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Effects of Salt Stress on the Growth, Physiological Responses, and Glycoside Contents of *Stevia rebaudiana* Bertoni

Jianwei Zeng, Aimeng Chen, Dandan Li, Bin Yi, and Wei Wu*

Agronomy College, Chengdu Campus, Sichuan Agricultural University, Wenjiang 611130, China

ABSTRACT: This study examined the effects of three different NaCl concentrations (60, 90, and 120 mM) on the growth, physiological responses, and steviol glycoside composition of *Stevia rebaudiana* Bertoni for 4 weeks. The results showed that the total dry weight decreased by 40% at 120 mM NaCl but remained the same at 60 and 90 mM NaCl. As salt concentration increased, chlorophyll contents decreased markedly by 10−70%, whereas the increments of the antioxidant enzyme activities were 1.0−1.6, 1.2−1.3, and 2.0−4.0 times, respectively, for superoxide dismutase, peroxidase, and catalase. The proline contents in salt-treated plants were 17−42 times higher than that in control. Moreover, leaf possessed significantly higher K^+ content and K^+/Na^+ ratio than stem and root for all salt treatments. In addition, 90−120 mM NaCl treatment notably decreased the content of rebaudioside A (RA) and stevioside (ST) by 16.2−38.2%, whereas the increment of the ratio of RA/ST of salt-treated plants was 1.1−1.4 times. These results indicate that *S. rebaudiana* is moderately tolerant to salt stress. Hypohaline soil can be utilized in the plantation of *S. rebaudiana* and may be profitable for optimizing the steviol glycoside composition.

KEYWORDS: *Stevia rebaudiana, growth, physiological response, glycoside contents, salt stress*

INTRODUCTION

As the global climate changes, water resources become more scarce in many regions of the world, especially irrigation water. A large amount of land that is irrigated with poor quality water has gradually turned into salty land.[1] Salt imposes a deteriorative impact on at least 800 million hectares of land in the world. It is estimated that about 20% of irrigated land, which produces one-third of the world's food, is salt-affected.[2] Exploiting saline soil becomes more urgent when saline degradation of plowland has proved to be a world problem. Selecting and culturing salt-tolerant crops in saline soil is an efficient strategy for better utilization of limited land resources. Salinity is a soil condition defined as a high content of soluble salts. NaCl is the most soluble and widespread salt contained in soil.[3]

Stevia rebaudiana Bertoni is a perennial shrub widely planted in many countries such as China, Japan, Korea, India, and certain countries of South America.[4] Stevioside (ST) and rebaudioside A (RA) synthesized in the leaves of *S. rebaudiana* are the main diterpenoid glycosides (Figure 1). ST has been applied as a substitute for saccharose, and in the treatment of diabetes mellitus, obesity, and hypertension and in caries prevention.[5] RA not only has the similar uses as ST but also possesses a more excellent flavor than ST, which may suggest a larger promising market of RA.[6] Thus, many plant culture practices aiming to increase the leaf yield and rebaudioside A content have been used.[7,8]

China not only holds the largest area of *S. rebaudiana* plantation but also is the biggest supplier of *S. rebaudiana* extracts in the world. The main plantation area of *S. rebaudiana* in China covers the provinces of Jiangsu, Shandong, Anhui, Helan, Zhejiang, and Xinjiang. However, with climate change and land clearing or irrigation, a large quantity of salt and alkali land have occurred in these areas, which possessed excellent light conditions for the growth and glycoside accumulation of *S. rebaudiana*. Therefore, it is necessary to understand the mechanisms of salt adaptation and tolerance of *S. rebaudiana*. Previous studies on plants under salt stress had revealed alterations in the biomass, antioxidant enzyme activities and osmolytes, mineral contents, and secondary metabolites.[9−11] However, as far as we know, salt effects on the survival and productivity of *S. rebaudiana* have rarely been reported.[12,13] The present study has been executed with the objectives of evaluating the effects of different NaCl concentrations on the growth, physiological responses, and main steviol glycoside composition of *S. rebaudiana*.

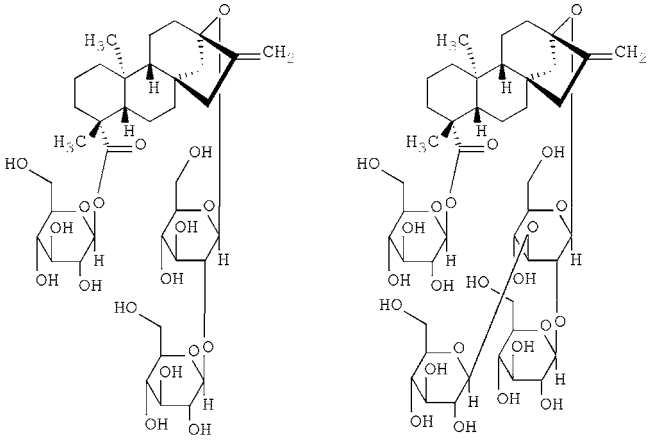

Figure 1. Chemical structures of stevioside and rebaudioside A.

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Table 1. Effects of Different NaCl Concentrations on the Growth Parameters of *Stevia rebaudiana* after 28 Days of Treatment[a]

	NaCl			
growth parameter	0 mM	60 mM	90 mM	120 mM
height (cm)	31.2 ± 1.8 a	24.1 ± 0.9 b	21.1 ± 1.5 c	21.6 ± 1.9 c
leaf number	42 ± 3 a	34 ± 2 b	27 ± 3 b	27 ± 6 b
branch length (cm)	18.73 ± 1.3 a	11.43 ± 0.6 b	10.30 ± 1.8 b	10.03 ± 1.1 b
total DW (g plant^{-1})	1.19 ± 0.11 a	1.11 ± 0.06 a	0.97 ± 0.19 a	0.71 ± 0.10 b
stem DW (g plant^{-1})	0.26 ± 0.02 a	0.22 ± 0.00 b	0.22 ± 0.01 b	0.19 ± 0.01 c
root DW (g plant^{-1})	0.12 ± 0.01 a	0.16 ± 0.02 a	0.14 ± 0.03 a	0.13 ± 0.03 a
leaf FW (g plant^{-1})	3.16 ± 0.11 a	2.82 ± 0.11 ab	2.53 ± 0.11 b	0.88 ± 0.27 c
leaf DW (g plant^{-1})	0.63 ± 0.07 a	0.63 ± 0.07 a	0.52 ± 0.13 b	0.33 ± 0.09 c
shoot/root ratio	8.61 ± 0.43 a	5.95 ± 0.53 b	6.03 ± 0.05 b	4.30 ± 0.82 c
shoot DW (g plant^{-1})	1.07 ± 0.10 a	0.95 ± 0.04 b	0.93 ± 0.06 b	0.55 ± 0.11c

[a]Data are the mean ± SE (n = 3). Different letters following values in the same row indicate significant difference among salt treatments using Duncan's multiple-range test at $p < 0.05$.

MATERIALS AND METHODS

Plant Materials. The experimental plants (SR-4) used in this study were provided by Chengdu Wagott Pharmaceutical Co., Ltd., and identified by Prof. Wei Wu of Sichuan Agricultural University as *Stevia rebaudiana* Bertoni. The experiment was conducted in a controlled chamber provided with lamplight, a humidifier, and and air conditioner on the Chengdu campus of Sichuan Agricultural University, Sichuan, China, from June to August 2012. In June, similarly sized plants were selected and cultured in a 72-hole culture tray (6 × 12). Plants were fixed with the mixture of quartz sand and white stone (1:1) and then placed in a stainless steel tray supplied with modified Hoagland's solution for adapting to the culture condition. Four lateral buds were retained per plant when the buds appeared largely in the adaptation period, which lasted a month. Two weeks after the adaptation period, the experiment was carried out in a randomized design with three replications. Salt treatments including four NaCl concentrations (0 (control), 60, 90, and 120 mM) were conducted by adding NaCl to modified Hoagland's solution to reach the designed salt concentration. The experiment lasted 4 weeks. *S. rebaudiana* plants were irrigated with 3 L of modified Hoagland's solution, which contained the designed content of NaCl. The experimental solution was renewed every 3 days. The daily evapotranspiration volume of each tray was compensated by an equal quantity of distilled water. The modified Hoagland's solution contained 1.00 mmol L^{-1} $NH_4H_2PO_4$, 6.00 mmol L^{-1} KNO_3, 6.00 mmol L^{-1} $Ca(NO_3)_2$, 2.00 mmol L^{-1} $MgSO_4$, 46.26 μmol L^{-1} H_3BO_3, 9.15 μmol L^{-1} $MnCl_2$, 0.77 μmol L^{-1} $ZnSO_4$, 0.32 μmol L^{-1} $CuSO_4$, 0.11 μmol L^{-1} H_2MO_4, 10.00 μmol L^{-1} $FeSO_4$, and 10.00 μmol L^{-1} Na_2EDTA at pH 5.8. The chamber conditions were as follows: average day and night temperatures of the entire treatment period set at 22 and 16 °C, respectively; 16/8 h photoperiod at 7500 lx; and relative humidity controlled at 70% by a humidifier.

Growth Parameters. Five plants were harvested from each tray after 28 days of NaCl treatments. Plants were uprooted carefully, and the root was washed with distilled water and then dried with blotting paper. After height, leaf number, and leaf fresh weight had been recorded, different organs including stem, root, and leaf were oven-dried at 80 °C to constant weights and their dry weights were also recorded, respectively.

Determination of Chlorophyll Contents. At the end of treatment (28 days), the second fully expanded leaves close to the shoot tip were cut into disks of 1 cm^2. After that, fresh disks (0.10 g) were bleached with 50 mL of mixed liquid of ethanol/acetone (1:1) at room temperature for 48 h under darkness. Then, the suspension was centrifuged at 10000g for 5 min, and the supernatant was used for chlorophyll assay. Three samples were analyzed for each treatment. The contents of chlorophylls a and b were determined according to the published method by using a spectrophotometer (Shimazu, UV2450).[14] Measurement wavelengths were set at 645 and 663 nm. The chlorophyll contents were calculated by using the extinction coefficients and the equations given by Aron.[14]

Extraction and Assay of Antioxidant Enzymes and Proline. After 16 days of salt treatment, antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) and proline (Pro) in the leaves of *S. rebaudiana* were extracted and assayed. The extraction and measurement of antioxidant enzymes were carried out according to the method of Xu.[15] Briefly, fresh leaves (0.1 g) were homogenized with a mortar and pestle in 10 mL of 50 mM sodium phosphate buffer (pH 7.8 for SOD, pH 6.0 for POD, and pH 7.0 for CAT) containing 1% polyvinylpyrrolidone (w/v). The homogenate was centrifuged at 15000g for 10 min, and the supernatant as enzyme extract was used for antioxidative enzyme assay. The whole extraction procedure was carried out at 4 °C. SOD activity was determined by monitoring its ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. One unit of enzyme activity was described as the amount of enzyme causing 50% inhibition in reduction of NBT. POD activity was determined on the basis of guaiacol oxidation at 470 nm. One unit of POD activity was determined by the variety of absorbance in 0.01 min^{-1}. CAT activity was determined by monitoring the destruction of H_2O_2 at 240 nm. One unit of CAT activity was determined by the variety of absorbance in 0.01 min^{-1}. With regard to the proline determination, the procedure was as follows: fresh leaf (0.1 g) fragments were extracted with 3% aqueous sulfosalicylic acid (5 mL) in boiled water for 10 min and then centrifuged at 5000g for 5 min. After that, acid-ninhydrin (4 mL) and glacial acetic acid (2 mL) were added to the homogenate (2 mL) in a test tube. The mixture was incubated at 100 °C for 1 h, and then the reaction was terminated by placing the test tube in cold water. Toluene (4 mL) was added to each test tube and vortexed for 30 s. Proline contents contained in organic toluene phase were quantified by using Bate's method at 520 nm.[16]

Ion Determination. Four weeks after salt treatment, the plants of each treatment were harvested and divided into root, stem, and leaf. These organs were cleared and oven-dried separately. Powdered material (0.50 g) of root, stem, and leaf was digested completely with 25 mL of mixture acid of nitric acid and perchloric acid (4:1) contained in a Teflon beaker on an 80 °C electrothermal board, respectively. Na^+ and K^+ contents were determined with a flame photometer (INESA, 6400A), whereas Ca^{2+} and Mg^{2+} contents were measured with atomic absorption spectrophotometer (Hitachi, Z-2000) after the addition of 1‰ $SrCl_2$ as releasing regent.[17]

Extraction and Determination of Steviol Glycosides. Four weeks after NaCl treatment, the leaves were harvested, oven-dried, and powdered by using a grinder. The extraction method of glycosides was based on the published method.[18] Briefly, for each sample, leaf powder (1.00 g) was first extracted with 50 mL of 80 °C distilled water for 3 h, with shaking once every hour. After that, the mixture was purified with 0.16 g of mixture of $FeSO_4$ and $CaCl_2$ (5:3) and centrifuged at 10000g for 10 min, and the supernatant (30 mL) was diluted to 50 mL with distilled water. Finally, the diluted supernatant (2 mL) was filtered through a filter of 1 μm pore size for measurement. The HPLC analysis of stevioside and rebaudioside A contents was detailed as the

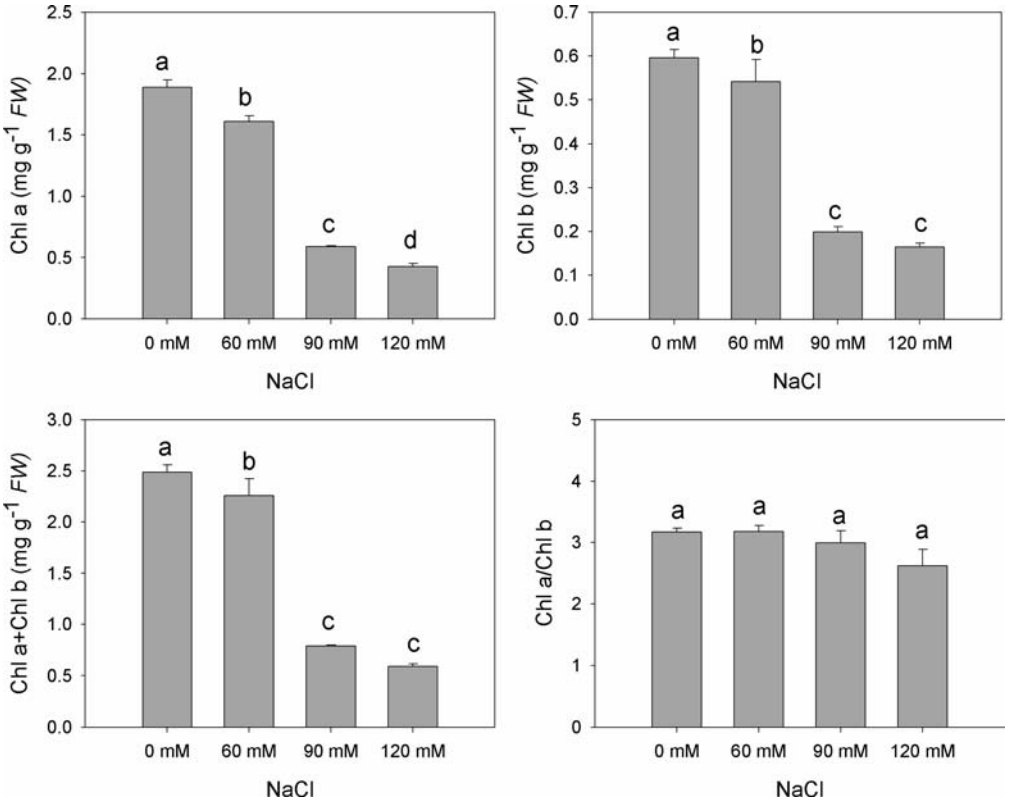

Figure 2. Effects of different NaCl concentrations on leaf chlorophyll contents of *Stevia rebaudiana* after 28 days of treatment. Values are expressed as the mean ± SE (n = 3). Bars carrying different letters are significantly different at $p < 0.05$ among NaCl treatments.

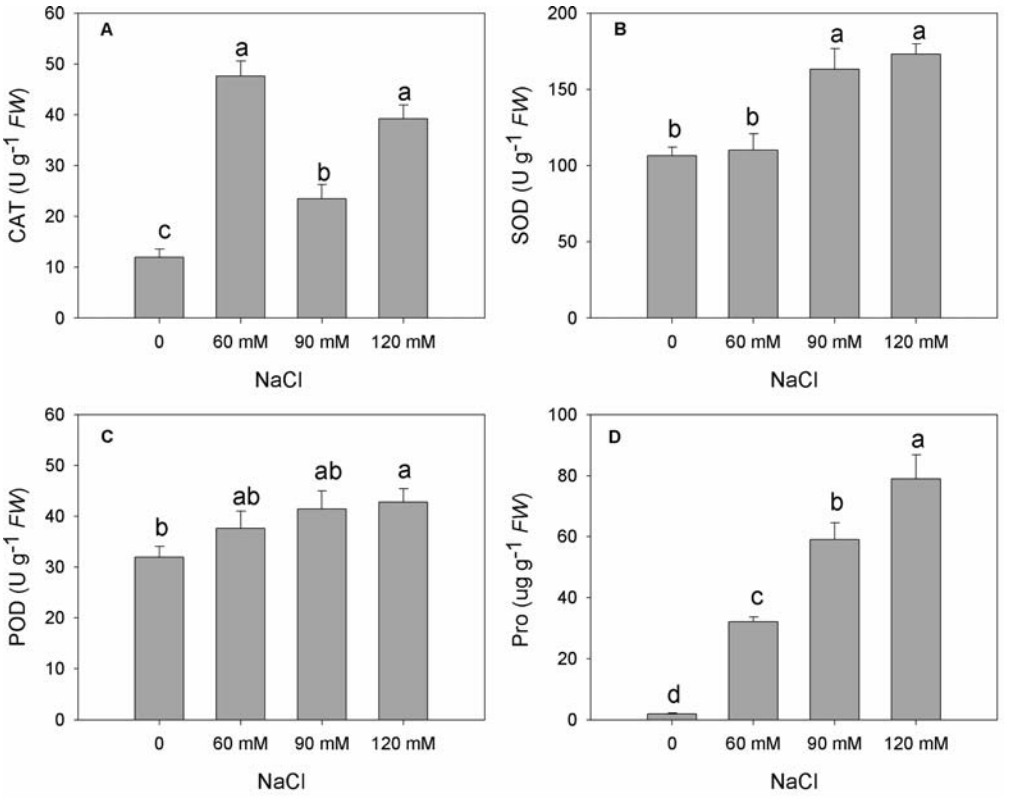

Figure 3. Effects of different NaCl concentrations on the activities of catalase (A), superoxide dismutase (B), and peroxidase (C) and proline contents (D) in the leaves of *Stevia rebaudiana* after 16 days of treatment. Bars are expressed as the mean ± SE (n = 3). Bars carrying different letters are significantly different at $p < 0.05$ among NaCl treatments.

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Table 2. Effects of Different NaCl Concentrations on Ion Contents in Different Organs of *Stevia rebaudiana* after 28 Days of Treatment[a]

ion parameter	NaCl (mM)	root	stem	leaf
K^+ (mg g^{-1} DW)	0	26.50 ± 0.77 a	21.83 ± 0.76 a	40.83 ± 1.26 ab
	60	21.17 ± 0.29 b	12.77 ± 0.25 d	41.33 ± 1.15 a
	90	16.33 ± 0.29 c	14.00 ± 0.00 c	38.50 ± 1.80 bc
	120	15.00 ± 0.00 c	16.33 ± 0.58 b	37.33 ± 0.58 c
Na^+ (mg g^{-1} DW)	0	3.53 ± 0.85 d	3.13 ± 0.12 d	0.53 ± 0.10 d
	60	27.23 ± 1.17 c	17.50 ± 0.50 c	10.75 ± 2.25 c
	90	34.40 ± 0.53 b	26.33 ± 0.58 b	34.00 ± 1.00 b
	120	37.03 ± 0.06 a	46.17 ± 1.26 a	54.00 ± 1.00 a
Ca^{2+} (mg g^{-1} DW)	0	12.79 ± 0.62 a	4.06 ± 0.39 b	5.21 ± 0.95 b
	60	6.41 ± 0.15 c	1.94 ± 0.29 c	2.56 ± 0.42 c
	90	9.82 ± 0.82 b	4.20 ± 0.19 b	3.52 ± 0.93 c
	120	9.46 ± 0.85 b	5.93 ± 0.87 a	8.02 ± 0.34 a
Mg^{2+} (mg g^{-1} DW)	0	0.81 ± 0.09 a	0.93 ± 0.05 b	1.58 ± 0.04 a
	60	0.60 ± 0.10 a	0.77 ± 0.02 c	1.40 ± 0.07 b
	90	0.72 ± 0.13 a	0.97 ± 0.01 b	1.44 ± 0.04 b
	120	0.66 ± 0.17 a	1.16 ± 0.06 a	1.55 ± 0.01 a
K^+/Na^+	0	9.63 ± 0.04 a	6.94 ± 0.08 a	76.92 ± 3.26 a
	60	0.78 ± 0.04 b	0.73 ± 0.01 b	3.85 ± 0.88 b
	90	0.47 ± 0.01 c	0.53 ± 0.01 c	1.13 ± 0.05 c
	120	0.41 ± 0.00 d	0.35 ± 0.01 d	0.69 ± 0.01 d

[a]Data are the mean ± SE (n = 3). Different letters following values in the same row indicate significant difference among salt treatments using Duncan's multiple-range test at $p < 0.05$.

method published in FAO JECFA Monograph 10 (2010).[19] Briefly, the HPLC (Agilent 1100, USA) analysis was carried out by a column of Luna 5 μm C18 (2) 100 A (250 × 4.6 mm, Phenomenex) maintained at 40 °C, and the flow rate was 1.0 mL min^{-1}. The mobile phase was acetonitrile/phosphate buffer, pH 2.6 (32:68). The UV detector was set to a wavelength of 210 nm. Each sample was assayed for 30 min. Identification and calculation of stevioside and rebaudioside A were carried out according to the method published in FAO JECFA Monograph 10 (2010).[19] The total glycoside content was calculated as the sum of the contents of stevioside and rebaudioside A.

Statistical Analysis. The data were subjected to analysis of variance (ANOVA), and comparisons between the mean values of treatment were made by using Duncan's multiple-range test in SAS 9.0 software ($p < 0.05$).

RESULTS

Plant Growth. The changes in the growth parameters of *S. rebaudiana* at the end of salt treatment are shown in Table 1. NaCl treatment significantly reduced all investigated growth parameters except for root dry weight. Plants treated with 90 and 120 mM NaCl showed significantly lower plant height, leaf number, branch length, stem dry weight, leaf fresh/dry weight, and shoot dry weight than control. However, leaf fresh/dry weight in 60 mM NaCl did not show significant difference from the control. The total dry weights of *S. rebaudiana* plants treated with 60 and 90 mM NaCl kept similar levels as the control. However, plants treated with 120 mM NaCl showed only 60% of the total dry weight of control. In addition, shoot/root ratios of salt-treated plants were inhibited markedly by 30−50% compared with that of the control.

Pigment Composition. Chl *a*, Chl *b*, and Chl (*a* + *b*) contents reduced significantly under 60 mM NaCl and further decreased drastically in 90 and 120 mM NaCl after 28 days of salt treatment (Figure 2). The lowest contents of Chl *a*, Chl *b*, and Chl (*a* + *b*), being only 22, 28, and 24% of control, were all observed in 120 mM NaCl, respectively. Consequently, Chl *a*/Chl *b* remained unchanged with elevated NaCl concentration.

Proline Accumulation and Activities of Antioxidant Enzymes. Sixteen days after salt treatment, leaves of plants treated with 60 mM NaCl showed significantly higher CAT activity (4-fold) than control, whereas the activities of SOD and POD under 60 mM NaCl maintained a similar level of control (Figure 3A−C). In 90 mM NaCl, CAT and SOD activities were enhanced markedly, but POD activity was not. When the NaCl dose was up to 120 mM, the activities of CAT, SOD, and POD all increased prominently. Moreover, proline content was enhanced significantly with the increase of NaCl concentration and was 17-, 31-, and 42-fold higher in 60, 90, and 120 mM NaCl than in the control, respectively (Figure 3D).

Ion Accumulation. Na^+ contents in root, stem, and leaf increased significantly with the increase of NaCl concentration, which accumulated to maximum concentrations in 120 mM NaCl expressed as 11-, 15-, and 102-fold higher than that in the control (Table 2), respectively. Root K^+ content decreased significantly with elevated NaCl concentration. However, K^+ content of stem was reduced significantly by NaCl but not consistently with increasing salt concentration. Moreover, K^+ content in leaf did not show significant difference between 60 or 90 mM NaCl treatment and control, whereas it decreased significantly in 120 mM NaCl. Therefore, K^+/Na^+ ratios in all organs were inhibited drastically with the increase of NaCl concentration, especially in the root and stem, which showed K^+/Na^+ ratios of <1. However, K^+/Na^+ ratios in leaf were >1 under 60 and 90 mM NaCl. Distribution of other macronutrients among plant organs varied with different mineral

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Table 3. Effects of Different NaCl Concentrations on Leaf Glycoside Contents of *Stevia rebaudiana* after 28 Days of Treatment[a]

	NaCl (mM)			
glycoside parameter	0 mM	60 mM	90 mM	120 mM
RA (mg g^{-1} leaf DW)	80.86 ± 0.94 b	83.86 ± 2.17 a	70.80 ± 1.60 b	50.83 ± 1.37 c
ST (mg g^{-1} leaf DW)	14.50 ± 0.83 a	11.76 ± 1.16 b	9.10 ± 1.20 c	8.10 ± 0.79 c
RA+ST (mg g^{-1} leaf DW)	95.36 ± 1.46 a	95.63 ± 2.06 a	79.90 ± 1.67 b	58.90 ± 2.15 c
RA/ST	5.59 ± 0.30 c	7.17 ± 0.75 ab	7.90 ± 1.15 a	6.31 ± 0.45 bc
RA/(RA+ST)	0.848 ± 0.007 c	0.877 ± 0.012 ab	0.886 ± 0.014 a	0.863 ± 0.008 bc
ST/(RA+ST)	0.152 ± 0.007 a	0.123 ± 0.012 c	0.114 ± 0.014 bc	0.137 ± 0.008 ab

[a]Data are the mean ± SE (n = 3). Different letters following values in the same row indicate significant difference among salt treatments using Duncan's multiple-range test at $p < 0.05$.

types. In general, Ca^{2+} content in all organs and Mg^{2+} content in aerial parts decreased significantly in 60 mM NaCl but then increased at different levels in different organs from 90 to 120 mM NaCl. Furthermore, compared with control, the Mg^{2+} content of root of salt-treated plants remained unchanged.

Glycoside Content. The ratios of RA/ST and RA/(RA +ST) in leaves of *S. rebaudiana* were enhanced under all salt treatments (Table 3). Under 60 mM NaCl, RA content and RA/(RA+ST) value were promoted significantly, whereas ST content and ST/(RA+ST) reduced significantly. In addition, total glycoside content had no distinct change in 60 mM NaCl. Plants treated with 90 and 120 mM NaCl showed significantly lower contents of RA, ST, and RA+ST than control. RA/ST and RA/(RA+ST) were enhanced notably by 90 mM but not by 120 mM NaCl. In contrast, ST/(RA+ST) decreased strikingly in 90 mM NaCl treatment while remaining unchanged at 120 mM NaCl.

DISCUSSION

Growth Inhibition. Plant growth inhibition is a phenomenon often occurring in glycophyte or salt-sensitive plant species under saline environment. This might be explained as the inadequate photosynthesis caused by stomatal closure and the reduction of carbon assimilation rate under salt stress.[9] In the present study, the biomass production of *S. rebaudiana* plants did not show significant reduction in lower and moderate salt concentration (60 and 90 mM NaCl, respectively), which indicated *S. rebaudiana* was more salt tolerant than safflower as previously reported by Harrathi, who found 50 mM NaCl could reduce the growth of safflower significantly.[20] However, high-salt treatment (120 mM NaCl) drastically decreased the total dry weight of *S. rebaudiana* by 40% in comparison to control. According to the view of Maas and Hoffman,[21] it was suggested that *S. rebaudiana* is a moderately salt-tolerant plant. However, *S. rebaudiana* could not survive in the environment of 120 mM NaCl for a month. Shoot growth was probably more susceptible to salt than root, which generally led to a lower shoot/root ratio in salt treatment than control.[22] The results obtained in the present experiment verified this conclusion by showing significantly lower shoot/root ratios in all NaCl treatments than in control.

Physiological Responses. Leaf is the main plant organ responsible for light absorption and transformation and plays an important role in providing energy and nutrients for the growth and development of plants. The growth inhibition caused by salt stress mainly originated from the damage to leaf function. In the present study, the number of fully expanded leaves decreased dramatically with increasing salt level, which could be seen as the combined effect of increasing the senescence rate of older leaves and decreasing the production of new leaves. Leaf chlorosis, wilting, and necrosis were found in plants treated with 90 and 120 mM NaCl for 28 days, respectively. Disruption of the thylakoid and stromal membranes may result in leaf chlorosis and necrosis,[23] which indicated that salt concentration above 90 mM NaCl could impair the chloroplast structure of *S. rebaudiana*. In accordance with this conclusion, the contents of Chl *a*, Chl *b*, and Chla (*a* + *b*) decreased notably with increasing NaCl concentration in the present study. In addition, the change of the Chl *a*/Chl *b* ratio could suggest the change of the contribution of photosystem II and photosystem I to photosynthesis.[24] It was suggested that the damage caused by the salt stress to the two photosystems in the leaves of *S. rebaudiana* was at a similar level because of the similar reduction of the contents of Chl *a* and Chl *b* and the unchanged Chl *a*/Chl *b* ratio.

Plants under salt stress might suffer oxidative damage induced by photoinhibition, along with the rise of the level of reactive oxygen species (ROS). This response would up-regulate the activities of some key enzymes such as SOD, CAT, and POD for modulating the ROS level. It has been clarified that CAT and POD functioned similarly in the protection of biomolecules from H_2O_2, whereas SOD catalyzed the reduction process from toxic superoxide to H_2O_2.[25] In the present study, 60 mM NaCl increased the activities of CAT and POD but not SOD, which suggested the excessive H_2O_2 induced by low salt stress was mainly from some pathways but not from SOD catalysis. Furthermore, CAT might be efficient in clearing the excess H_2O_2 induced by low salt stress with significantly increased activity and slightly increased POD activity but without change in SOD activity. Besides H_2O_2 stress, salt stress might simultaneously enhance superoxide production in cells. Our results showed that SOD activities increased significantly in the moderate and high salt treatments, which suggested the superoxide burst in the *S. rebaudiana* leaves. Compared with low and moderate salt treatments, it was likely that the excessive H_2O_2 and superoxide under high salt treatment joined together and inhibited the normal metabolism for the growth of *S. rebaudiana*. A similar deduction had been confirmed in maize.[26] In addition, plants also synthesized low molecular weight organic compounds, such as proline, betaine, and free amino acids in the cytoplasm, to prevent cytoplasm dehydration induced by salt stress.[27] We found that proline accumulation increased significantly in response to salt stress, especially at high salt concentration, which indicated that high salt stress might result in a high level of osmotic stress to *S. rebaudiana*, and the proline was involved in the osmotic adjustment of water deficit caused by salt stress and thus enhanced the tolerant ability of *S. rebaudiana* to the osmotic stress.

Ion toxicity of high salt concentration could result in plant growth inhibition. The increasing NaCl concentration to the roots of plants may lead to higher Na^+ content in leaves and thus inhibited photosynthesis.[28] In the present experiment, the Na^+ of the organs of plants treated with 0−90 mM NaCl most accumulated in root, followed by stem and leaf, whereas it showed an opposite distribution pattern in plants treated with 120 mM NaCl, which exactly confirmed the negative effects of high Na^+ content in leaves on plant growth. K^+ accumulation under salt stress could be considered as one character of the plant adaptation to salinity.[29] However, membrane systems would be disrupted by high Na^+ content, increasing Na^+ influx while decreasing K^+ influx, which would result in the acceleration of senescence.[3,30] In the present study, K^+ contents in all organs of *S. rebaudiana* decreased drastically under high salt treatment, which suggested a damaging effect of high salt stress to the membrane systems of *S. rebaudiana*. Besides, K^+ content in different plant organs might respond differently to high salt stress. The obtained data showed that the leaf was characterized by having higher K^+ content (at least 2-fold) than stem and root under all salt treatments, which indicated better adaptation ability to salt stress than shown by stem and root. Wyn Jones proposed that a K^+/Na^+ ratio of >1 in plant organs was a minimum value suggested for the normal functioning of most mesophytes under salt stress.[31] On the basis of the obtained results, stem and root under all salt treatments possessed a K^+/Na^+ ratio of <1, whereas leaf under 90 mM NaCl still had a K^+/Na^+ ratio of >1, which also testified to the better adaptation ability of leaf to saline condition than that of stem and root. In addtion, Ca^{2+} content in all organs and Mg^{2+} content in aerial parts decreased significantly in 60 mM NaCl but then increased at different levels in different organs from 90 to 120 mM NaCl. This result was similar to the previous study on the Salt Range (an ecotype of *Cynodon dactylon* (L.) Pers.), which concluded that increased Ca^{2+} contents of shoot and root were an adaptation strategy to high salinities.[32] To the best of our knowledge, limited studies are published on the roles of ions in the growth of salt-treated Asteraceae species. Thus, our study enriched the understanding of ion response of Asteraceae species to salt stress. However, studies based on the mechanisms of ion absorption and transport of *S. rebaudiana* under salt stress are interesting and need to be continued.

Steviol Glycoside Composition. Stevia plants have gained importance as sweeteners because of their ST and RA contents. The ratio of RA content, RA/ST, and RA/(RA+ST) increased, whereas ST content and ST/(RA+ST) decreased in the present experiment under all salt treatments. Stevioside is the substrate for the synthesis of rebaudioside A,[33] which probably leads to *S. rebaudiana* plants high in rebaudioside A but low in stevioside. The results indicated that NaCl could modulate the composition of steviol glycosides for its function of promoting the transformation of stevioside to rebaudioside A. A previous study had discovered that *UGT76G1* played the key role in the transformation process.[34] It was suggested that NaCl stress could enhance the transcription of *UGT76G1*. Thus, the relationship between salt stress and the transcription level of *UGT76G1* is interesting and needs to be further studied. Lower salt concentration in soil could alter the composition of steviol glycosides by distinctly improving RA content. Similar results were also obtained by Harrathi, who found that 50 mM NaCl could alter the composition of the fatty acid content in *Carthamus tinctorius* L.[21] However, the content of ST+RA decreased prominently under relatively higher salt concentration (90 and 120 mM NaCl). Similar results were obtained for the total content of essential oil of *Salvia officinalis* L. under 100 mM NaCl.[11] When plants lived under high salt stress conditions, energy was first allocated to the process of maintaining metabolic homeostasis, such as enhancing activities of antioxidant enzymes and synthesis of simple osmolytes,[9,35] rather than the synthesis of complex secondary metabolites.

AUTHOR INFORMATION

Corresponding Author

*(W.W.) Phone: +86-28-86290970. Fax: +86-28-86290870. E-mail: ewuwei@gmail.com.

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