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ORIGINAL ARTICLE

Tetraploid exhibits more tolerant to salinity than diploid in sugar beet (*Beta vulgaris* L.)

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Abstract

Soil salinity is one of the major environmental stress factors limiting crops growth, development, and productivity worldwide. The aim of this study was to compare differences of salinity tolerance between diploid (cv. TY03209) and tetraploid (cv. TY03410) seedlings of sugar beet (*Beta vulgaris* L.) treated with various concentrations (0, 50, 100, 200, and 300 mM) of NaCl. Our results indicated that fresh weight (FW) and dry weight (DW) of shoot in tetraploid were remarkably higher than those in diploid when subjected to various concentrations of NaCl (except for FW under 200 mM). At 200 and 300 mM NaCl, tetraploid obviously accumulated less Na^+ in its shoots and roots compared with diploid. However, there were no differences in K^+ accumulation between tetraploid and diploid under salinity stress. Our results also showed tetraploid displayed a smaller Na^+/K^+ ratio and a stronger selective capacity for K^+ over Na^+ than diploid when exposed to high-salt stress (300 mM). Furthermore, it was observed that tetraploid possessed a bigger net K^+ uptake rate and a smaller net Na^+ uptake rate compared to diploid at high-salt condition. We also investigated the relative expression levels of six genes related to K^+ and Na^+ transport in roots of diploid and tetraploid by qRT-PCR method, and found that *BvHKT1;1, BvNHX1, BvSKOR*, and *BvSOS1* were induced by additional 50 mM NaCl, and their transcript abundances in tetraploid were relatively higher than those in diploid. The expression level of *BvAKT1* was down-regulated in tetraploid during 3–48 h of salt treatment, whilst basically remained unchanged in diploid. It was observed that the transcript abundance of *BvHAK5* in diploid displayed the reduced trend with the prolonging of salt treatment time compared to tetraploid. In addition, soluble sugars contents were obviously higher in tetraploid than in diploid exposed to 100, 200, and 300 mM NaCl. Taken together, these results suggested that tetraploid exhibited more tolerant to salinity stress than diploid in sugar beet by accumulating less Na^+ and more soluble sugars, and by maintaining lower Na^+/K^+ ratio and greater capacity of selective absorption for K^+ over Na^+. The results of this study provide insights into physiological and molecular consequences of polyploidization in sugar beet.

Keywords Sugar beet · Diploid · Tetraploid · Na^+ toxicity · Na^+ and K^+ transporters · Salinity tolerance

Abbreviations

AKT1	*Arabidopsis* K^+ transporter 1
ANOVA	Analysis of variance
BT	Before treatments
Ct	Cycle threshold
DW	Dry weight
FW	Fresh weight
HAK5	High-affinity K^+ transporter 5
HKT1	High-affinity K^+ transporter 1
KT	K^+ transporter
KUP	K^+ uptake protein
RFW	Root fresh weigh
NXH1	Tonoplast Na^+/H^+ antiporter 1
qRT-PCR	Quantitative reverse transcription-polymerase chain reaction
SA	Selective absorption for K^+ over Na^+
SE	Standard error
SKOR	Shaker-like K^+ outward rectifying channel
SOS1	Salt overly sensitive 1
ST	Selective transport for K^+ over Na^+
TEA	Tetraethylammonium
UV	Ultra violet
XPCs	Xylem parenchyma cells

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Introduction

Soil salinity is one of the major environmental stress factors limiting crops growth, development, and productivity worldwide, because most of plant species are sensitive to high level of salinity (Kronzucker and Britto 2011; Zhang and Shi 2013; Mishra and Tanna 2017). It has been estimated that about one-fifth of the world's lands and approximately half of the irrigated lands are adversely affected by salinity (Rozema and Flowers 2008; Zhang et al. 2012, 2014). In China, over one million acres of the cultivated lands are damaged by salt because of irrigated water with high soluble salinity (Yuan et al. 2016). It was shown that high salinity can cause water deficits, membrane alterations, ionic toxicity, nutrient deficiency, and free radical production, and induce deleterious metabolic disorder, resulting in molecular damages, thereby affecting the growth, morphology, and survival in glycophytes and even some halophytes (Zhu 2001; Golldack et al. 2014; Zhang et al. 2014; Lu et al. 2017). Some plants, such as halophytes or natrophilic crops, have evolved a variety of mechanisms to adapt to salt conditions or prevent salt stress (Roy et al. 2014; Shabala et al. 2014; Gao et al. 2015; Pan et al. 2016; Arzani and Ashraf 2016). One of the effective adaptations is reduction of Na^+ accumulation and enhancement of K^+ accumulation in cytosol to cope with Na^+ toxicity and, thus, to maintain cellular and whole-plant K^+/Na^+ homeostasis in saline environments (Chen et al. 2014; Pan et al. 2016). There are evidences that plants adopt several physiological strategies, including the control of Na^+ influx into roots, exclusion of Na^+ to the apoplastic space, control of xylem loading of Na^+, and sequestration Na^+ into the vacuoles, to mitigate Na^+ toxicity (Kumari et al. 2015; Mishra and Tanna 2017). Therefore, control of Na^+ influx and maintenance of ion homeostasis may enhance salt resistance in plants.

Polyploidization has occurred during evolutionary history of various plant species and is thought to be one of the major driving forces for genome evolution in plants (Stupar et al. 2007; Chao et al. 2013; Yang et al. 2014a). It has been documented that polyploidy may either combine the genomes of two different species (allopolyploidy) or double the genome of a single species (autopolyploidy) (Stupar et al. 2007; Wang et al. 2013a). Most of polyploid plants exhibit a lot of novel variation, morphologies, or anatomies relative to their parental species (Romero-Aranda et al. 1997; Stupar et al. 2007; Wang et al. 2011; Sattler et al. 2016; Dong et al. 2017; Xue et al. 2017). It was also observed that physiological functions or gene expression was changed by polyploidy in higher plant species such as maize (*Zea mays*), potato (*Solanum phureja*), wheat (*Triticum aestivum*), and *Paulownia australis* (Stupar et al. 2007; Riddle et al. 2010; Yang et al. 2014a; Dong et al. 2017). Polyploidy has also been considered as an important tool to enhance salt tolerance in plants (Xue et al. 2015; Ruiz et al. 2016). There are evidences that tetraploid black locust (*Robinia pseudoacacia*) plants display higher salt tolerance than diploid plants (Wang et al. 2013a), and the salt tolerance of tetraploid apple (*Malus domestica*) plants was shown to be stronger than that of diploid ones (Xue et al. 2015). In *Arabidopsis thaliana*, tetraploid plants were shown to have elevated K^+ and reduced Na^+ accumulation in leaves compared with diploid ones exposed to salinity stress (Chao et al. 2013). In bread wheat, synthetic and natural hexaploid cultivars have also been found to accumulate more K^+ and less Na^+ in leaves, and display higher salt tolerance than tetraploid ones (Schachtman et al. 1992; Yang et al. 2014a). These results implied that the improved tolerance of ploidy to salinity was related to both the elevated K^+ and the reduced Na^+ accumulation, thereby maintaining ion homeostasis in plant tissues (Chao et al. 2013; Yang et al. 2014a).

So far, several kinds of K^+ and Na^+ transport systems have been documented to play key roles in ion homeostasis under saline condition (Kronzucker and Britto 2011; Yamaguchi et al. 2013; Deinlein et al. 2014). Of these, inward-rectifier K^+ channel AKT1 (*Arabidopsis* K^+ transporter 1), SKOR (shaker-like K^+ outward rectifying channel), HKT1 (high-affinity K^+ transporter 1), HAK5 (high-affinity K^+ transporter 5), NHX1 (tonoplast Na^+/H^+ antiporter 1), and SOS1(salt overly sensitive 1) have drawn particular attention due to their capacity of transporting K^+ and/or Na^+ across cellular membranes (Munns and Tester 2008; Wu et al. 2011; Guo et al. 2012; Yamaguchi et al. 2013; Hu et al. 2016; Ma et al. 2017; Huang et al. 2018). It has been shown that AKT1 was a key component for both low- and high-affinity K^+ uptake, and the *AKT1* gene was expressed preferentially in root hairs, endodermis, epidermis, and cortex of mature root, where cell types specialized in K^+ absorption (Maathuis and Sanders 1994; Li et al. 2014; Xu et al. 2014). In halophytes, *Suaeda salsa* and *Puccinellia tenuiflora*, AKT1 has been shown to mediate salinity resistance by maintaining stronger selectivity for K^+ over Na^+ during salinity stress (Duan et al. 2015; Wang et al. 2015). In xerophyte *Zygophyllum xanthoxylum*, ZxAKT1 not only plays an important role in K^+ absorption, but also functions in regulating Na^+ absorption and transport systems (Ma et al. 2017). SKOR has been documented to control the delivery of K^+ from stelar cells to xylem sap in the roots, a key step in the long-distance K^+ transport from roots to shoots of plants (Liu et al. 2006; Demidchik 2014; Hu et al. 2016). *SKOR* is expressed predominantly in root hairs and other cell types (Liu et al. 2006; Huang et al. 2018). In *A. thaliana, atskor* mutants showed both smaller K^+ concentration and

smaller xylem sap K^+ content compared to wild-type (WT) plants (Gaymard et al. 1998). Overexpression of *CmSKOR* from melon (*Cucumis melo*) remarkably improved tolerance to salinity by increasing K^+ accumulation in transgenic *A. thaliana* plants (Huang et al. 2018). There are evidences that several members of HKT family can mediate Na^+ absorption and are involved in controlling K^+/Na^+ homeostasis in plants exposed to salt stress (Garciadeblás et al. 2003; Hamamoto et al. 2014). It was indicated that AtHKT1;1 controlled retrieval Na^+ from the xylem vessels to the xylem parenchyma cells (XPCs), thereby decreasing Na^+ accumulation in leaf tissues, and the *AtHKT1;1*-overexpressed in the mature root steles of *A. thaliana* reduced Na^+ contents in the shoots (Davenport et al. 2007; Møller et al. 2009). It was suggested that HAK5, one member of the KUP/HAK/KT family, played important roles in maintaining high-affinity K^+ absorption and normal growth in plants under salinity stress (Nieves-Cordones et al. 2010; Li et al. 2018). In rice (*Oryza sativa*), *OsHAK5* is expressed predominantly at both the phloem of root vascular tissues and the xylem parenchyma, particularly under K^+ deficiency conditions, and its inactivation decreases both K^+ contents and K^+ efflux rates in the xylem sap (Yang et al. 2014b). It was also showed that OsHAK5 mediates K^+ accumulation in XPCs to enable SKOR to release efficiently K^+ into the xylem sap driven by the electrochemical gradient of K^+ (Gaymard et al. 1998; Li et al. 2018). NHX1 has been documented to be responsible for Na^+ compartmentation into the vacuoles under saline condition and plays important roles in controlling cellular pH and ion homeostasis (Yamaguchi et al. 2013). The overexpression of *NHX1* significantly enhanced salt resistance in various plant species such as common buckwheat (*Fagopyrum esculentum*) (Chen et al. 2008) and peanut (*Arachis hypogaea*) (Banjara et al. 2012). SOS1 functions not only in restricting Na^+ influx by enhancing Na^+ exclusion at the plasma membrane (PM) of root cells but also in loading Na^+ from the xylem parenchyma cells into the xylem in roots (Shi et al. 2002a, b). It was reported that SOS1 controlled long-distance transport of Na^+ from the roots to the shoots (Shi et al. 2002b; Ma et al. 2014). Overall, these transporters play vital functions in maintaining ion homeostasis and enhancing salinity resistance in plants.

Sugar beet (*Beta vulgaris* L.), one of the Chenopodiaceae family, is a major sugar crop providing nearly 30% of the world's sugar production (Dohm et al. 2014; Monteiro et al. 2018). It is not only used in the industry of food but also for the bioethanol production as a source of renewable energy (Magaña et al. 2011; Hossain et al. 2017). It is well known that sugar beet is thought to be a typically natrophilic crop (Skorupa et al. 2016). Our previous studies indicated that the relatively higher tolerance of sugar beet cultivar "Gantang7" to salt stress was associated with the abilities of plant to accumulate more soluble sugars and proline and maintain smaller Na^+/K^+ ratio (Wu et al. 2013). Our further studies suggested that the application of 50 mM NaCl can stimulate the growth of plants and enhance the tolerance to drought stress in sugar beet (Wu et al. 2015a). Most of sugar beet cultivars are diploid with $2n = 2x = 18$ chromosomes (Dohm et al. 2014), but many polyploidy cultivars also exist (Beyaz et al. 2013). The previous studies have compared enzyme activities, total chlorophyll and protein content between diploid and polyploid sugar beet varieties (Spettoli et al. 1976; Beyaz et al. 2013). However, little is known about the physiological and molecular differences of salt tolerance between diploid and tetraploid sugar beet cultivars.

The objective of this study was to compare sugar beet cultivars at different ploidy levels with respect to the growth of plant, Na^+ and K^+ concentrations, net uptake rates of Na^+ and K^+, soluble sugars contents, and expression levels of genes related to Na^+ and K^+ transport under salinity stress to elucidate if tetraploidy can enhance the tolerance to salinity in sugar beet. The results of this study have provided insights into physiological and molecular consequences of polyploidization in sugar beet.

Materials and methods

Plant materials, growth conditions, and treatments

Seeds of two sugar beet (*B. vulgaris* L.) cultivars, "TY03410" (tetraploid, 36 chromosomes) and "TY03209" (diploid progenitor, 18 chromosomes), were provided kindly by Prof. Hua-Zhong Wang from Heilongjiang University, China, in mid-August 2014. Seed was sterilized with 75% ethanol (*v*/*v*) for 1 min and rinsed four times with distilled water, and then germinated at 25 °C on the filter paper in the dark for 3 days. The uniform seedlings were transplanted into a plugged hole in plastic containers (5 cm × 5 cm × 5 cm; two seedlings/container), filled with sterilized vermiculite, and watered with the modified Hoagland nutrient solution containing 1 mM $NH_4H_2PO_4$, 2.5 mM KNO_3, 0.5 mM $MgSO_4$, 0.5 mM $Ca(NO_3)_2$, 92 μM H_3BO_3, 60 μM Fe-Citrate, 1.6 μM $ZnSO_4 \cdot 7H_2O$, 18 μM $MnCl_2 \cdot 4H_2O$, 0.7 μM $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 0.6 μM $CuSO_4 \cdot 5H_2O$. Solutions were changed every 3 days. Seedling was grown in a chamber where the temperature was 20/25 °C (night/day), the relative humidity was about 65–75%, the daily photoperiod was 8/16 h (night/day), and the flux density was approximately 550–600 μmol m^{-2} s^{-1} during the light period. Four-week-old seedlings were used for the following treatments. (1) Seedlings were treated with modified Hoagland solution supplemented with additional 0, 50, 100, 200, and 300 mM NaCl, the concentration of which was increased incrementally by 50 mM each day to the final concentrations. (2) Seedlings were treated with modified Hoagland nutrient

solution supplemented with additional 50 mM NaCl and harvested at 0, 12, 24, 48, 72, 96, and 120 h after treatment. Treatment solutions were changed every 3 days to maintain a constant ion concentration.

Determination of growth and concentrations of Na^+ and K^+

After salt treatments, plants were separated into roots and shoots. Fresh weight (FW) was determined immediately and samples oven dried at 80 °C for 48 h to obtain dry weight (DW). Na^+ and K^+ concentrations were determined according to the method of Wang et al. (2007). Briefly, Na^+ and K^+ were extracted from dried samples in 100 mM acetic acid at 90 °C for 2 h. Na^+ and K^+ analysis was performed using an atomic absorption spectrophotometer (2655-00, Cole-Parmer Instrument Co., Vernon Hills, USA).

Calculation of Na^+/K^+ ratio and net uptake rate

Na^+/K^+ ratio was assayed according to the method described by Yue et al. (2012). Net Na^+ or K^+ uptake rate was calculated according to the methods described by Wang et al. (2009) and Guo et al. (2015).

Calculation of SA and ST values

The capacity of selective absorption (SA) and selective transport (ST) for K^+ over Na^+ were investigated according to the method described by Wang et al. (2009).

Determination of soluble sugar contents

Soluble sugars were assayed using the anthrone ethyl acetate reagent according to the methods described by Wu et al. (2015a). Soluble sugars contents were tested using an UV-3000PC spectrophotometer (Mapada, Shanghai, China) at 630 nm and determined from calibration curve using Sucrose (Sangon, Shanghai, China) as a standard and expressed as mg/g DW.

qRT-PCR analysis

Four-week-old sugar beet plants were exposed to the modified Hoagland nutrient solution with additional 50 mM NaCl, and the roots were harvested at 0, 3, 6, 12, 24, and 48 h after treatment. Total RNA was extracted from roots samples using UNlQ-10 Column Trizol Total RNA Isolation Kit (Sangon, Shanghai, China) following the manufacturer's instructions. First-strand cDNA was synthesized from 1 μg of total RNA using PrimeScript™ RT Master Mix Kit (Takara, Dalian, China) following the manufacturer's instructions. The reverse-transcribed cDNAs were used for qRT-PCR analysis following the manufacture's protocol. Briefly, each qRT-PCR reaction was carried out in a 20 μL reaction system with 10 μL TB Green™ Premix Ex Taq™ II mix (Takara, Dalian, China) and 0.8 μL of each PCR primer at 10 μM. RT-PCR was conducted on a MA-6000 Real-Time PCR System (Molarray, Suzhou, China) as follows: 95 °C for 30 s, and 40 cycles of 95 °C for 5 s and 60 °C for 60 s. The relative expression levels of six genes related to Na^+ and K^+ transport (*BvAKT1, BvHAK5, BvHKT1;1, BvNHX1, BvSKOR*, and *BvSOS1*) were analyzed by qRT-PCR method. Primers used for qRT-PCR are listed in Table 1. *BvACTIN1* was used as an internal reference to normalize the expression data. Each of the treatment groups consisted of three biological replicates, and the experiments were repeated at least three times. Relative expression levels of genes were calculated using the $2^{-\Delta\Delta Ct}$ (cycle threshold) method by described by Livak and Schmittgen (2001).

Statistical analysis

All the data were presented as means with standard errors (SE). Data analyses were performed by one- and two-way analysis of variance (ANOVA) using the SPSS statistical software (Version 19.0, SPSS Inc., Chicago, IL, USA), respectively. Duncan's multiple range tests were used to analyze differences among means at a significant level of $P < 0.05$.

Table 1 Primers used in qRT-PCR

Gene name	Accession number	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)
BvACTIN1	KF214784	ACTGGTATTGTGCTTGACTC	ATGAGATAATCAGTGAGATC
BvAKT1	XM_010672404	GACACATTCCAGAATTCCCT	TTGTAACCTCGCTCTTTTCC
BvSKOR	XM_010671906	ATCCAAGTTATTATGACGCC	TTGAGGATTTCAGCCGTTCC
BvHAK5	XM_010672399	CCACTACTAGCTAGTTGGAG	AGGTCAAGAGAATCATGACG
BvHKT1;1	XM_010691955	TAGCAGCTTAGGAGAATCCC	GGAAGTAGTGAAGGTTGGTC
BvSOS1	XM_010681801	GCCAGCTATGGCAGCTTATC	ATCCAAGGCCAATGCCGATG
BvNHX1	XM_010674170	TCGATGATTCTTTCATGAGG	GCCAACTGCCTCATACTCTG

Results

Biomass in response to salt stress and comparison of diploid and tetraploid sugar beet cultivars

To investigate the observed differences in the growth response, FW and DW of tetraploid and diploid were compared at 7 days after salt treatments. It was showed that FW of shoots and roots in tetraploid cultivar were clearly higher than those in diploid cultivar when exposed to 50, 100, and 300 mM NaCl, respectively. However, no clear difference was found between tetraploid and diploid at 200 mM NaCl (Fig. 1a, b). DW of shoots in tetraploid were 33.7%, 18.1%, and 51.5% higher than those in diploid subjected to 100, 200, and 300 mM NaCl (Fig. 1c), respectively. In addition, DW of roots in tetraploid were 10.2% and 32.3% higher than those in diploid exposed to 100 and 300 mM NaCl (Fig. 1d), respectively. In contrast, DW of roots in tetraploid were 16.9% lower than those in diploid exposed to 50 mM NaCl (Fig. 1d). The results of two-way ANOVA showed that interactive effects of salinity and ploidy were significant for either FW or DW of both shoots and roots, although the effects of ploidy were not statistically significant for both FW and DW of roots (Table 2).

To further observe the difference of plant growth at the time, FW and DW were investigated in both cultivars subjected with 50 mM NaCl over a 120-h period, respectively. FW and DW of shoots in both diploid and tetraploid followed the increased trends with the increasing of treatment time, while the increased magnitude of tetraploid was significantly higher than that of diploid after 72 h of NaCl treatment (Fig. 2a, c). DW of roots in tetraploid was remarkably higher than that in diploid at 24, 72, 96, and 120 h after salt treatment (Fig. 2d).

Cation accumulation and Na^+/K^+ ratio in response to salt stress

To test the observed differences in ion accumulation, Na^+ and K^+ concentrations in tetraploid and diploid were also

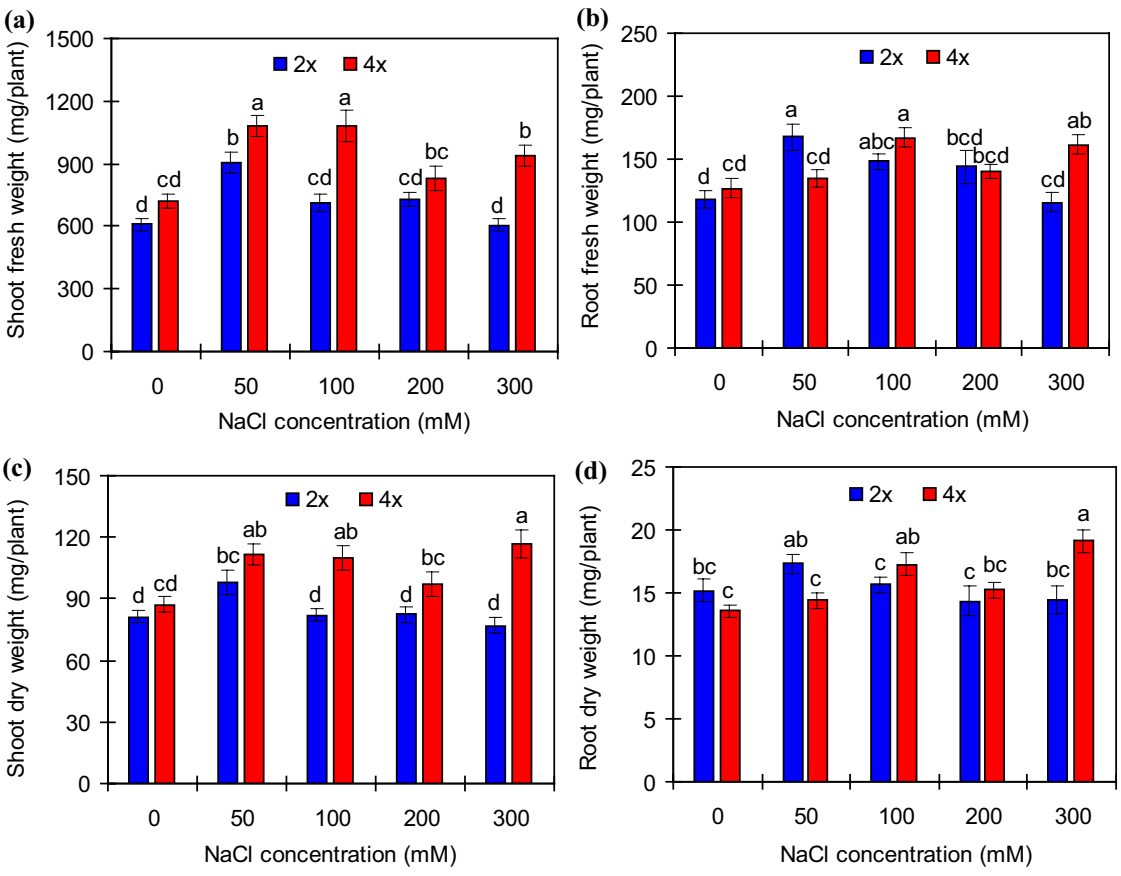

Fig. 1 Fresh (**a, b**) and dry (**c, d**) weights of shoot and root in diploid (2*x*) and tetraploid (4*x*) sugar beet seedlings exposed to 0, 50, 100, 200, and 300 mM NaCl for 7 days. Two plants were pooled in each replicate ($n=8$). Values are means ± SE and bars indicate SE. Columns with different letters indicate significant differences at $P<0.05$ (Duncan's test)

Table 2 Results of a two-way ANOVA test for the effect of salinity and ploidy and their interactions with various physiological parameters in sugar beet

Parameters	Salinity	Ploidy	Salinity × ploidy
Shoot FW	14.024***	52.5278***	3.517*
Shoot DW	5.056**	43.454***	3.728**
Root FW	5.409***	1.994^{ns}	6.331***
Root DW	3.145*	0.949^{ns}	6.300***
Shoot Na^+ concentration	478.127***	19.154***	5.280***
Root Na^+ concentration	187.353***	30.445***	4.409**
Shoot K^+ concentration	20.622***	4.445*	2.487^{ns}
Root K^+ concentration	12.991***	4.586*	0.950^{ns}
Shoot Na^+/K^+ ratio	209.776***	5.286*	7.257***
Root Na^+/K^+ ratio	121.997***	4.284*	3.074*
SA value	181.342***	5.743*	10.608***
ST value	31.452***	0.0432^{ns}	0.620^{ns}
Net Na^+ uptake rate	113.235***	3.627^{ns}	2.977*
Net K^+ uptake rate	18.820***	1.112^{ns}	6.170***
Shoot soluble sugars content	11.423***	2.230^{ns}	2.084^{ns}
Root soluble sugars content	3.593*	0.822^{ns}	4.386**

*, ** and *** indicate significant differences at $P<0.05$, $P<0.01$, and $P<0.001$, respectively

ns non-significant difference

compared after 7 days treated with different concentrations of NaCl. It was showed that Na^+ concentrations of both shoots and roots in tetraploid and diploid significantly increased after 7 days of the growth in 50–200 mM NaCl in comparison with the control, the degree of increase was always higher in roots than in shoots at NaCl treatments (Fig. 3a, b). Under high salt (200 and 300 mM), Na^+ concentrations of shoot and root in tetraploid were 9.1%, 20.8%, and 16.1%, 20.7% lower than those in diploid (Fig. 3a, b), respectively. It was investigated that the effect of salinity, ploidy, and salinity × ploidy interaction was significant for Na^+ concentrations of both shoot and root, respectively (Table 2). The increase of NaCl concentrations in medium solution decreased K^+ accumulation of shoots and roots in two cultivars (Fig. 3a). Although K^+ concentrations of shoot in diploid were clearly higher than those in tetraploid at 0 and 50 mM NaCl, there were no differences between tetraploid and diploid at 100–300 mM NaCl (Fig. 3c). Results of two-way ANOVA showed that the effect of either salinity or ploidy alone was significant for K^+ concentration of both shoot and root, while the salinity × ploidy interaction effect was not statistically significant (Table 2).

To determine the differences of tissue's ion accumulation at time course, concentrations of Na^+ and K^+ were observed in both tetraploid and diploid subjected with additional 50 mM NaCl over a 120-h period. Na^+ concentrations of shoot and root in tetraploid and diploid displayed the increased trends with the prolonging of treatment time (Fig. 4a, b). However, at 120 h of 50 mM NaCl treatment, tetraploid accumulated less Na^+ in shoots compared to diploid (Fig. 5a). At 72, 86, and 120 h after treatment of NaCl, tetraploid also accumulated less Na^+ in root than diploid (Fig. 5b). In addition, K^+ concentrations of shoot and root showed the decreased trends with the prolonging of treatment time after 24 h of salt treatment (Fig. 5c, d). Diploid accumulated more K^+ in roots than tetraploid from 48 to 120 h of NaCl treatment (Fig. 5d).

We further observed Na^+/ K^+ ratio of shoot and root in tetraploid and diploid exposed to various concentrations of NaCl. It is clear that Na^+/ K^+ ratios of shoot and root in tetraploid were 23.8% and 21.1% lower than those in diploid at 300 mM NaCl (Fig. 6a, b), respectively. It was also found that effect of salinity, ploidy, and salinity × ploidy interaction was significant for Na^+/K^+ ratios of both shoots and roots, respectively (Table 2).

SA and ST values in response to salt stress

At 300 mM NaCl, SA values were remarkably higher in tetraploid than in diploid (Fig. 7a). However, there was no clear difference in ST values between tetraploid and diploid exposed to 50–300 mM NaCl (Fig. 7b). The results of two-way ANOVA showed that the effect of ploidy and salinity × ploidy interaction on SA value was significant, while the effect on ST value was not significant (Table 2).

To further estimate the observed differences for the selective absorption and transport capacity for K^+ over Na^+ at time course, values of SA and ST in both diploid and tetraploid were investigated at additional 50 mM NaCl over a 120-h period. Value of SA in tetraploid and diploid showed the sharply declined trends with the prolonging of treatment time (Fig. 8a). At 72 and 96 h after treatment of NaCl, value of SA in tetraploid was remarkably higher than that in diploid (Fig. 8a). At 24 and 120 h after NaCl treatment, value of ST in tetraploid was significantly higher than that in diploid (Fig. 8b).

Net uptake rates of Na^+ and K^+ in response to salt stress

Net Na^+ uptake rates in both tetraploid and diploid displayed the clearly increased trends with the increase of salt concentrations (Fig. 9a). No remarkable differences were found between tetraploid and diploid exposed to 50–200 mM NaCl (Fig. 9a). However, net Na^+ uptake rate in tetraploid was 19.9% smaller than that in diploid exposed to 300 mM NaCl (Fig. 9a). Furthermore, net K^+ uptake rates in both tetraploid and diploid displayed the drastically reduced trends with the

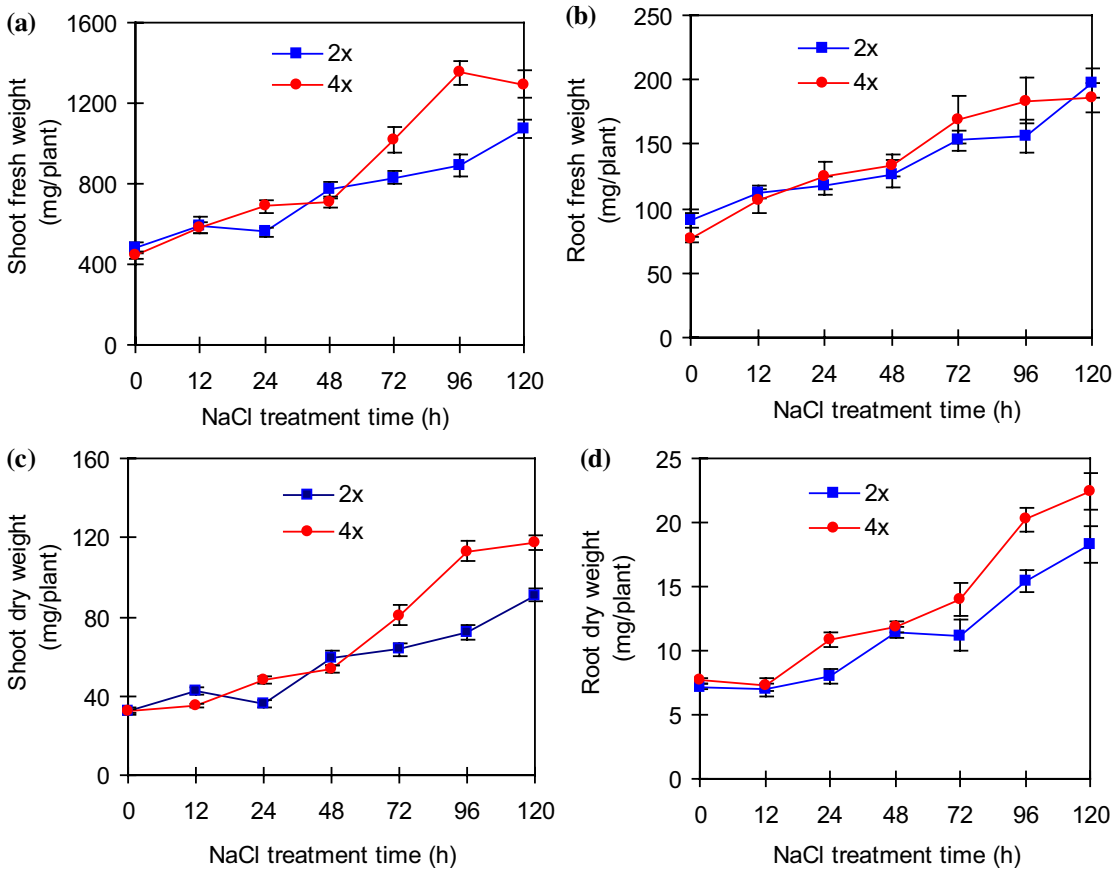

Fig. 2 Fresh (**a, b**) and dry (**c, d**) weights of shoot and root in diploid (2*x*) and tetraploid (4*x*) sugar beet seedlings during 0-120 h treatment with 50 mM NaCl. Two plants were pooled in each replicate ($n=8$). Values are means ± SE and bars indicate SE

increasing NaCl in the nutrient solution (Fig. 9b). There was no significant difference in net K^+ uptake rate between tetraploid and diploid treated with 50–200 mM NaCl (Fig. 9b). However, net K^+ uptake rate in tetraploid was 54.5% higher than that in diploid exposed to 300 mM NaCl (Fig. 9b). Our further analysis showed that although effects of ploidy on net uptake rate of both Na^+ and K^+ were not significant, interactive effect of salinity and ploidy was significant (Table 2).

Soluble sugars in response to salt stress

In comparison with the corresponding control, 50 mM NaCl did not obviously affected shoot soluble sugars contents in both diploid and tetraploid. At 100 and 200 mM NaCl, soluble sugars contents of shoots remarkably reduced by 17.1% and 42.3% in diploid, respectively, whereas remained unchanged in tetraploid (Fig. 10a). Moreover, 300 mM NaCl observably reduced shoot soluble sugar accumulation in both diploid and tetraploid compared to the corresponding control, but greater reduction was observed in diploid (Fig. 10a). In addition, 50 and 100 mM NaCl remarkably increased root soluble sugars contents by 27.6% and 26.2% in tetraploid, respectively (Fig. 10b). It was also found that interactive effect of salinity and ploidy was significant for soluble sugars in root but not shoot (Table 2).

Expression levels of genes related to ion transport response to salt treatment

To determine the differences of genes related to Na^+ and K^+ transport at the transcriptional level, the expression abundances of six genes were analyzed by qRT-PCR method in roots of both tetraploid and diploid plants treated with 50 mM NaCl for 0, 3, 6, 12, 24, and 48 h. Our results showed that the expression level of *BvAKT1* was evidently lower in tetraploid than in diploid at 3, 12, and 48 h of salt treatment (Fig. 11a). However, *BvHAK5* displayed the clearly higher transcript abundance in tetraploid than in diploid at 6 and 24 h of salt treatment (Fig. 11b). The expression level of *BvHKT1;1* in tetraploid was also remarkably higher than that in diploid at 3, 6, and 12 h of salt treatment (Fig. 11c). In addition, the transcript level of *BvNHX1* in tetraploid was

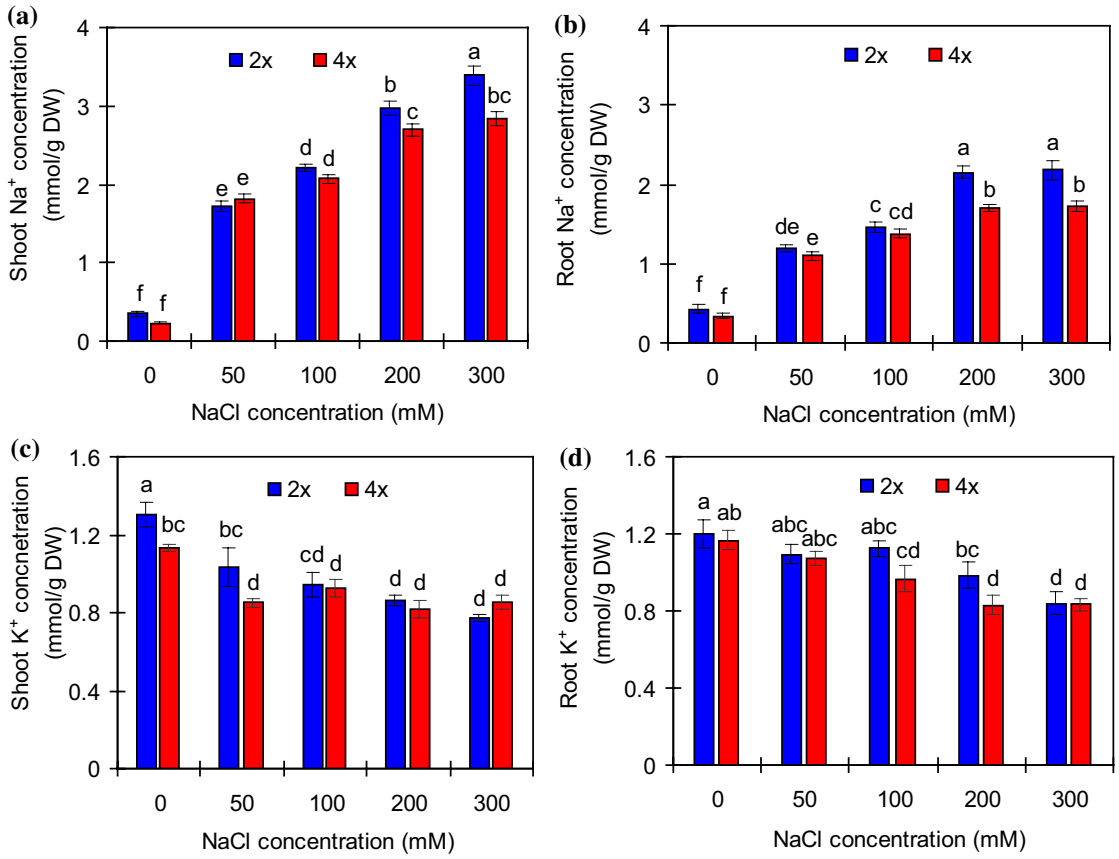

Fig. 3 Na^+ (**a, b**) and K^+ (**c, d**) concentration of shoot and root in diploid (2*x*) and tetraploid (4*x*) sugar beet seedlings exposed to 0, 50, 100, 200, and 300 mM NaCl for 7 days. Two plants were pooled in each replicate ($n=8$). Values are means ± SE and bars indicate SE. Columns with different letters indicate significant differences at $P<0.05$ (Duncan's test)

obviously higher than that in diploid at 6 and 48 h after salt treatment (Fig. 11d). Moreover, *BvSKOR* had significantly higher expression level in tetraploid than in diploid at 6, 12, and 48 h of salt treatment (Fig. 11e). Furthermore, the relative expression level of *BvSOS1* in tetraploid was observably higher than that in diploid at 3, 6, and 48 h after salt treatment (Fig. 11f).

Discussion

There are evidences that the differences between tetraploid and diploid plants include morphological, physiological, biochemical, and cellular and molecular aspects (Beyaz et al. 2013; Tu et al. 2014). In general, tetraploid plants exhibit bigger stomata and cells compared to diploid ones, thereby leading to larger and thicker leaves, greater flowers, fruits, and seeds (Xue et al. 2015, 2017). In this study, we compared differences of salinity tolerance between tetraploid and diploid sugar beet cultivars subjected with salinity stress, and found that salinity tolerance of tetraploid cultivar was significantly higher than that of diploid.

Tetraploid accumulated greater biomass than diploid in sugar beet under salt stress

It is accepted that some plants, including glycophytes and halophytes, displayed great difference in salinity tolerance, as reflected in different responses of their growth (Flowers and Colmer 2010). Biomass is often thought to be an important criterion for identifying salt tolerance in plants (Bao et al. 2009; Wu et al. 2013). In tetraploid, NaCl concentrations of 50, 100, 200, and 300 mM tremendously increased shoot fresh weigh and dry weight by 14.8–50.3% and 11.9–33.8% compared to those of control plants (Fig. 1a, c), respectively. However, in diploid, alone NaCl concentration of 50 mM significantly increased shoot FW and DW by 47.9% and 20.6% compared to corresponding control plants (Fig. 1a, c), respectively. These data further supported results of Wu et al. (2015a), who reported that suitable concentrations of salt were required for the normal growth and development in sugar beet plants.

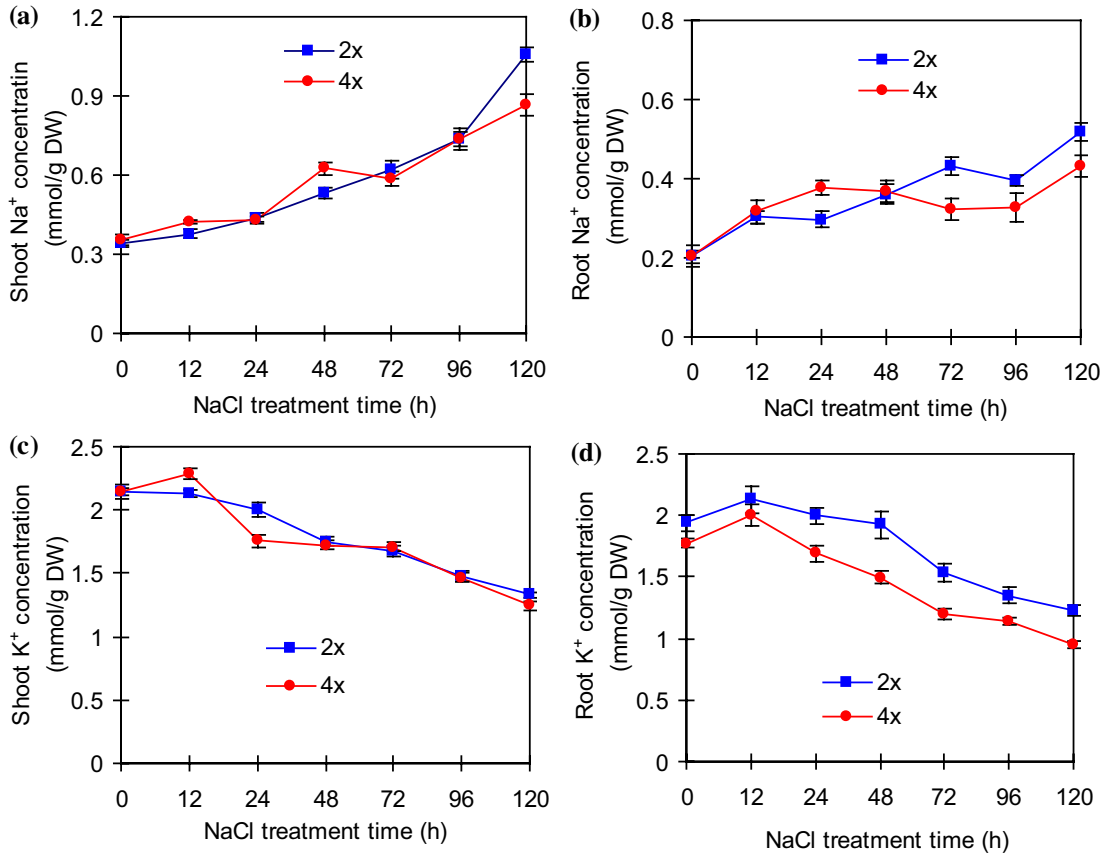

Fig. 4 Na^+ (**a, b**) and K^+ (**c, d**) concentration of shoot and root in diploid (2*x*) and tetraploid (4*x*) sugar beet seedling during 0–120 h treatment with 50 mM NaCl. Two plants were pooled in each replicate ($n = 8$). Values are means ± SE and bars indicate SE

Importantly, the present study observed that shoot FW and DW in tetraploid are remarkably higher than those in diploid when exposed to various concentrations of NaCl (except for FW at 200 mM) (Fig. 1a, c). Similarly, it was found that tetraploid had greater FW and DW of shoots in comparison with diploid at 72, 96, and 120 h after 50 mM NaCl treatment (Fig. 2a, c). However, no significant difference was recorded between tetraploid and diploid under the control condition (Fig. 1a–d). Similar results were also observed in honeysuckle (*Lonicera japonica*) as described by Yan et al. (2015), who reported that tetraploid plants displayed greater photosynthetic capacity and accumulated higher biomass than diploid ones when exposed to salinity. In rice, FW of root in tetraploid genotype was obviously higher than in diploid one under salt stress (Tu et al. 2014). These results implied that tetraploid possessed stronger salinity tolerance than diploid in sugar beet.

Tetraploid accumulated less Na^+ and maintained lower Na^+/K^+ under salt stress

It was well documented that the capacity to maintain intracellular K^+ and Na^+ homeostasis, especially higher K^+/Na^+ ratio, is thought as an important indicator of salt resistance in plants (Tu et al. 2014; Guo et al. 2015). In this study, Na^+ concentrations of both shoot and root exhibited the drastically increasing tendency in both diploid and tetraploid with the increase of salt concentrations, but the increased degrees in shoots were greater than those in roots (Fig. 3a, b). We further estimated Na^+ relative distribution of different plant parts and found that 81.0–86.2% of total Na^+ amounts were accumulated in shoot (data not shown). The higher Na^+ accumulation in shoots may be beneficial by helping plants maintain cell turgor, thereby maintaining capacity of uptake water. Similar results were recorded in the halophytes such as genus *Atriplex* (Bose et al. 2015; Pan et al. 2016) and *Suaeda maritima* (Wang et al. 2007), and the xerophytes such as *Z. xanthoxylum* (Ma et al. 2012) and *Haloxylon ammodendron* (He et al. 2018). It was accepted that Na^+ is considered as a beneficial element for many species of Chenopodiaceae family, including spinach (*Spinacia oleracea*) and sugar beet (Kronzucker et al. 2013). However, excessive Na^+ in the cytosol has been demonstrated to be deleterious to the cells by disrupting acquisition of K^+, inhibiting functional enzymes and protein synthesis,

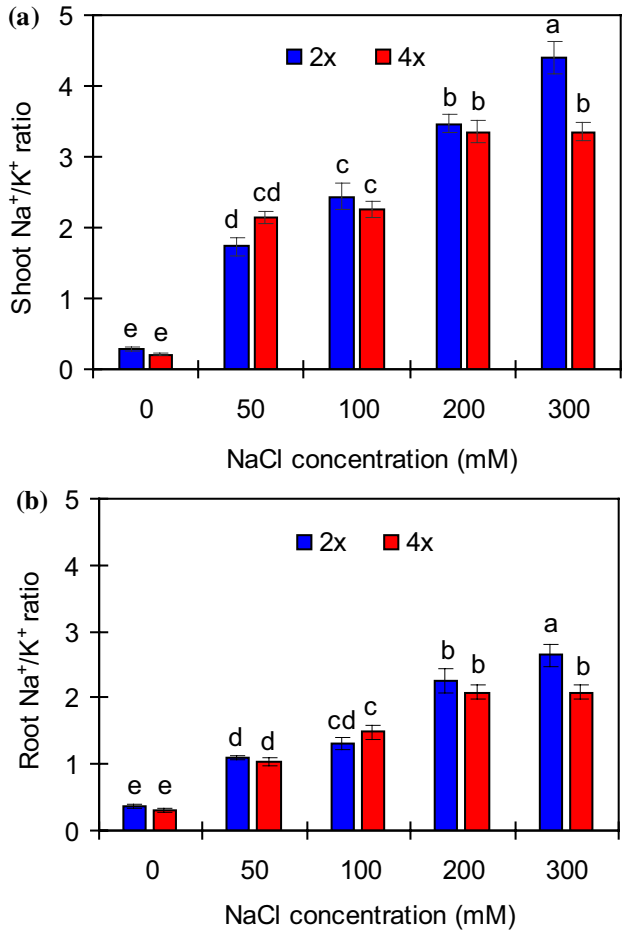

Fig. 5 Na^+/K^+ ratio of shoot (**a**) and root (**b**) in diploid (2*x*) and tetraploid (4*x*) sugar beet seedling exposed to 0, 50, 100, 200 and 300 mM NaCl for 7 days. Two plants were pooled in each replicate ($n=8$). Values are means ± SE and bars indicate SE. Columns with different letters indicate significant differences at $P<0.05$ (Duncan's test)

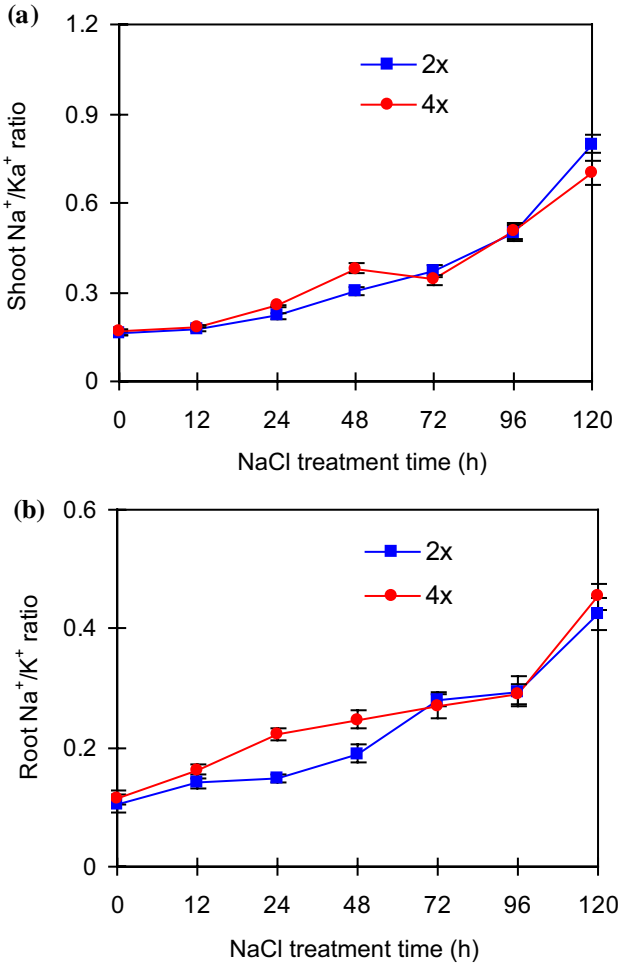

Fig. 6 Na^+/K^+ ratio of shoot (**a**) and root (**b**) in diploid (2*x*) and tetraploid (4*x*) sugar beet seedlings during 0–120 h treatment with 50 mM NaCl. Two plants were pooled in each replicate ($n=8$). Values are means ± SE and bars indicate SE

and resulting in oxidative stress in plants (Kronzucker et al. 2013). Thus, the maintenance of less Na^+ concentration in the cytoplasm is crucial for plants adapting to saline environments (Munns and Tester 2008; Flowers and Colmer 2010; Guo et al. 2015). In this study, it was noteworthy that Na^+ concentrations of both shoot and root were pronouncedly lower in tetraploid than in diploid when challenged by 200 and 300 mM NaCl (Fig. 3a, b). Similar results were reported in *A. thaliana* (Chao et al. 2013), rice (Tu et al. 2014), and *Citrus macrophylla* (Ruiz et al. 2016). A lower net Na^+ uptake rate was found in tetraploid compared with diploid subjected to high concentration of NaCl (Fig. 9a), which may be partly due to reduce Na^+ influx into roots and/or enhance Na^+ efflux to the growth medium in tetraploid. These results implied that tetraploid is able to efficiently restrict Na^+ transport to shoots from roots, and further avoiding the excessive accumulation of Na^+ in the shoots damaging the photosynthetic tissues.

Furthermore, our results showed that K^+ concentrations of shoots and roots displayed the slightly decreased trends in both tetraploid and diploid with the increase of NaCl concentrations (Fig. 3c, d). Although there are no obvious differences in K^+ accumulation of shoots and roots between tetraploid and diploid (Fig. 3c, d), net K^+ uptake rate was obviously higher in tetraploid than in diploid when challenged by 300 mM NaCl (Fig. 10b). In *A. thaliana*, however, tetraploids had elevated K^+ concentration of leaf compared with diploids (Chao et al. 2013). Interestingly, our data showed that tetraploid exhibited greater capacity of selective absorption for K^+ over Na^+ compared to diploid (Fig. 7a), thereby contributing to maintain a smaller Na^+/K^+ ratio of shoots and roots in tetraploid than diploid exposed to high-salt stress (Fig. 5a, b). It was documented that the selective capacity for K^+ over Na^+ is a critical determinant of salinity resistance, and it relies on the characteristics of ion transporters that mediate uptake of Na^+ and K^+ (Kronzucker and

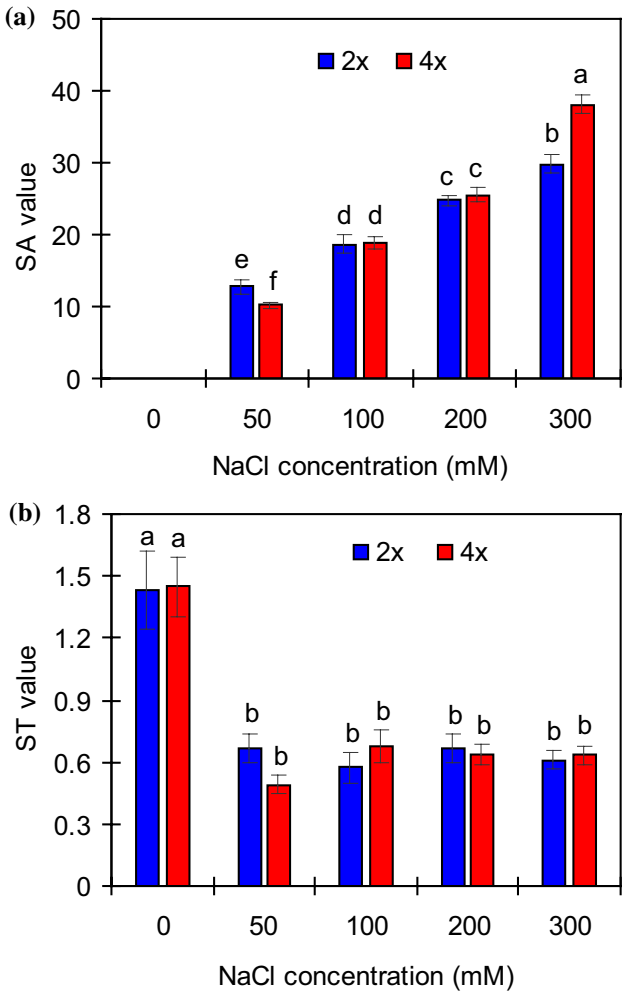

Fig. 7 SA (**a**) and ST (**b**) value in diploid (2*x*) and tetraploid (4*x*) sugar beet seedlings exposed to 0, 50, 100, 200, and 300 mM NaCl for 7 days. Two plants were pooled in each replicate ($n=8$). Values are means ± SE and bars indicate SE. Columns with different letters indicate significant differences at $P<0.05$ (Duncan's test)

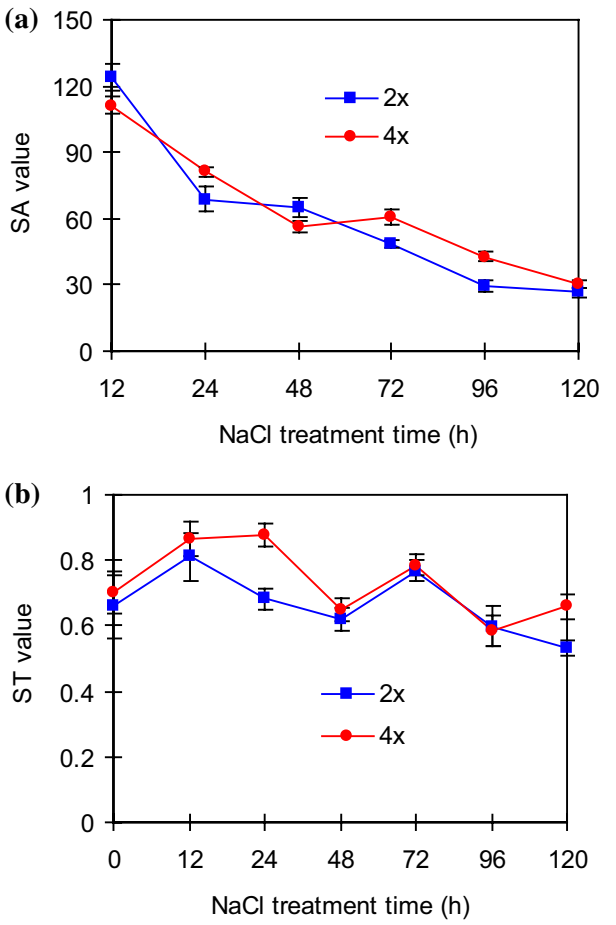

Fig. 8 SA (**a**) and ST (**b**) value in diploid (2*x*) and tetraploid (4*x*) sugar beet seedlings during 0–120 h treatment with 50 mM NaCl. Two plants were pooled in each replicate ($n=8$). Values are means ± SE and bars indicate SE

Britto 2011; Guo et al. 2015). AKT1, a shaker-like family K^+ channel, has been shown to be mediator of K^+ absorption by roots from micromolar to millimolar concentrations (Alemán et al. 2011; Nieves-Cordones et al. 2014). Overexpression of *Puccinellia tenuiflora PtAKT1* leads to not only increase K^+ accumulation but also reduce Na^+ concentration in *A. thaliana* plants (Ardie et al. 2010). This suggested that AKT1 exhibits a high capacity of selective absorption for K^+ over Na^+ (Ardie et al. 2010; Wang et al. 2015). In this study, the transcript level of *BvAKT1* was down-regulated in both diploid and tetraploid cultivars at 6 and 48 h after 50 mM NaCl treatment (Fig. 11a). Similar expression patterns in *AKT1* were recorded in rice (Fuchs et al. 2005), *A. thaliana* (Kaddour et al. 2009), and *P. tenuiflora* (Wang et al. 2015). It was found that transcript level of *BvAKT1* in tetraploid was significantly lower than that in diploid at 3, 12, and 48 h of salt treatment (Fig. 11a). In rice cultivars with different salt resistance, the transcript level of *OsAKT1* in the resistant cultivar "Pokkali" was lower than that in the sensitive cultivar "IR29" in response to salt stress (Golldack et al. 2003). Our previous studies indicated that K^+ absorption was sensitive to Ba^{2+} or Cs^+, whereas insensitive to TEA^+, suggesting that AKT1 may mediate K^+ absorption in sugar beet plants (Wu et al. 2015b). The results of present studies further demonstrated that BvAKT1 plays a vital function in the selective absorption for K^+ over Na^+ in tetraploid cultivar subjected to salt treatment. In *A. thaliana*, K^+ absorption from the soil was mediated by AKT1 and HAK5 transporters (Pyo et al. 2010; Nieves-Cordones et al. 2010; Li et al. 2018). Overexpression of *OsHAK5* enhanced K^+/Na^+ ratios in shoot tissues and salinity resistance, whilst loss-of-function of *OsHAK5* reduced K^+/Na^+ ratios in shoot tissues, leading to sensitive to salinity stress (Yang et al. 2014b). In this study, *BvHAK5* displayed higher transcript abundance in tetraploid than in diploid at 6 and 24 h of salt

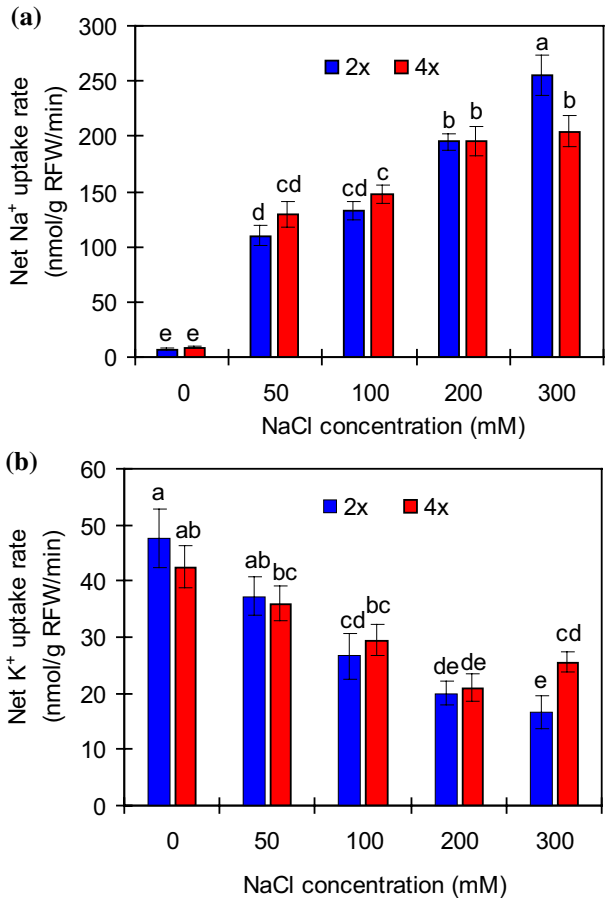

Fig. 9 Net Na^+ (**a**) and K^+ (**b**) uptake rate in diploid (2*x*) and tetraploid (4*x*) sugar beet seedlings exposed to 0, 50, 100, 200, and 300 mM NaCl for 7 days. Two plants were pooled in each replicate ($n=8$). Values are means ± SE and bars indicate SE. Columns with different letters indicate significant differences at $P<0.05$ (Duncan's test)

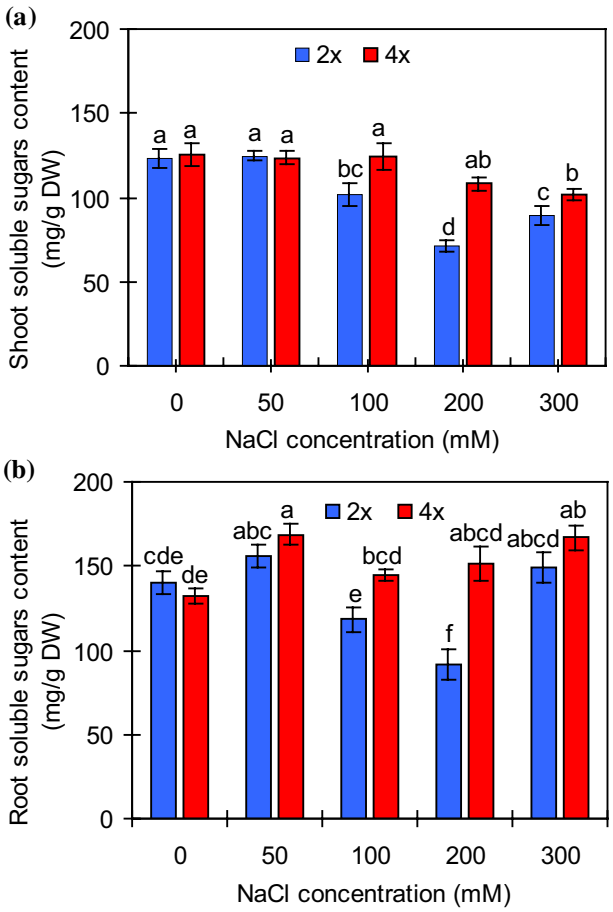

Fig. 10 Soluble sugar content of shoot (**a**) and root (**b**) in diploid (2*x*) and tetraploid (4*x*) sugar beet seedlings exposed to 0, 50, 100, 200, and 300 mM NaCl for 7 days. Two plants were pooled in each replicate ($n=8$). Values are means ± SE and bars indicate SE. Columns with different letters indicate significant differences at $P<0.05$ (Duncan's test)

treatment (Fig. 11b). It was shown that HKTs were involved in controlling K^+ and Na^+ transporter and maintaining ion homeostasis in plants under salt stress (Zhang et al. 2010, 2012; Hamamoto et al. 2014). AtHKT1;1 has been shown to selectively unload Na^+ directly from the xylem vessels to the xylem parenchyma cells and thus decrease accumulation of excessive Na^+ in the xylem vessels in shoots and roots, thereby preventing shoots from Na^+ toxicity (Sunarpi et al. 2005). Similar results were recorded for OsHKT1;5 that involved in salinity tolerance by retrieving Na^+ from the ascending xylem sap in the roots, thus, limiting Na^+ levels in the shoots of rice plants (Ren et al. 2005). *OsHKT1;5* and *AtHKT1;1* are both expressed preferentially in the parenchyma cells surrounding the xylem vessels of rice and *A. thaliana*, respectively, and their expression levels were up-regulated by salt treatment (Ren et al. 2005; Sunarpi et al. 2005). Our results also showed that the expression level of *BvHKT1;1* was up-regulated alone in tetraploid during 3–24 h of salt treatment (Fig. 11c), implying that BvHKT1;1 plays an important role in retrieving Na^+ from the xylem sap to the parenchyma cells in the roots of tetraploid. Similar results were found in the roots of hexaploidy wheat, where *TaHKT1;5-B* and *TaHKT1;5-D* showed a significant up-regulation when plants were subjected with salt condition (Yang et al. 2014a). It was well known that sequestering Na^+ into vacuoles is a crucial strategy that the plant cells employ for the mitigation of excessive accumulation of Na^+ in the cytoplasm (Kronzucker and Britto 2011; Yamaguchi et al. 2013). NHX1 plays vital in sequestering Na^+ into the vacuoles to maintain Na^+ homeostasis, and, thus, to improve salinity resistance in plants (Chen et al. 2008; Wu et al. 2011). Our previous studies showed that *BvNHX1* was induced by salt, and its transcript abundance was clearly higher in the shoots than in the roots of sugar beet plants (Wu et al. unpublished data). In the present study, the expression level of *BvNHX1* displayed the obviously increased trends in roots of both

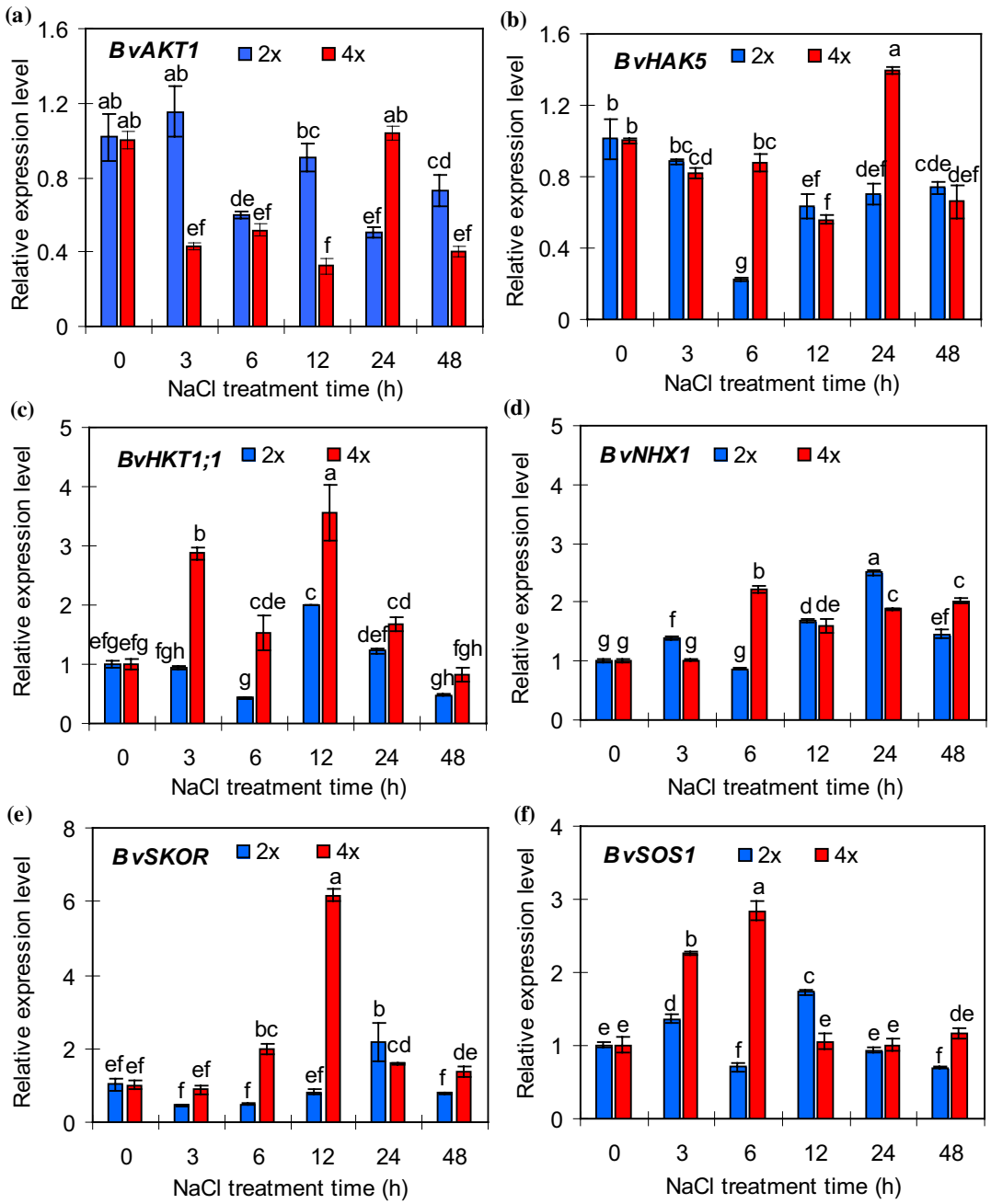

Fig. 11 Relative expression level of *BvAKT1* (**a**), *BvHAK5* (**b**), *BvHKT1;1* (**c**), *BvNHX1* (**d**), *BvSKOR* (**e**), and *BvSOS1* (**f**) in root of sugar beet seedlings exposed to 50 mM NaCl for 0, 3, 6, 12, 24, and 48 h. *BvACTIN1* was used as an internal control. Experiments were repeated at least three times. Values are means ± SE and bars indicate SE. Columns with different letters indicate significant differences at $P < 0.05$ (Duncan's test)

diploid and tetraploid with the prolonging of salt treatment time (Fig. 11d). Compared with diploid, the transcript abundances in tetraploid were remarkably lower at 3 and 24 h, whereas observably higher at 6 and 48 h of salt treatment (Fig. 11d). Furthermore, SOS1 plays key roles in the long-distance Na^+ transport (Shi et al. 2002b). It was observed that *AtSOS1* was expressed preferentially in the roots and induced obviously by salt stress (Shi et al. 2002a, b). Similar expressional patterns were recorded for *P. tenuiflora PtSOS1* (Ardie et al. 2010; Guo et al. 2012), *Z. xanthoxylum ZxSOS1* (Ma et al. 2014), and wheat *TaSOS1* (Zhou et al. 2016). It was shown that AtSOS1 was involved in loading Na^+ into the xylem of roots for regulating Na^+ transport to the shoots and storage in the leaf mesophyll cellsin plants under mild

salinity (Shi et al. 2002b). In the present study, *BvSOS1* was remarkably up-regulated in both two cultivars at the short-term of NaCl treatment (3–12 h), and its expression level was obviously higher in tetraploid than in diploid at 3 and 6 h after 50 mM NaCl treatment (Fig. 11f). These results indicated BvSOS1 may function in transport Na^+ to the shoots by loading Na^+ into the xylem sap in tetraploid in the initial period of salt treatment. Moreover, in the present study, tetraploid displayed a greater net K^+ uptake rate and a smaller net Na^+ uptake rate than those of diploid under saline conditions (Fig. 9a, b), which contributed to maintain a stronger capacity of selective absorption for K^+ over Na^+. Taken together, these results indicated that tetraploid can control K^+ and Na^+ homeostasis by improving capacity of selective absorption for K^+ over Na^+ under salt stress.

Tetraploid accumulated higher level of soluble sugars than diploid under salinity stress

It is well known that plants also suffer from osmotic stress when subjected to salinity (Apse and Blumwald 2002). To cope with osmotic stress caused by salinity, the maintenance of normal turgor pressure by accumulating organic compounds, such as soluble sugar and protein, is one of most common strategies (Apse and Blumwald 2002; Zhang et al. 2012; Li et al. 2017). Soluble sugar has been documented to be key osmolyte contributing to osmotic adjustment under saline condition (Radić et al. 2013; Li et al. 2017). It can also enhance salt resistance by stabilizing cell membranes and by protecting enzymes when plants were subjected to salt stress (Gupta and Huang 2014). Therefore, soluble sugar may be considered as an important parameter for assessment of salt tolerance in crops (Apse and Blumwald 2002; Wu et al. 2013). In the present study, additional 50 and 300 mM NaCl in the medium notably enhance root soluble sugars in tetraploid (Fig. 10b). Similar results were found in halophyte *Thellungiella halophila*, where salinity stress significantly increased accumulation of sucrose and total sugars in leaves (Wang et al. 2013b). In this study, although no obviously difference in soluble sugar contents of shoots and roots was observed between tetraploid and diploid at 0 and 50 mM NaCl, soluble sugar contents were obviously greater in tetraploid than diploid at 100 and 200 mM NaCl (Fig. 10a, b). Tu et al. (2014) reported that polyploidization resulted in a similar increase in soluble sugars accumulation in different rice cultivars under salinity stress, and the differences were pronounced between tetraploid and diploid rice exposed to salinity stress. Furthermore, tetraploid also clearly accumulated more soluble sugars in shoots but not in roots compared to diploid under 300 mM NaCl (Fig. 10b). Our results suggested that accumulation of larger amounts of soluble sugars might be the key mechanism that confers greater tolerance to salinity in tetraploid sugar beet cultivar.

In conclusion, our results suggested that tetraploid exhibits higher salinity tolerance than diploid in sugar beet by accumulating less Na^+ and more soluble sugars and by maintaining smaller Na^+/K^+ ratio and greater selectivity for K^+ over Na^+. The results of the present study provide insights into physiological and molecular consequences of autopolyploidization in sugar beet.

Author contribution statement G.-Q. Wu designed the research, and wrote and revised the article. L.-Y. Lin and Q. Jiao conducted the research. S.-J. Li analyzed the data. All authors read and approved the final manuscript.

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