

ABSTRACT

To investigate the effects of osmotic stress on plant growth, and ions and compatible solutes accumulations of sugar beet (*Beta vulgaris* L.), in the present study, two-month-old plants were subjected to different degrees of osmotic stress (–0.5, –1.0, and –1.5 MPa) induced by sorbitol for 7 days. The results showed that fresh weight and water content in both leaf blade and leaf petiole significantly decreased by osmotic stress. With the increase of osmotic stress, Na^+ concentration in leaf blade showed the significantly increasing trend. However, osmotic stress significantly reduced K^+ concentration in lateral root. It was observed that osmotic stress of –1.5 MPa remarkably increased sucrose accumulation in storage root compared to control. In addition, plants accumulated more sucrose and fructose in storage root than in other tissues. Proline concentrations in leaf blade, leaf petiole and storage root significantly increased by osmotic stress of –1.0 MPa and –1.5 MPa; in leaf blade it was to a higher degree than in leaf petiole and storage root. These results suggested that sugar beet plants can adapt to osmotic stress by accumulating more osmolytes, such as Na^+, sucrose and proline.

Keywords: drought; salinity; glucose; extreme climate; Na^+/K^+ ratio

Drought is one of the most important growth-restricting environmental factors for crops species in arid and semi-arid regions of the world (Chaves and Oliveira 2004, Ben Hassine et al. 2010). Crop losses resulting from abiotic stresses such as drought or salinity can reduce crop yield by as much as 50% (Chaves and Oliveira 2004). Climate changes largely exacerbate this situation due to the increasing incidence of more extreme climate events. Lack of water can inhibit growth and development of plants, mainly by decreasing the photosynthesis, leaf turgor and transpiration rates (Gong et al. 2005, Tahi et al. 2007). Plants have evolved a series of adaptive mechanisms to maintain cellular optimal environment for ensuring normal growth of plants under drought stress (Ludlow and Muchow 1990). One of the responses to drought in plants is osmotic adjustment (Ma et al. 2012), which was mainly achieved by accumulating more organic solutes and inorganic ions within cells (Sonobe et al. 2010). The accumulation of organic solutes, such as proline, soluble sugar and betaine, can adjust cellular osmotic potential for better uptake of water in plants from external adverse conditions (Morgan 1984, Serraj and Sinclair 2002, Martínez et al. 2004, Szabados and Savouré 2010, Wu et al. 2014, 2015). Besides, it was reported that Na^+ can largely accumulate in leaf in response to water stress in xerophyte *Zygophyllum xanthoxylum* under drought stress, indicating that Na^+ plays a critical role on the contribution of osmotic adjustment in leaf tissue (Wang et al. 2004). Similar results were also observed in the halophytes such as *Atriplex halimus* and *Sesuvium portulacastrum* (Martínez et al. 2004, 2005, Slama et al. 2007). Therefore, it is clear that Na^+ is a bud-

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get molecule, which is involved in adjusting cellular osmotic potential under drought stress.

Sugar beet (*Beta vulgaris* L.) is one of the important commercial crops that supplies approximately 35% of the worlds sugar and is widely cultivated in arid and semi-arid regions of northern China (Liu et al. 2008). Wu et al. (2015) reported that low concentrations of NaCl (5–50 mmol/L) can effectively improve performance of sugar beet by accumulating more Na^+ in shoot to adjust osmotic potential. However, physiological traits and adaptive capacity to change and adverse drought conditions in sugar beet plants is little known. These knowledge gaps restricted our capacity to properly evaluate and predict the performance of sugar beet in response to drought conditions, especially the physiological response in different tissues of sugar beet plants.

In the present study, we investigated the changes of growth, ions, soluble sugars and proline accumulation in different tissues of sugar beet plants exposed to osmotic stress. This knowledge will expand the information of physiologically adaptive mechanisms for coping with osmotic stress in sugar beet plants.

MATERIAL AND METHODS

Seeds of sugar beet (*B. vulgaris* L. cv. Gantang7) were provided by the Wuwei Sannong Seed Technology, Co. Ltd. Gansu province, China. Seeds were surface sterilized for 1 min in 75% ethanol (*v*/*v*) and rinsed 3 times with distilled water, soaked in distilled water for 1 day and then germinated at 25°C in the dark for 3 days. Uniform seedlings were carefully transferred to plastic container (5 cm × 5 cm × 5 cm; two seedlings/container) filled with vermiculite and irrigated with the modified Hoagland nutrient solution containing 2.5 mmol/L KNO_3, 0.5 mmol/L $MgSO_4$, 0.5 mmol/L $Ca(NO_3)_2$, 1 mmol/L $NH_4H_2PO_4$, 92 μmol/L H_3BO_3, 60 μmol/L Fe-citrate, 18 μmol/L $MnCl_2{\cdot}4\ H_2O$, 0.7 μmol/L $(NH_4)_6Mo_7O_{24}{\cdot}4\ H_2O$, 1.6 μmol/L $ZnSO_4{\cdot}7\ H_2O$, and 0.6 μmol/L $CuSO_4{\cdot}5\ H_2O$. Solutions were renewed every 3 days. All the plants were grown in growth chamber under 16 h light (25°C)/8 h dark (20°C) photoperiod, 70% relative humidity and 600–800 μmol/m^2/s photon flux density. After 20 days, seedlings were carefully transferred to plastic pots (11 cm height × 10 cm diameter) for continuous cultivation. Two-month-old plants were treated with the modified Hoagland solution supplemented with 0, 160, 320, and 480 mmol/L sorbitol for 7 days (Wu et al. 2012). Osmotic potentials of treatment solutions were 0, –0.5, –1.0, and –1.5 MPa, respectively. Each treatment had three replicates. The treatment solutions were renewed every 3 days to maintain a constant osmotic potential.

At the end of treatments, plants were washed twice using distilled water to remove surface salts, and the roots were placed in ice-cold 20 mmol/L $CaCl_2$ for 8 min to exchange cell wall-bound Na^+. Then, plants were rapidly separated into four sections: leaf blade (LB); leaf petiole (LP); storage root (ST), and lateral root (LT). Fresh weight (FW) was measured immediately and samples were dried at 80°C for 72 h to determine dry weight (DW). Tissue water content (WC) was calculated according to the method as described by Wu et al. (2015).

Na^+ and K^+ concentrations were determined according to the method as described by Wu et al. (2014, 2015). Na^+/K^+ ratios were calculated according to the method as described by Yue et al. (2012). The sucrose, fructose, and glucose contents were determined according to the methods described by Liu et al. (2008). Proline was assayed using the ninhydrin reagent according to the method of Bates et al. (1973).

The data were performed by one-way analysis of variance (ANOVA) using the statistical software SPSS (19.0, Chicago, USA). Duncan's multiple range tests were used to detect significant difference between means at a significant level of $P < 0.05$.

RESULTS

Effects of osmotic stress on growth in sugar beet plants. With the increase of osmotic stress, fresh weight in leaf blade and leaf petiole showed a significantly decreasing trend ($P < 0.05$), while remained unchanged in storage root and lateral root (Figure 1a). Furthermore, osmotic stress significantly reduced water content in leaf blade and leaf petiole ($P < 0.05$), but did not significantly affect storage root and lateral root (Figure 1b).

Effects of osmotic stress on Na^+ and K^+ accumulations in sugar beet plants. Regardless of control or osmotic stress, the tissues Na^+ concentrations presented the following pattern from big to small: leaf blades > leaf petioles > lateral roots > storage roots (Figure 2a). Compared with

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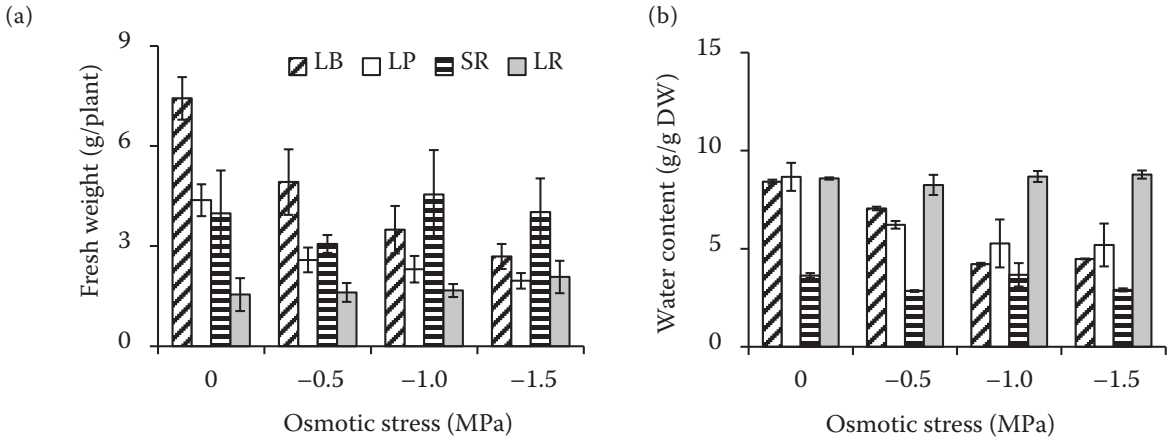

Figure 1. (a) Fresh weight and (b) water content in leaf blade (LB); leaf petiole (LP); storage root (SR), and lateral root (LR) of two-month-old sugar beet (*Beta vulgaris* L.) plants grown under osmotic stress for 7 days. Values are means ± standard error based on three replicates. Columns with different letters indicate significant differences at $P < 0.05$ (Duncan's test); DW – dry weight

control, osmotic stress of –0.5 MPa and –1.0 MPa significantly enhanced Na^+ concentration in leaf blade, whereas osmotic stress of –1.5 MPa had no significant effect. It was also observed that Na^+ concentration in leaf petiole increased by 45% at osmotic stress of –1.5 MPa ($P < 0.05$) (Figure 2a). However, Na^+ concentration in storage root and lateral root maintained stable state under osmotic stress (Figure 2a). In contrast, osmotic stress significantly decreased K^+ concentration in lateral root compared with control ($P < 0.05$) (Figure 2b). It was also found that Na^+/K^+ ratios in leaf blade and lateral root were increased by osmotic stress of –0.5 MPa and –1.0 MPa ($P < 0.05$) (Figure 2c).

Effects of osmotic stress on sugars and proline accumulations in sugar beet plants. Compared to other tissues, storage root accumulated more sucrose and fructose under either control or os-

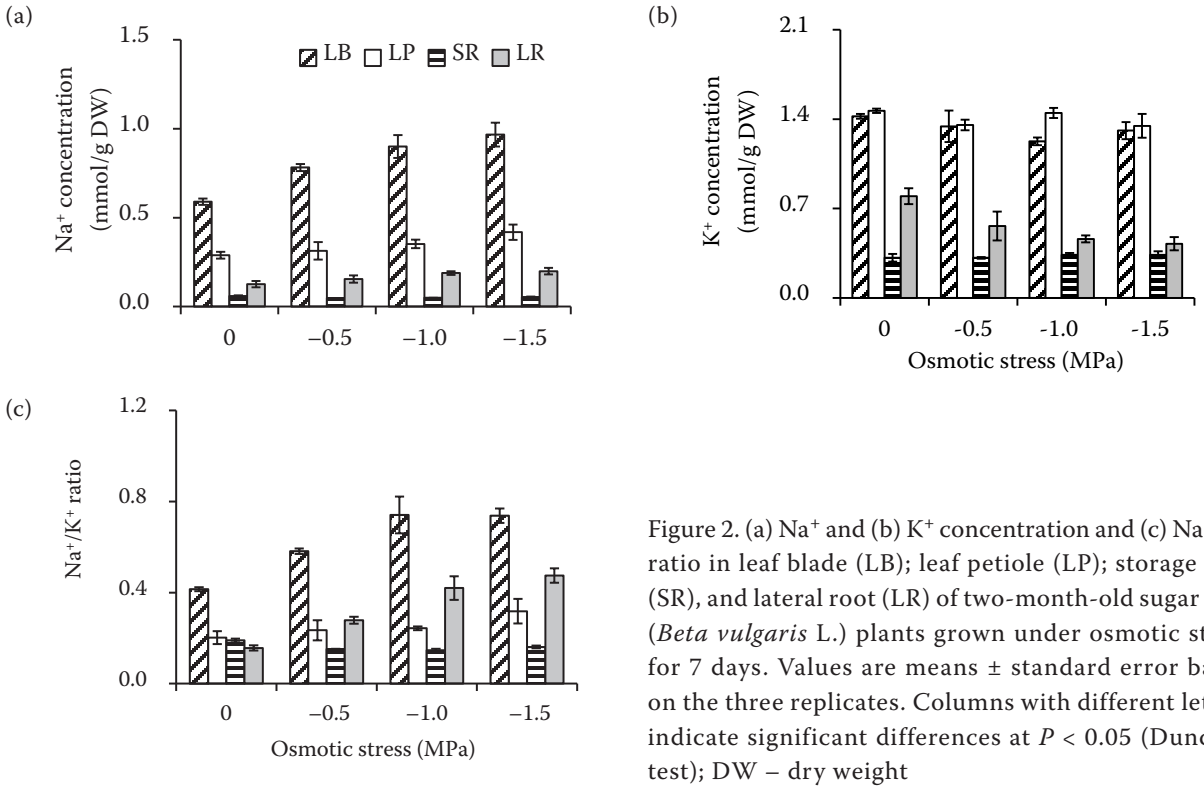

Figure 2. (a) Na^+ and (b) K^+ concentration and (c) Na^+/K^+ ratio in leaf blade (LB); leaf petiole (LP); storage root (SR), and lateral root (LR) of two-month-old sugar beet (*Beta vulgaris* L.) plants grown under osmotic stress for 7 days. Values are means ± standard error based on the three replicates. Columns with different letters indicate significant differences at $P < 0.05$ (Duncan's test); DW – dry weight

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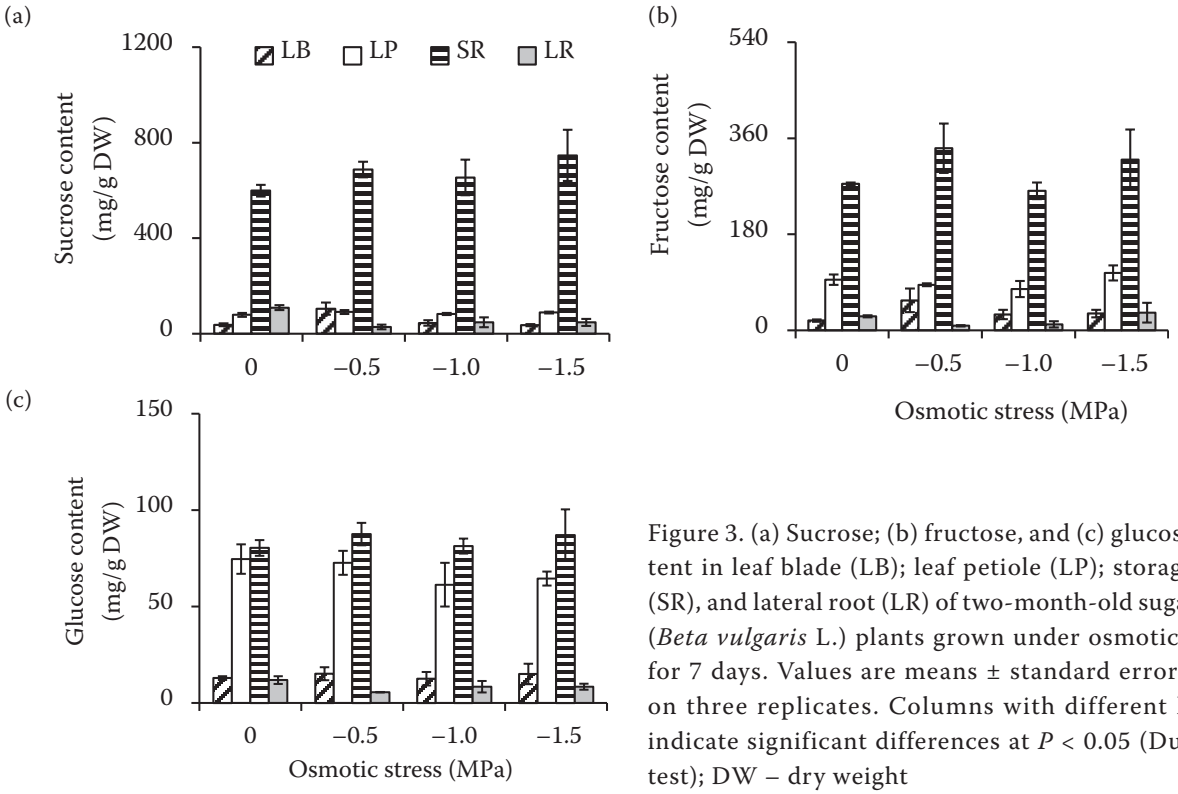

Figure 3. (a) Sucrose; (b) fructose, and (c) glucose content in leaf blade (LB); leaf petiole (LP); storage root (SR), and lateral root (LR) of two-month-old sugar beet (*Beta vulgaris* L.) plants grown under osmotic stress for 7 days. Values are means ± standard error based on three replicates. Columns with different letters indicate significant differences at $P < 0.05$ (Duncan's test); DW – dry weight

motic stress (Figure 3a,b). It was also observed that leaf petiole and storage root accumulated more glucose compared with leaf blade and lateral root ($P < 0.05$) (Figure 3c). In storage root, sucrose content was significantly higher than fructose and glucose ($P < 0.05$) (Figure 3a). Compared with control, osmotic stress of –1.5 MPa triggered a significant increase of sucrose content in storage root by 24.6% ($P < 0.05$) (Figure 3a).

Proline concentration in leaf blade, leaf petiole, and storage root showed a gradually increasing trend with the increase of osmotic stress. For example, osmotic stress of –1.5 MPa significantly increased proline level in leaf blade, leaf petiole, and storage root by 260, 145, and 352% ($P < 0.05$), respectively, compared to control (Figure 4).

DISCUSSION

Drought is regarded as a major environmental limiting factor of sugar beet production (Pidgeon et al. 2001), which also adversely affected yield formation. For the maintenance of yield under drought, it is necessary to identify limiting steps in the yield formation process of sugar beet (Hoffmann 2010). In the present study, sugar beet plants were subjected to osmotic stress two months after sowing. It was observed that osmotic stress significantly reduced FW and WC in leaf blade and leaf petiole, while storage root and lateral root remained unchanged (Figure 1a). Our previous results indicated that WC in both shoot and root was significantly

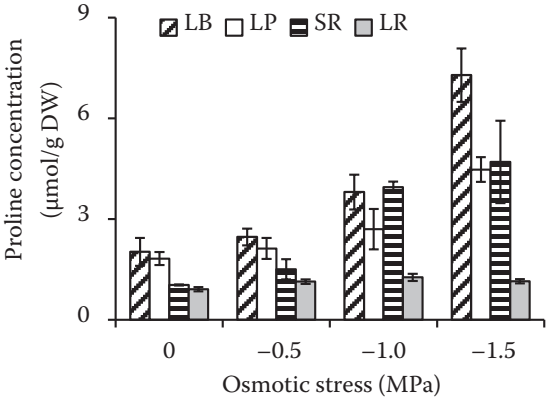

Figure 4. Proline concentration in leaf blade (LB); leaf petiole (LP); storage root (SR), and lateral root (LR) of two-month-old sugar beet (*Beta vulgaris* L.) plants grown under osmotic stress for 7 days. Values are means ± standard error based on three replicates. Columns with different letters indicate significant differences at $P < 0.05$ (Duncan's test); DW – dry weight

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reduced by drought stress in 4-week-old sugar beet seedlings, and their growth suffered largely from effect at osmotic stress of –1.0 MPa (Wu et al. 2014). These results suggested that two-month-old sugar beet plants display the drought tolerance to some extent, which can be due to increased ability for maintaining adequate water uptake by lateral root. There was also evidence that water deficit of leaf can inhibit the activity of photosystem II and the rate of CO_2 assimilation (Bloch et al. 2006, Monti et al. 2006), which in turn could decrease photosynthesis. Therefore, the growth of sugar beet plants inhibited by drought was probably associated with the reduction of photosynthesis rate, and loss of cellular turgor in leaf under osmotic stress.

The accumulation of osmolyte compounds was often proposed as a solution to overcome the negative effects of water deficits in crop production (Serraj and Sinclair 2002), which can decrease cellular osmotic potential to maintain optimal environment for the uptake of water. One of the osmolytes is Na^+, which can largely accumulate in leaf for coping with drought stress in plants (Wang et al. 2004, Ma et al. 2012, Yue et al. 2012). In our previous work, it was observed that 50 mmol/L NaCl improved the ability of sugar beet plants to adapt to osmotic stress by increasing Na^+ accumulation in shoot (Wu et al. 2015). In the present study, Na^+ concentration in leaf blade significantly increased when plants suffered from osmotic stress (Figure 2a). Furthermore, plants accumulated more Na^+ in leaf blade compared with other tissues under either control or osmotic stress (Figure 2a). In contrast, K^+ concentration displayed a gradually decreasing trend in lateral root with the increase of osmotic stress, but remained unchanged in other tissues (Figure 2b). Similar results were reported by Yue et al. (2012), who found that osmotic stress significantly increases Na^+ concentration in leaf, whereas K^+ concentrations remained unchanged in all tissues. It was also suggested that the accumulation of larger quantities of Na^+ over K^+ in leaf may be used to osmotic adjustment in xerophytes *H. ammodendron* and *Z. xanthoxylum* (Wang et al. 2004). These results suggested that Na^+ ions in leaf may play an important role in osmotic adjustment in sugar beet plants.

It is well documented that storage root exhibited important alterations under long-term drought stress, mainly including the reduction of diameter and dry weight, cell division and cell expansion, and the rate of sucrose accumulation (Hoffmann 2010). Furthermore, it was pointed out that cell expansion in storage root is very important for the yield formation process in sugar beet, which can compartmentalize more sucrose and compatible solutes into vacuole to maintain a low water potential (Milford 1973, Hoffmann 2010). In the present study, accumulation of sucrose, fructose, and glucose in storage root was significantly higher than in other tissues under osmotic stress (Figure 3a–c). In addition, sugar beet plants accumulated more glucose in leaf petiole than in leaf blade and lateral root under normal condition and osmotic stress (Figure 3c). Furthermore, compared for control, osmotic stress of –1.5 MPa significantly enhanced the content of sucrose in storage root, but fructose and glucose remained unchanged (Figure 3a–c). These results indicated that short-term osmotic stress can increase sucrose accumulation in storage root of sugar beet plants.

A key osmoprotective function of free proline was emphasized in numerous plants (Verbruggen and Hermans 2008, Szabados and Savouré 2010, Wu et al. 2014). In addition to osmotic adjustment, proline has certain regulatory functions, acts as a signal molecule, and protects and stabilizes the structure of membranes and enzymes under environmental stress (Szabados and Savouré 2010). In the present study, concentrations of proline in leaf blade, leaf petiole, and storage root were significantly increased by osmotic stress of –1.0 MPa and –1.5 MPa (Figure 4). Moreover, plants accumulated more proline in leaf blade than in other tissue under osmotic stress of –1.5 MPa (Figure 4), which was probably showed to adjust cellular osmotic potential for water uptake.

In conclusion, our results showed that osmotic stress remarkably reduced the fresh weight and water content in leaf blade and leaf petiole, whereas it remained unchanged in storage root and lateral root. It is clear that osmotic stress enhanced Na^+ and proline accumulation in leaf blade, and maintained stable state of K^+ in leaf blade and leaf petiole of sugar beet plants.

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Corresponding author:

Dr. Guo-Qiang Wu, Lanzhou University of Technology, School of Life Science and Engineering, 287 Langongping Road, 730 050 Lanzhou, P.R. China; e-mail: wugq08@126.com