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Seawater Stress Differentially Affects Germination, Growth, Photosynthesis, and Ion Concentration in Genotypes of Jerusalem Artichoke (*Helianthus tuberosus* L.)

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Abstract Two Jerusalem artichoke (*Helianthus tuberosus* L.) genotypes, NY-1 and NY-7, were subjected to different seawater concentrations (0, 10, 20, 30, 40, and 50%) for various periods of time to determine the effects on seedling growth, ion content, and photosynthetic productivity in a greenhouse. Under different seawater concentrations, sprouting rates varied greatly among the genotypes. The differences in relative growth rate (RGR), leaf chlorophyll content, total leaf area (TLA), plant dry weight (PDW), photosynthetic rate (A), stomatal conductance (g_s), and efficiency of the light harvesting of photosystem II (F_v'/F_m') were significant between NY-1 and NY-7 after 12 days of stress at 40 and 50% seawater. Seawater treatments resulted in the reduction of almost all the growth parameters and coincident increases of Na^+ and Ca^{2+} concentrations in plant tissues. Our results indicate that there is great variability for seawater tolerance among *H. tuberosus* varieties, and that greater photosynthesis capacity, higher RGR, and relatively higher tissue Na^+ accumulation at high seawater concentrations appears to be associated with seawater tolerance in *H. tuberosus* varieties.

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Introduction

Water is a renewable resource, but its availability is variable and limited. Nearly every country in the world experiences water shortages during certain periods of the year (Gleick 1993), and more than 80 countries now suffer from serious water shortages (Jin and others 2007). To cope with the scarcity of fresh water for the sustainable development of agriculture, there is increasing interest among agricultural scientists and planners in the utilization of seawater (at least diluted) for irrigation of crops (Jin 1999; Liu and others 2003). The high salt concentration in seawater is the main limiting factor for using seawater in agriculture (Long and others 2008). Salinity affects many morphological, physiological, and biochemical processes, including seed germination, plant growth, and water and nutrient uptake. Several studies demonstrated that germination of brassicas (*Brassica* spp. L.), wheat (*Triticum aestivum* L.), and other species is delayed and reduced by low water potential of the growth medium (Rao and Dao 1987; Hao and De Jong 1988), high salinity (Romo and Haferkamp 1987), and combinations of these factors (Willenborg and others 2004; Zhao and others 2007). Salinity affects plants by causing osmotic stress, mineral deficiencies, and physiological and biochemical alterations (Zhao and others 2007).

In contrast, Jerusalem artichoke (*Helianthus tuberosus* L.) is a salt-tolerant and drought-tolerant species that is easily grown in coastal and semiarid areas (Newton and others 1991; Long and others 2008). It is mainly a biomass crop for ethanol production that commonly yields around 7

and potentially up to 14 t ha^{-1} of carbohydrates (Long and others 2008). However, it is not known whether different genotypes of *H. tuberosus* differ in tolerance to salinity and whether diluted seawater can be used for growing them to preserve precious fresh water resources.

The objective of this study was to determine the effects of different concentrations and durations of seawater stress on sprouting, seedling growth, photosynthesis, and ion content in different genotypes of *H. tuberosus*. In addition, our study was aimed at increasing our understanding of the role of specific physiological processes in seawater tolerance of *H. tuberosus*.

Materials and Methods

Preliminary Sprouting Experiment

A preliminary sprouting test was conducted to choose two genotypes that differ in salt tolerance for further study. Eight *H. tuberosus* genotypes (designated NY-1 to NY-8) chosen were previously grown with diluted seawater for six generations (6 years). In the present study, genotypes were subjected to six levels of salinity in a factorial design with four replicates. Tuber slices with buds were surface-sterilized with $HgCl_2$ (1.0 g L^{-1}) for 8 min, rinsed thoroughly with distilled water, and sprouted on moist acid-washed quartz sand in pots with perforated bottoms placed in a plastic basin containing 400 ml of treatment solution, which was aerated for 8 h daily and renewed every other day. The treated solution included six seawater concentrations (0, 10, 20, 30, 40, and 50%, corresponding to the following ECs: 1.14, 4.56, 7.17, 10.44, 13.43, and 16.30 dS m^{-1}), made by adding calculated amounts of crude seawater salt to the half-strength Hoagland's nutrient solution (Hoagland and Arnon 1938). The crude salt was produced by evaporation from seawater in Laizhou Bay (118°32′-119°37′E, 36°25′-37°19′N). The ion composition in Laizhou Bay seawater included 0.13 g L^{-1} HCO_3^-, 3.87 g L^{-1} SO_4^{2-}, 17.33 g L^{-1} Cl^-, 0.79 g L^{-1} Ca^{2+}, 1.03 g L^{-1} Mg^{2+}, 0.60 g L^{-1} K^+, and 9.48 g L^{-1} Na^+. Plants were grown in a glasshouse with a daily photoperiod of 12 h at a photon flux density of 392-415 μmol m^{-2} s^{-1} and maximum/minimum temperature of 25/18°C. The relative humidity was 65-80%. The basin was checked daily and additional solution was added if necessary.

The number of sprouted seedlings was checked daily and the final number was determined at 10 days after treatment. A seed with a radicle exceeding 5 cm in length was regarded as sprouted. At the final count, shoot and root lengths of each seedling were measured. The ratio of shoot-to-root length was calculated.

Sand Culture Experiment

Plant Material and Growth Conditions

Based on the result of the sprouting test, the two genotypes that differed the most in salt tolerance (salt-tolerant NY-1 and salt-sensitive NY-7) were chosen for further work. A 2 × 6 factorial experiment, arranged in a completely randomized design with eight replications, was conducted from March to June 2006 and ran for the second time from March to June 2007. Two-liter pots filled with silica sand were used for the study. Four seeds were planted in each pot and thinned to two plants per pot after emergence. Pots were irrigated with distilled water for 4 days after emergence and then for an additional 14 days with salt-free half-strength Hoagland's solution (Hoagland and Arnon 1938). Pots were randomized weekly to minimize variations in the greenhouse during the experiment. At 18 days after seedling emergence, salinity treatments of 0, 10, 20, 30, 40, and 50% seawater salt were imposed in the half-strength Hoagland's solution. Care was taken to make sure that each pot received the same volume of the planned solution for every application and that there was no moisture stress.

Growth Analysis

Dry matter samples were taken at 4, 8, and 12 days after salt application (DASA). Shoots and roots were cut, cleaned thoroughly with tap water, and then dried at 70°C for 72 h to determine dry weight. The relative growth rate (RGR) was calculated according to Kingsbury and others (1984) as

$$\mathrm{RGR} = (\ln w_2 - \ln w_1)/(t_2 - t_1)$$

where w_1 and w_2 are dry weights of shoots and roots in grams at times t_1 and t_2 (in days). At 12 DASA, all leaf blades were removed from the shoot and leaf area was measured with an Area Meter model Li-3000.

Leaf greenness was measured on the second topmost fully expanded leaf of two plants per pot with a chlorophyll meter (SPAD-502, Minolta Camera Co. Ltd., Japan) and expressed as the average of three readings from the base, middle, and tip of the leaf blade at 2-day intervals after stress application.

Photosynthetic Parameters

An infrared, open gas exchange system (LI-6400, LI-COR Inc., Lincoln, NE) coupled with an integrated fluorescence chamber head (FMS-2 leaf chamber fluorometer) was used to measure photosynthetic rate (A) and stomatal conductance (g_s) on the same leaf used for the leaf chlorophyll measurement. The area of each leaf in the photosynthetic meter chamber was determined manually. During the

measurement, sufficient light (1180 μmol m^{-2} s^{-1}) was provided. Data were logged manually when gas exchange and chlorophyll fluorescence parameters became stable. Fluorescence parameters were measured on leaves that were dark-adapted for 5 min before measurements. The equations of Genty and others (1989) were used for fluorescence parameters. The efficiency of energy harvesting by open reaction centers of photosystem II for leaves was calculated as

$$F'_v/F'_m = (F'_m - F'_0)/F'_m$$

where F'_v is the variable fluorescence, F'_0 is the minimal fluorescence of a momentarily darkened leaf, and F'_m is the maximal fluorescence during a saturating flash of light (3000 μmol m^{-2} s^{-1}). Photochemical quenching (qP) was calculated as indicated by the manufacturer's manual:

$$qP = (F'_m - F_s)/(F'_m - F'_0)$$

where F_s is the steady-state fluorescence.

Ion Concentration Measurement

Oven-dried plant samples (aboveground dry matter) harvested at 12 DASA were ground into a fine powder and digested according to Long and others (2008). The Na^+, K^+, and Ca^{2+} concentrations were determined with a flame atomic absorption spectrometer (Z-8000).

Statistical Analysis

All data were subjected to analyses of variance for each run of the experiment using the general linear model procedures of SAS. Mean treatment differences were separated by the least significant difference ($LSD_{0.05}$) test if F tests were significant ($p \leq 0.05$) (Fisher's protected test). The regression analysis was performed between the sprouting rates or the relative growth rates and seawater concentrations using Microsoft Excel 2003 (Microsoft Corp., Redmond, WA).

Results

Preliminary Sprouting Experiment

Among the tested genotypes, six *H. tuberosus* sprouted well (>90% sprouting rate) under no salt stress conditions (Fig. 1); sprouting rates of the remaining two genotypes (NY-4 and NY-6) were 60% or less (data not shown), probably because of poor tuber quality. These genotypes were therefore excluded from the analysis.

Salinity inhibited tuber sprouting in all tested genotypes (Fig. 1). Sprouting rate, seedling growth, and shoot-to-root

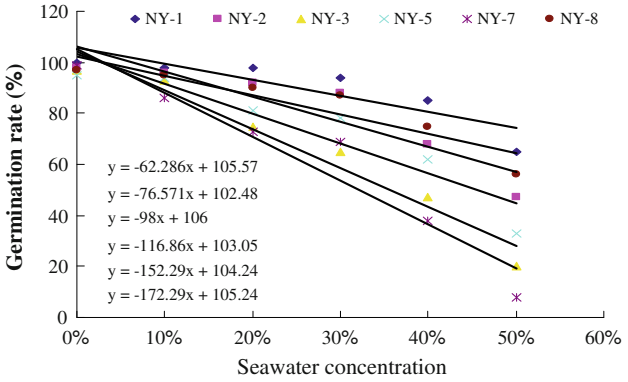

Fig. 1 Regression analysis between sprouting rates and seawater concentrations for each cultivar

length ratio declined with an increase of seawater concentrations (data not shown). The regression equations for NY-1, NY-2, NY-3, NY-5, NY-7, and NY-8 between the sprouting rate and seawater concentration were $y = -62.286x + 105.57$, $y = -98.0x + 106$, $y = -152.29x + 104.24$, $y = -116.86x + 103.05$, $y = -172.29x + 105.24$, and $y = -76.57x + 102.48$, respectively (Fig. 1), where y is the sprouting rate (%) and x is the seawater concentration. The r^2 values were 0.76, 0.84, 0.96, 0.93, 0.88, and 0.86, respectively. The coefficient gradient of NY-1 was significantly less than the other cultivars. In the 50% seawater treatment, sprouting of NY-7 was completely inhibited, whereas the sprouting rate for NY-1 was reduced to 69% (Fig. 1). Under 50% seawater treatment, reduction in germination of NY-8 was also significantly lower than that of NY-2, NY-3, and NY-5. Salinity stress not only affected germination rate, but also delayed the germination process. Under salt stress, it took more days to reach the germination peak (when the majority of seeds were germinating).

Sand Culture Experiment

For most parameters measured in the greenhouse study with salt-tolerant NY-1 and salt-sensitive NY-7, effects of genotype and saline treatments in the second run of the experiment followed the same pattern as in the first run, although the numerical differences in the second run were sometimes smaller than those in the first run. There were no significant run × genotype or run × treatment effects. Unless otherwise specified, the following presentation is focused on the first run of the experiment.

Response of Plant Growth to Seawater

The main effects of genotype and seawater treatment were both significant ($p < 0.01$) for relative growth rate (RGR) starting from 4 DASA. There was a significant genotype × salinity interaction at day 4 and day 8 out of

Table 1 Mean square of different variation sources and coefficient of variation for parameters determined on day 12 after seawater stress application

Source	RGR × 10^{-3}			SPAD			TLA	PDW	*A*	*Gs*	F_v'/F_m'	qP	Na^+	K^+	Ca^{2+}
	Day 4	Day 8	Day12	Day 4	Day 8	Day 12	$\times 10^{-3}$			$\times 10^{-3}$					
First run															
Genotype (G)	1.3**	7.3**	5.2*	135**	215**	642**	0.43	0.12*	32.4**	8.2**	4.0	26.0**	32.0	324.6**	54.8*
Seawater (S)	5.7**	14.2**	21.3**	75**	754**	2466**	26.4**	2.31**	187.9**	34.0**	76.8**	98.0**	9864.0**	1242.8**	754.2**
G × S	0.5**	1.1*	0.8	32	85	186**	0.35	0.03	0.9	4.6*	7.0**	4.6	423.8**	142.0**	31.0*
Error	0.2	0.4	0.6	25	64	24	0.26	0.03	2.4	2.4	3.2	3.8	76.8	14.6	8.9
CV (%)	3.9	13.6	31.7	11.5	21	14	23.8	21.4	32.4	8.0	8.6	24.6	21.0	16.8	31.8
Second run															
Genotype (G)	2.6**	8.9**	9.5**	78	64	176**	0.67*	0.32**	15.2**	14.8**	5.0	44.0**	2.8	546.9**	432.6**
Seawater (S)	4.3**	13.2**	23.6**	94**	52	976**	15.3**	18.2**	184.8**	32.4**	72.0**	78.4**	3642.8**	742.8**	214.8**
G × S	0.3	0.3	1.8**	38	43	74**	2.32**	0.32**	5.4*	4.6**	10.4**	2.0	108.6***	8.8**	18.6**
Error	0.2	0.2	0.2	26	26	32	0.34	0.05	2.3	3.8	4.8	3.4	2.0	1.7	1.0
CV (%)	6.9	6.1	11.8	16	16.6	21	23.1	11.3	17.8	24.6	21.0	21.0	4.2	3.6	8.6

RGR relative growth rate, *SPAD* leaf chlorophyll content, *TLA* total leaf area, *PDW* plant dry weight, *A* photosynthetic rate, g_s stomatal conductance, F_v'/F_m' efficiency of light harvesting of photosystem II

* Significant at 0.05 probability level

** Significant at 0.01 probability level

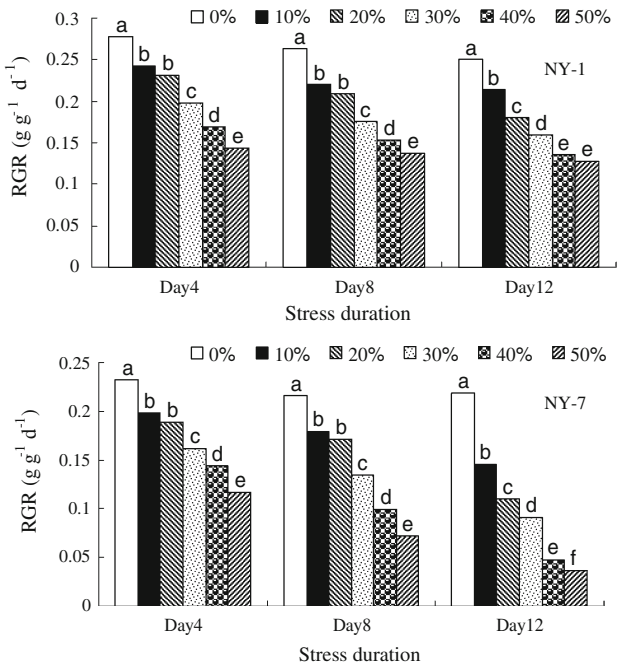

Fig. 2 Effect of increasing seawater concentration on the relative growth rate (RGR) of shoots for two *Helianthus tuberosus* varieties. Bars with different letters are significantly different by an *F*-protected $LSD_{0.05}$ test

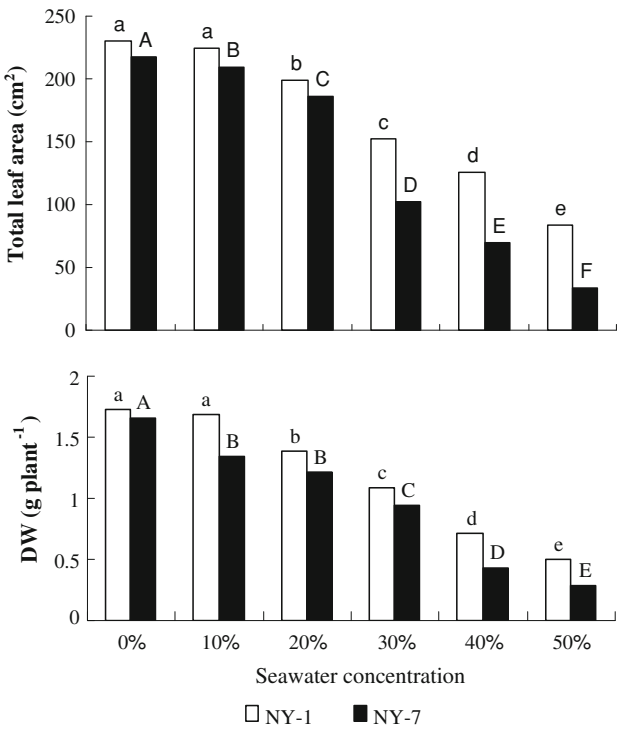

Fig. 3 Effect of increasing seawater concentration on total leaf area and plant shoot dry weight (DW) of two *Helianthus tuberosus* cultivars 12 days after seawater application. Within each variety bars with different letters are significantly different by an *F*-protected $LSD_{0.05}$ test

12 days of measurements (Table 1). In the second run of the experiment, however, significant genotype × salinity interactions for RGR did not occur until day 12, whereas the main effects of both genotype and salinity remained the same as those in the first run of the experiment. The RGR was greatly reduced as the seawater concentration and stress duration increased. Differences in responses to salinity levels were also significant with prolonged stress.

Significant differences between the two genotypes were observed from day 8, with the salinity level of 40% seawater or higher. Relative growth rate was decreased more for NY-7 than for NY-1 during day 8 and day 12 in the first run. In the second run of the experiment, in contrast with the 40 and 50% seawater stress at 12 DASA, the RGR for NY-1 was reduced by 46 and 49%, respectively (Fig. 2). With 40 and 50% seawater stress, RGR for NY-7 was reduced, respectively, by 54 and 66% at 8 DASA and 78 and 83% at 12 DASA (Fig. 2). The regression equations for NY-1 and NY-7 between the relative growth rate and times measured (at 4, 8, and 12 DASA) were $y = -0.0075x + 0.151$ and $y = -0.04x + 0.1553$, respectively, where y is the relative growth rate and x is time measured. The r^2 values were 0.9868 and 0.9948, respectively. The coefficient gradient of NY-1 was less than that of NY-7.

Total leaf area was significantly decreased with increased seawater concentration (Table 1). With 40 and 50% seawater stress, total leaf area was reduced by 45 and 63% for NY-1 and by 68 and 84% for NY-7, respectively, at 12 DASA (Fig. 3). Although the genotype effect was not

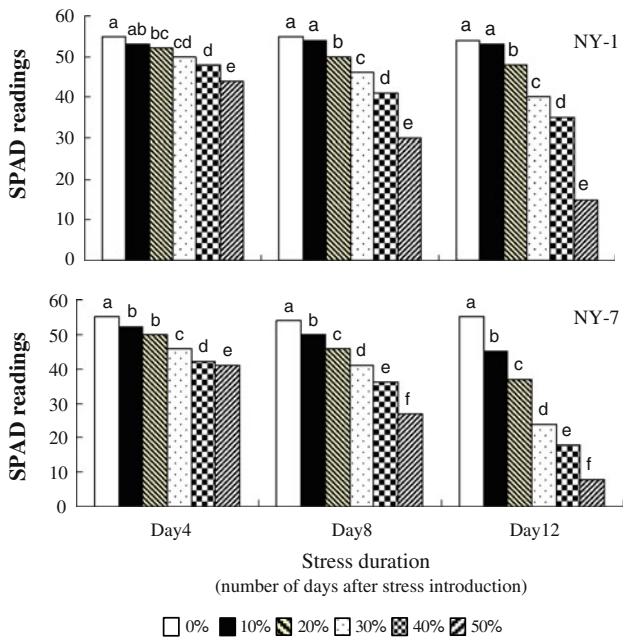

Fig. 4 Effect of increasing seawater concentration on leaf chlorophyll content of NY-1 and NY-7 12 days after seawater application. Bars with different letters are significantly different by an *F*-protected $LSD_{0.05}$ test

significantly different in the first run of the experiment, large differences in total leaf area between the two genotypes were observed at 30, 40, and 50% salinity levels of seawater (Fig. 3). Total leaf area of NY-7 was 44% smaller than that of NY-1 at the 40% seawater salinity treatment.

Plant dry weight was also greatly reduced with increased seawater concentration (Fig. 3), causing a 59, 71, 74, and 83% decrease at the 40 and 50% seawater treatments at 12 DASA for NY-1 and NY-7, respectively. A significant difference between the two genotypes was not observed until stress reached 40 and 50% seawater. Plant dry weight of NY-7 was decreased by 39 and 44% than that of NY-1 under 40 and 50% seawater stress, respectively.

Under seawater stress, leaf chlorophyll content of *H. tuberosus* was greatly reduced ($p < 0.01$) at all sampling dates, except for the measurements on day 8 in the second run of the experiment (Table 1). With prolonged salt stress, differences between the two genotypes at various salinity concentrations and the interaction between genotype and salinity became significant at 12 DASA (Table 1). As shown in Fig. 4, at 12 DASA leaf greenness in the 50% seawater treatment was reduced by 72% for NY-1 and by 81% for NY-7 compared with the control. The results in Fig. 4 clearly show that effects intensified with the treatment duration. The coefficients of regression between the leaf chlorophyll content and times measured (at 4, 8, and 12 DASA) under 50% seawater stress were -14.5 for NY-1 and -16.5 for NY-7. The r^2 values were 0.9996 and 0.9924, respectively.

Response of Plant Photosynthesis to Salinity

Under salinity stress the leaf photosynthetic rate was reduced significantly ($p < 0.01$) as seawater concentration was increased in both runs of the experiment, although there was a significant genotype × salinity interaction ($p \leq 0.05$) in the second run (Table 1). A significant reduction in the photosynthetic rate was observed even at low seawater concentrations (Fig. 5). The photosynthetic rate decreased by 79% for NY-1 and by 91% for NY-7 at 50% seawater treatment at 12 DASA. Concurrently, stomatal conductance was also reduced with an increase in seawater concentration (Fig. 5), for example, by 72 and 88% for NY-1 and NY-7, respectively, at 50% seawater stress compared with the control (Fig. 5).

Up to the 20% seawater level, the efficiency of light harvesting of photosystem II, as measured by F_v'/F_m', was not significantly affected (Fig. 5). When salinity concentration increased to 30% seawater or higher, a sharp reduction in light harvesting efficiency was observed, with reduction in F_v'/F_m' by 42% for NY-1 and 52% for NY-7 at 50% seawater treatment.

Photochemical quenching (qP) (Fig. 5) showed a similar trend as F_v'/F_m', and a significant decrease was observed at

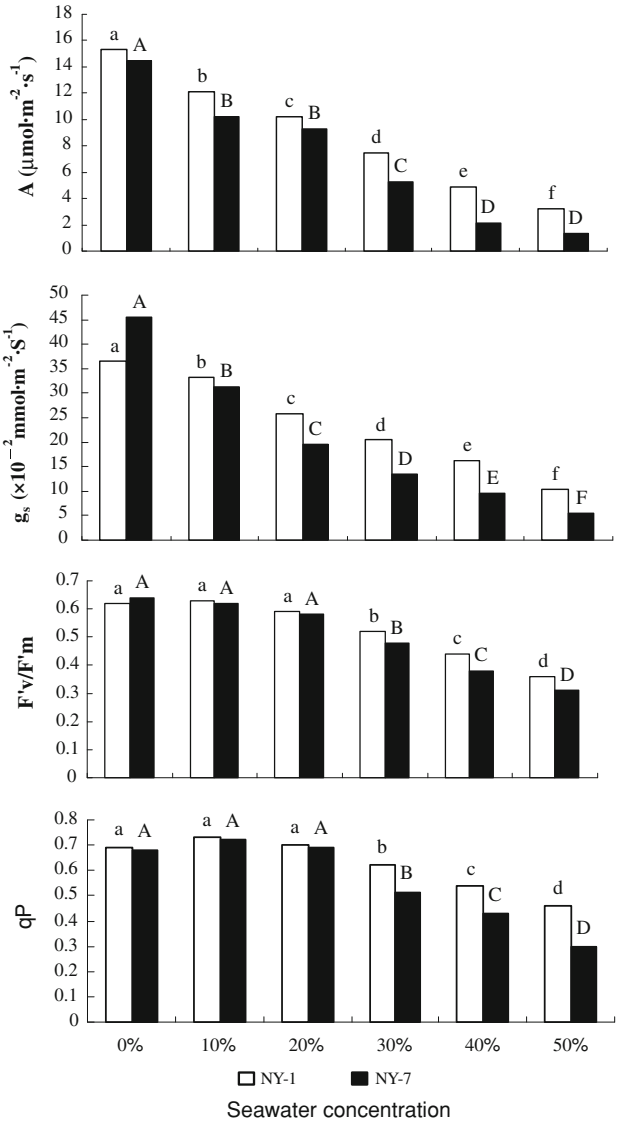

Fig. 5 Effect of increasing seawater concentration on photosynthetic rate, stomatal conductance, F_v'/F_m', and qP of two *Helianthus tuberosus* varieties 12 days after seawater application. Within each variety bars with different letters are significantly different by an *F*-protected $LSD_{0.05}$ test

30, 40, and 50% seawater treatments. Significant differences between the two genotypes were obtained at the 30, 40, and 50% seawater concentrations. At 30% seawater stress, the qP value for NY-1 was significantly higher than for NY-7, whereas similar values were recorded for both genotypes under the 40% seawater treatment. The qP values dropped significantly in the 50% seawater treatment.

Response of Tissue Ion Concentrations to Salinity

At 12 DASA shoot tissue Na^+ concentration increased significantly ($p < 0.01$) with increasing seawater concentration levels. Compared with the control, plant Na^+

accumulation increased from 5.0- to 18.5-fold with an increase in seawater concentration. However, there was a significant genotype × salinity interaction in both runs of the experiment (Table 1). The Na^+ concentration increased with increasing seawater concentration. Compared with the control, Na^+ concentration was increased about 11.1- and 14.0-fold for NY-1 and 17.6 and 24.6-fold for NY-7 with 40 and 50% seawater stress, respectively, at 12 DASA. Differences in Na^+ concentration between the two genotypes were observed at each seawater concentration level (Fig. 6). In general, NY-1 had significantly higher levels than NY-7 at high seawater concentration levels such as 40 and 50%.

A significant interaction between genotype and salinity treatment was also observed for K^+ (Table 1). Increasing seawater concentrations led to a significant reduction in plant K^+ concentration (Fig. 6). Compared with the control, plant K^+ concentration was decreased by 27 and 34% for NY-1 and by 28 and 45% for NY-7 in the 40 and 50% seawater stress treatments, respectively. The K^+ concentration of NY-7 was 19% lower than that of NY-1 at 50% seawater treatment (Fig. 6). However, the differences between the two genotypes were small to nonexistent in the treatments with 0 to 40% seawater.

Tissue Ca^{2+} concentration increased significantly with an increase in seawater concentration. There was a significant genotype × salinity interaction (Table 1). Compared with the control, plant Ca^{2+} concentration was increased from 0.4- to 3.2-fold with an increase in seawater concentration. For example, the Ca^{2+} concentration with 40 and 50% seawater stress at 12 DASA was increased about 2.5- and 3.1-fold for NY-1 and 2.7- and 3.2-fold for NY-7, respectively. NY-1 had higher Ca^{2+} tissue concentrations than NY-7 at all seawater concentrations (Fig. 6).

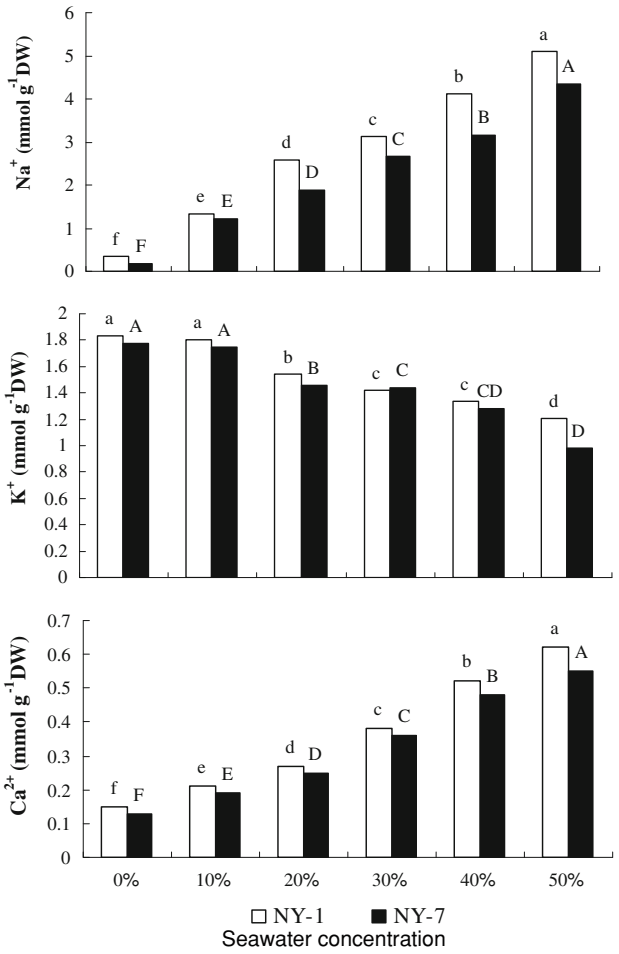

Fig. 6 Effect of increasing seawater concentration on shoot Na^+, K^+, and Ca^{2+} content of two *Helianthus tuberosus* varieties 12 days after seawater application. Within each variety bars with different letters are significantly different by an *F*-protected $LSD_{0.05}$ test

Discussion

The treatments tested in the present study were chosen to reproduce conditions of some natural coastal zones, as characterized by infiltration of saline waters. Under these conditions, salinity is the major abiotic stress for plants. Some plant species have the ability to adapt to various environmental changes. *H. tuberosus* is generally considered to be moderately tolerant to salinity and to exhibit genotypic variability in salinity tolerance (Newton and others 1991; Long and others 2008). The sprouting screening in our study indicated that different genotypes had different sensitivities. There also was a distinct effect of the seawater treatments on the growth of the two genotypes (Table 1; Fig. 2). The above observation agreed well with the results of previous studies (Meneguzzo and others 1999).

Plant RGR was reduced greatly with increased salt concentration and stress duration, but the reduction in RGR for the salt-tolerant NY-1 was smaller than that for the salt-sensitive NY-7 (Fig. 2). At the whole-plant level, the reduction in RGR could be attributed to photosynthesis-related morphological changes (Hunt 1990). The results from the present study indicate that the RGR of the two genotypes was related to their photosynthetic rate and leaf area, suggesting that both leaf expansion and photosynthetic rate are the growth-limiting factors under salinity stress. A decrease in leaf area may be attributed to early senescence and death, reduced growth rate, or both (Bernstein and others 1993). The greatest difference in RGR between the two genotypes was observed at the 10% seawater level (Fig. 2). At this level, the reduction in photosynthetic rate and total leaf area was not as great as at the higher seawater stress levels. Reduction in plant dry weight may have resulted from reduced or inhibited tillering at lower salinity levels (data not shown).

The negative impact of seawater on total leaf area development was evident at the lowest seawater concentration (10%) (Fig. 3) and increased linearly with increasing seawater concentration. However, El-Hendawy and others (2005) reported that under salinity stress, the decrease in RGR in wheat was related to only the photosynthetic rate, not to the total leaf area. A significant reduction in the photosynthetic rate and stomatal conductance under seawater stress was observed in this study. It is most likely that the decrease in photosynthesis was causally related to decreased stomatal conductance. These results are in agreement with the report by Netondo and others (2004), who found a positive correlation between stomatal conductance and CO_2 assimilation rate under salinity stress, suggesting stomatal conductance was a primary factor limiting photosynthesis in sorghum under salt stress.

Leaf greenness or chlorophyll content was also affected by seawater stress (Fig. 4). Salinity can affect chlorophyll content through inhibition of chlorophyll synthesis or acceleration of its degradation (Reddy and Vora 1986). The chlorophyll content of *H. tuberosus* decreased with increasing seawater concentration and stress duration in this study. The difference between genotypes was significant at 50% seawater stress at 12 DASA. Similar results were reported in alfalfa (Winicov and Seemann 1990), sunflower (Ashraf and others 1986), and wheat (El-Hendawy and others 2005) exposed to salinity stress.

A significant difference in F_v'/F_m' for NY-1 and NY-7 occurred at 30, 40, and 50% seawater concentrations (Fig. 5). This is in agreement with the findings of Netondo et al. (2004), who reported a significant reduction in F_v'/F_m' for sorghum when plants were subjected to 250 mM salt stress. The change in qP was similar to that in F_v'/F_m' in *H. tuberosus*, and significant differences were observed at 40 and 50% seawater concentrations (Fig. 5). However, Jiang and others (2006) reported that although qP showed some differences among barley (*Hordeum vulgare* L.) genotypes, salinity did not significantly affect this parameter. Indeed, Lu and Zhang (1998) reported that photosystem II in sorghum was highly resistant to salinity stress, and its thermostability was increased.

Accumulation of inorganic solutes such as cations Na^+ and K^+ can play a role independently or in combination with other mechanisms in maintaining the osmotic imbalance caused by salt stress and influence the osmotic potential adjustment of plant cells (Bayuelo-Jiménez and others 2003). As salinity concentration increased, tissue Na^+ content in *H. tuberosus* increased and no saturation was observed (Fig. 6). These results indicate that high concentrations of seawater can influence ion distributions so that they can contribute to the osmotic potential and increase protection against osmotic stress. A variable response in Na^+ concentration to changes in the seawater concentration was observed in NY-1 and NY-7. This was consistent with the reports of El-Hendawy and others (2005) and Houshmand and others (2005) in wheat.

Salinity not only caused high Na^+ accumulation in plants but also influenced the uptake of essential nutrients such as K^+ and Ca^{2+} through the effects of ion selectivity. The superior K^+ retention and efficient use of compatible solutes are crucial components of osmotic adjustment for salt tolerance (Bayuelo-Jiménez and others 2003; Peng and others 2004). A significant decrease in K^+ accumulation was observed even at the lowest salinity concentration in this experiment (Fig. 6). A decline in K^+ accumulation because of salinity stress has been reported for Swiss chard (*Beta vulgaris* L.) (Hessini and others 2005), barley (Jiang and others 2006), naked oat (*Avena sativa* L.) (Zhao and others 2007), and other species. However, salt-tolerant NY-1 maintained higher Na^+ and K^+ concentrations compared with salt-sensitive NY-7. Micromolar amounts of compatible solutes are sufficient for salt-tolerant cultivars to survive in severe salinity.

It is interesting to note that Ca^{2+} concentration in *H. tuberosus* was increased under salinity stress (Fig. 6). This was in contrast with the findings of El-Hendawy and others (2005) that salinity stress significantly decreased Ca^{2+} content in some wheat and sorghum genotypes. However, an increase of Ca^{2+} concentration under salinity stress was also reported in rapeseed (*Brassica napus* L.) (Procelli and others 1995) and in rice (Alpaslan and others 1998). Houshmand and others (2005) reported that Ca^{2+} concentration was not significantly affected by salinity in durum wheat (*Triticum durum* Desf.). Ca^{2+} does not always completely ameliorate the inhibition of growth by Na^+, and salinity can disturb normal functions without disturbing overall Ca^{2+} tissue concentrations, especially in the early growth stages. Therefore, Ca^{2+} concentration in plants has not typically been proposed as a useful trait for the screening for salt tolerance (Cramer 2002).

In the present experiment, NY-1 showed significantly higher Na^+, Ca^{2+}, and K^+ concentrations than NY-7. However, the results were less clear regarding the cation ratios. The genotypic differences in K^+/Na^+ and Ca^{2+}/Na^+ were significant at control and low salinity levels, but both varieties showed similar values at 40 and 50% seawater concentrations (data not shown). Given that the K^+/Na^+ ratio is an indicator of salinity tolerance (Houshmand and others 2005), further work to elucidate the relationship between the cation ratios in *H. tuberosus* genotypes differing in salt tolerance is warranted.

In summary, salinity stress significantly inhibited *H. tuberosus* growth by reducing total leaf area, chlorophyll content, photosynthetic rate, stomatal conductance, qP, and F_v'/F_m'. Both Na^+ and Ca^{2+} concentrations increased and K^+ concentrations decreased with increasing

seawater concentration. The two genotypes differed more in Na^+ and Ca^{2+} concentrations than in K^+ tissue concentration. It is worth noting that salt-tolerant NY-1 and salt-sensitive NY-7 have different tuber characteristics. NY-1 has larger tubers than NY-7. Nevertheless, it is unclear whether this difference is related to the differential salt tolerance of the two genotypes. Our data suggest that selection for greater photosynthetic productivity and higher RGR under saline conditions will be a good strategy for improving *H. tuberosus* genotypes for better tolerance to seawater. Furthermore, pyramiding regulatory genes that control various aspects of salt tolerance (that is, ionic and osmotic homeostasis) in a single genotype is expected to yield a high tolerance to seawater and similar stresses. However, the effects of stresses with respect to plant ontogeny should be assessed at realistic stress pressures that occur naturally in the field.

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