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EFFECT OF SALINITY ON GROWTH AND PHYSIOLOGY OF *THELLUNGIELLA HALOPHILA* L. ECOTYPES

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Abstract

The influence of salinity on seed germination, growth, and water relations of four Chinese [Hebei (HB), Henan (HN), Shandong (SH), or Jiangsu (JS)] and four North American [Cracker Creek (CC), Colorado (CO), Dillibrough (DB), or Yukon (YK)] ecotypes of *Thellungiella halophila* was investigated. The goal of this study was to identify superior or inferior ecotypes, related to the mean population, for eventual identification of the growth and physiological basis for salt tolerance in this new model species. Germination of seeds was completely diminished in all ecotypes above 200 mM NaCl. Seedlings of CC, CO, DB and SH had higher root growth at 100 mM than other ecotypes. North American ecotypes showed less shoot dry weight% in control compared to Chinese ecotypes. Colorado showed maximum shoot and root% of control compared to all other ecotypes. Chinese ecotypes showed less increase in leaf specific weight compared to North American ecotypes. In case of relative water content and leaf succulence an increasing trend was observed up to 400 mM NaCl. North American ecotypes showed decreasing, but Chinese showed increasing trend at low salt concentration (200 mM) and decreasing at higher level of salt (600 mM). Shoot osmotic potential decreased with increasing concentration of salt from 0-300 mM but an increasing trend was observed by increasing salt concentration in the medium between root and leaves.

Key words: Growth, Sodium chloride, Stress, Water relation.

Introduction

Soil salinity is a significant environmental factor limiting productivity of crops throughout the world and estimates for increasing areas of the world affected by salinity are alarming (Munir *et al*., 2021). It is estimated that of the current 230 Mha of land under irrigation, 20% are affected by salt and of the 1500 Mha of dry land agriculture, 2% are salt effected (Anon., 2018). Overall, it is estimated that the world is losing at least 3 ha of arable land every minute because of soil salinity (Anon., 2018). High concentrations of salt in the soil negatively affect the growth and development of crops and therefore cause serious problems for world food production (Khanom, 2016).

A variety of glycophyte and halophyte has been used to study the effect of salinity in plants; however, the mechanisms of salt tolerance are still not well understood (Hasegawa *et al*., 2000; Gupta & Huang, 2014). To date, *Arabidopsis thaliana* is the best model organism to study the molecular basis of salt tolerance in plants (Vinocur & Altmann, 2005) and several genes linked to salt stress responses have been identified (Zhu, 2000). However, *A. thaliana* is a true glycophyte, it reveals little information about salt tolerance in halophytes and unable to complete full life cycle even at moderate salinity (~100 mM NaCl) (Zhu, 2000; Bressan *et al.*, 2001). It is suggested to study some novel process or mechanisms related with salt tolerance that exist in salt tolerant plants, rather than in plants that are salt sensitive (Wang *et al*., 2003). In recent time, the halophyte *T. halophila* (salt cress) has been used as a model organism for studying salt resistance in plants (Bressan *et al*., 2001; Zhu, 2001; Volkov *et al*., 2003; Kant *et al.*, 2006; Ghars *et al*., 2008; Xiao-Jing *et al.*, 2015). *T. halophila* is an extremophile that can survive low temperature, drought, and high salt up to 500 mM (Zhu, 2001; Mrah *et al.*, 2006; Ghars *et al*., 2008). *T. halophila* is considered as close relative of *A. thaliana* since the morphology and sequence identity in both plants are similar thus genetic information of *Arabidopsis* can be used to investigate salt stress response in *Thellungiella* (Taji *et al.*, 2004).

Salinity restricts the plants growth by nutritional imbalance, specific ion toxicity, and osmotic imbalance (Lauchli & Epstein, 1990; Rehman *et al*., 2021). Osmotic stress impacts on leaves growth immediately after the salt concentration around the root increases. By contrast the impact of Na^+ toxicity on leaves growth is delayed (Munns & Tester, 2008). *T. halophila* does not produce salt glands or other complex morphological alteration either before or after salt adaptation (Mrah *et al*., 2006; Zang *et al*., 2012) and it suggested that its salt tolerance ability is likely the result of some basic physiological mechanisms. The physiological information obtained by the study of this salt tolerant plant will represent a key step in the discovery of genes involved in salt tolerance of other halophytes and glycophytes.

Keeping these all information in view, we studied the response of eight ecotypes [Cracker Creek (CC), Colorado (CO), Dillibrough (DB), Yukon (YK), Hebei (HB), Henan (HN), Shandong (SH), or Jiangsu (JS)] of *T. halophila* to NaCl (0-800 mM) during vegetative growth (after 30 days of salt treatment) to determine if different ecotypes behave differently under stress conditions then what is the possible physiological mechanism that make one ecotype more salt tolerant than the other one ? So, such type of study might be helpful to answer this question. In this respect an extensive analysis of water relations and plant growth behavior in *T. halophila* under salt stress conditions was carried out during this investigation.

Materials and Methods

Plant materials and growth conditions: For each experiment, seeds of all ecotypes (obtained from Center for Environmental Stress physiology, Purdue University

Indiana USA) were surface sterilized by soaking for one minute in 70% ethanol followed by several washing with sterile distilled water and then for 3 minutes in 1% sodium hypochlorite and then again several washing with sterile distilled water. To break dormancy seeds were stratified in water in micro-centrifuge tubes at 4ºC for 8 days.

Seed germination: Germination rate of each ecotype on petri-plates (100 × 15 mm) containing Murashige and Skoog (MS, Murashige & Skoog, 1962) medium (20 mL) with 3% sucrose alone or supplemented with different concentrations of NaCl (0, 50, 100, 200, 300, or 400 mM) was recorded daily. The petri-plates were sealed with parafilm and incubated at 25°C temperature and 16 hours photoperiod (30 µmol/m^2/s). A seed was regarded as germinated when the radicle protruded through the seed covering structures which was scored using binocular microscope (Olympus, Tokyo Japan).

Seedling growth: Seeds of each ecotype were germinated and grown on petri-plates (100 × 15 mm) containing MS basal medium for one week at same temperature and light conditions described previously. Uniformly sized seedlings (roots 1.5-2.0 mm long) of each ecotype were then transferred to plates with MS media containing NaCl from 0–400 mM concentrations. The time of transfer of seedling defined as day zero. To prevent the roots from coiling up, the petri-plates were slightly inclined (angle from the vertical: 15%). Root growth was measured every 7 days for 3 weeks by scanning petri-plates and root length was determined by Image J program (Rosband, 2008). After 21 days of growth, shoot was weighed for fresh and then dried at 70ºC to a constant weight to obtain shoot dry weight.

Plant growth and water relations: For growth and water relations study, seeds were sown onto 164 mL SC10 Ray Lech "Cone-tainers" (Stuewe & Sons, Inc., Corvalis, OR) containing Profile® calcined clay (Profile Products LLC, Buffalo Grove, IL). Cone-tainers were placed under a mist system (10 second after every 10 minutes) for 15 days after sowing. Once germinated, Cone-tainers were moved to a greenhouse with mean day and night temperatures of 28ºC and 16ºC, respectively. Day-length was extended to 15 hours with a combination of fluorescent and incandescent supplemental lighting at bench level ca. 30 µmol/m^2/s. Plants were sub-irrigated as needed by putting the rack for one hour in bins having 40 L salt solution. At each irrigation, plants were fertilized with (in mg/L) 200 N, 29 P, 167 K, 67 Ca, 30 Mg, and micronutrients. Nutrients were supplied from 1 g/L 15-5-15 commercial fertilizer formulation (Miracle Gro© Excel© Cal-Mag; the Scotts Co., Marysville, OH). Adjustment of pH to 5.7-6.0 and alkalinity reduction was achieved via 93% sulfuric acid (Ulrich Chemical, Indianapolis) at 0.08 mL/L. Fertilizer water was supplemented with sodium chloride ranging from 0 to 800 mM. Plants were grown for 50 days before salinity was initiated. Salinity was increased in 100-200 mM increments with each watering. Plants were irrigated with their respective salinity levels for 21 days. EC, pH and osmotic potential was recorded at each salinity treatment by taking 50 mL sample from well mixed salt solution.

Water relations: For all physiological measurements, recently-expanded, young and fully mature leaves were used. At the conclusion of each experiment, 2-3 leaves per plant were removed, photographed, and immediately placed in a pre-weighed vial containing deionized water. The difference in vial weight before and after addition of the leaf material was recorded as the fresh weight (FW) of the leaves. After 24 hours, the leaves were removed, blotted dry and weighed to obtain turgid weight (TW). Leaves were then dried at 70ºC to a constant weight to obtain dry weight (DW). Relative water content was calculated as:

$$\frac{\mathrm{FW}-\mathrm{DW}}{\mathrm{TW}-\mathrm{DW}}\times 100$$

Succulence was calculated as:

$$\frac{FW}{DW}$$

Leaf area (LA) was calculated by digitally analyzing the photograph using Image J software. Specific leaf weight (SLW) was calculated as:

$$\frac{DW}{LA}$$

Osmotic potential: For measurement of osmotic potential, 2-3 leaves per plant were removed, placed into a Costar Spin-X centrifuge tube filter (Sigma-Aldrich) with a 0.45 µm pore size, and immediately placed in liquid nitrogen. The filter was placed in a microfuge tube and kept on ice until returning to the lab. Tubes were stored at -4ºC. Tubes were thawed, weighed, and centrifuged at 15,000 rpm for five minutes to extract cell sap. The sap was immediately placed in a Wescor 5500 vapor pressure osmometer (Wescor. Inc, Logan, Utah). Similarly for root osmotic potential, root tips ca. 5cm was used after washing with deionized water. Solute concentration was converted to osmotic potential (ψs) using the Van't Hoff equation:

$$\psi s = -RTC$$

where R is the gas constant, T is absolute temperature, and C is the molar solute concentration. Osmotic potential at full turgor (ψs=100) was estimated as:

$$Y_\pi\ (100) = \frac{Y_\pi(RWC - RWC_a}{1 - RWC_a}$$

where RWCa is the correction factor for dilution by apoplastic water (Campbell *et al*., 1979).

The fresh weight of the leaf tissue was calculated by subtracting the average weight of tubes and inserts from the weight of the tube including the tissue. Shoot root dry weights. After removing leaves for other measurements (relative water content, succulence, shoot osmotic potential and osmotic potential at full turgor), the remaining portion of the shoot was removed and dried at

70ºC to a constant weight to obtain shoot dry weight. The sum of all dry weights from previously described measurements represents the total shoot dry weight. Roots were washed by immersing in deionized water to remove Profile and dried at 70ºC to a constant weight to obtain root dry weight.

Statistical analysis

Four independent experiments were performed to collect the data. For germination and seedling growth 3 plates with 10 seeds/plate were inoculated for each ecotype and salinity level. A randomized complete block design with 3 blocks 3 replicate/block of each ecotype for each salinity level completely randomized among tubes for the study of physiology and for the study of growth 3 block 5 replicate/block of each ecotype for each salinity level. Data was analyzed using Tukey's Honestly Significantly Difference Test and Dunnett's test.

Results

Germination of *T. halophila* on various NaCl concentrations: All ecotypes germinated 100% except SH on MS medium with 0 mM NaCl and full germination was obtained within 10 days. Low concentration of salt (50 mM) did not inhibit germination of CC, CO, DB, YK, HB, or HN ecotypes however, it showed significant negative effect on SH and JS germination. *T. halophila* ecotypes DB, SH, or JS did not germinate at 100 mM NaCl and YK exhibited best germination response within this level of salt (Fig. 1). North Americans ecotypes (CC, CO and YK) showed more germination response compared to the Chinese ecotypes at 100 mM NaCl. JS and SH were severely affected by all concentrations of salt. No germination occurred in any ecotypes at 200, 300, or 400 mM NaCl.

Seedling growth: Seedlings of all ecotypes grew very well on MS medium with 0 mM NaCl and maximum root length was recorded in ecotype DB and minimum in YK. Low concentration of salt (50 mM) significantly inhibited seedling growth of all ecotypes and maximum reduction in growth was recorded in HB and HN. At 100 mM salt concentration ecotypes DB and HB showed maximum reduction in root growth. North American ecotypes showed better response at 100 mM NaCl concentration. *T. halophila* all ecotypes showed 0% root growth on 200, 300, or 400 mM as in case of seed germination. In general a decrease in seedling root growth was observed by increasing the concentration of salt in MS medium in all ecotypes (Fig. 2). It was observed that seedling fresh/dry weights were decreased by increasing salt in the MS medium. Seedling fresh and dry weight was highest in CC ecotypes at 100mM salt as compared to others. Seedling fresh/dry weight in North American ecotypes was more pronounced at higher salt concentration (Fig. 3).

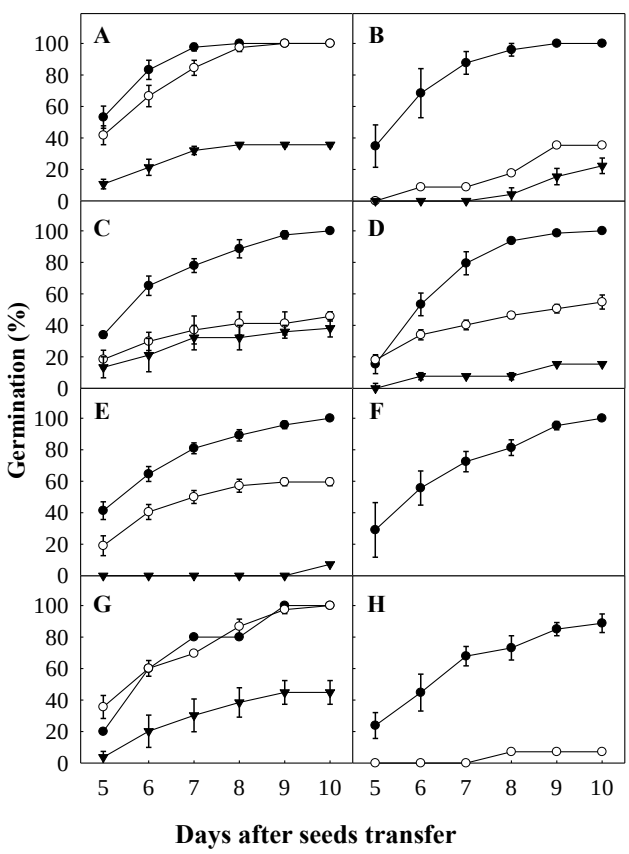

Fig. 1. Seed germination of *T. halophila* [CC (A), HB (B), CO (C), HN (D), DB (E), JS (F), YK (G), and SH (H)] on MS medium supplemented with 0 (●), 50 (○), or 100 (▼) mM NaCl. Vertical bars represent ± standard error of three replicates.

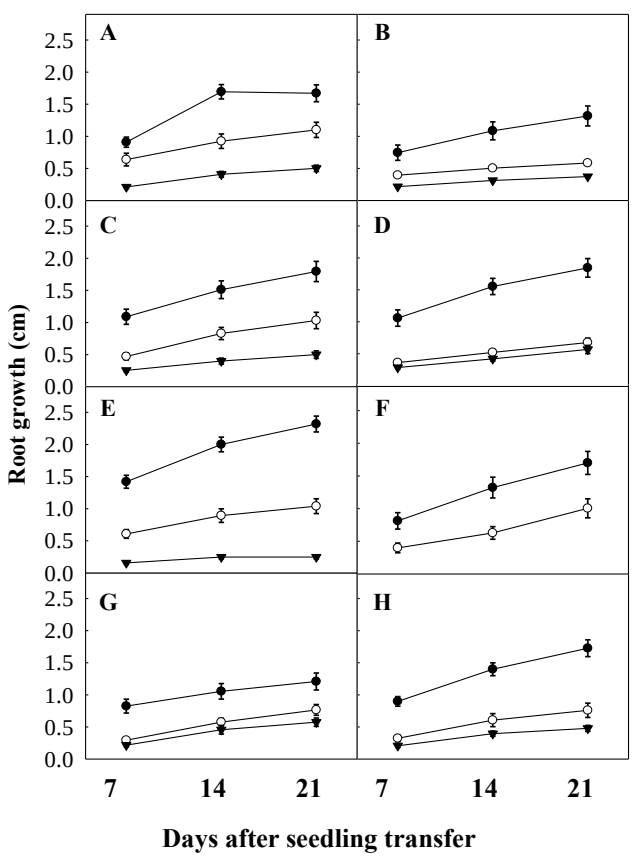

Fig. 2. Cumulative root growth of *T. halophila* seedling [CC (A), HB (B), CO (C), HN (D), DB (E), JS (F), YK (G), or SH (H)] on MS medium supplemented with 0 (●), 50 (○), or 100 (▼) mM NaCl. Vertical bars represent ± standard error of three replicates.

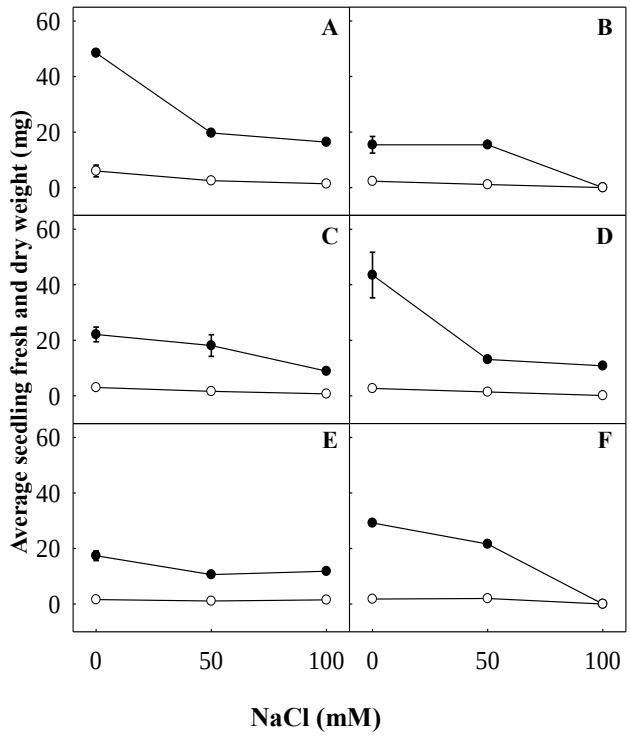

Fig. 3. Average seedling fresh (●) and dry weight (○) of *T. halophila* seedling [CC (A), HB (B), CO (C), HN (D), DB (E), or SH (F)] on MS medium supplemented with 0, 50, or 100 mM NaCl. Vertical bars represent ± standard error of three replicates.

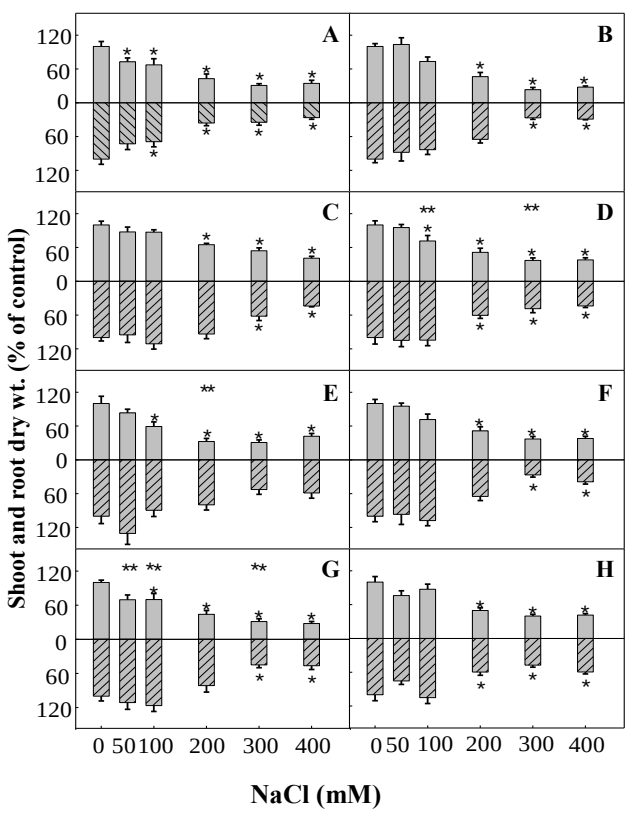

Fig. 4. Shoot (filled column) and root dry weight % of control (lined column) of *T. halophila* [CC (A), HB (B), CO (C), HN (D), DB (E), JS (F), YK (G), or SH (H)] at different concentrations of NaCl 0, 50, 100, 200, 300, or 400 mM. Vertical bars represent ± standard error of five replicates.

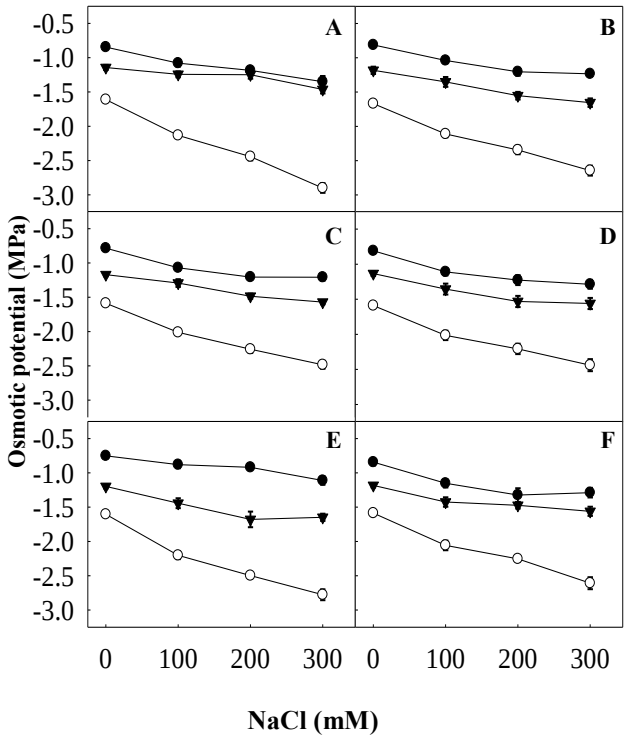

Fig. 5. Shoot osmotic potential (○), root osmotic potential (●), osmotic potential at 100% (▼) at different concentration (0, 100, 200, or 300 mM) of NaCl in *T. halophila* ecotypes CC (A), HB (B), CO (C), HN (D), DB (E), or SH (F). Vertical bars represent ± standard error of five replicates.

Shoot and root dry weight: When plants of *T. halophila* were grown without additional NaCl, all the ecotype showed maximum shoot dry weight. At 50 mM shoot dry weight was not affected except in CC and YK but above this salt treatment (100, 200, 300, or 400 mM), shoot dry weight decreased progressively in all the ecotype and hence, there was a significant difference between shoot/root dry weight at different salt levels. North American ecotypes (CO, CC, YK, or DB) showed less shoot dry weight% of control compared to Chinese ecotypes (SH, XJ, HB, or HN). In case of shoot dry weight % of control, a significant difference was observed between all the ecotypes at 50, 100, 200, 300, or 400 mM NaCl concentrations. At lower level of salt (50 mM), HB showed highest shoot dry weight and at higher level (100, 200, or 300 mM) of salt CO showed maximum shoot dry weight compared to other ecotypes. Similarly salt treatment resulted in a decrease of root dry weight of *T. halophila* and hence, there was a significant difference between all the ecotypes at all salt levels. Maximum dry weight (% of control) was recorded at 50 mM NaCl level in DB ecotype and minimum in CC. At 400 mM NaCl maximum root dry weight was recorded in DB and minimum in CC. Root dry weight was less affected by the adverse effect of salinity compared to shoot. North American ecotypes showed better growth as compared to Chinese (Fig. 4).

Leaf specific weight % of control was significantly increased by increasing the concentration of salt in the medium (Table 1). DB, YK, or HN adversely affected by salt compared to the other ecotypes. On the other hand Chinese ecotypes showed less increase in case of leaf specific weight compared to North Americans ecotypes.

Table 1. Specific leaf weight (% of control) of *T. halophila* ecotypes exposed to 0, 200, 400, or 600 mM NaCl (n=5).

	Salinity (mM)				Contrasts	
Ecotype	0	200	400	600	lin	quad
Colorado	100	142^{a*}	146^{ab*}	138^{abc*}	***	**
Cracker Creek	100	118^{bc}	123^{cd}	132^{bcd*}	**	n.s.
Dillibrough	100	108^{c}	140^{abc*}	121^{cd*}	***	**
Hebei	100	127^{ab*}	151^{a*}	158^{a*}	***	n.s.
Henan	100	110c	118^{d}	113^{d}	n.s.	n.s.
Shandong	100	139^{a*}	154^{a*}	144^{ab*}	***	**
Xinjiang	100	120^{bc*}	128^{bcd*}	124^{cd*}	**	*
Yukon	100	105^{c}	110^{d}		n.s.	
Sig^{z}		***	***	***		
China	100	124.2	138.3	133.3		
N. America	100	118.2	131.7	131.3		
Sig^{z}		n.s.	n.s.	n.s.		

^{z}Ecotypes are significantly different from one another at $p \leq 0.05$ (*), 0.01 (**), or 0.001 (***).
Values with the same letter within the same column are not different from each other, based on Tukey's Honestly Significantly Difference Test
Values with * within the same row are significantly different from the control (0 mM) based on Dunnett's test ($p \leq 0.05$)

Table 2. Relative water content (% of control) of *T. halophila* ecotypes exposed to 0, 200, 400, or 600 mM NaCl (n=6).

	Salinity (mM)				Contrasts	
Ecotype	0	200	400	600	lin	quad
Colorado	100	97.7^{ab}	96.9^{a}	101.9^{a}	n.s.	n.s.
Cracker Creek	100	91.2^{ab*}	95.5^{a}	84.4^{ab*}	***	n.s.
Dillibrough	100	90.8^{ab}	87.3^{b*}	73.8^{b*}	***	n.s.
Hebei	100	88.7^{b}	86.0^{b}	84.1^{ab}	*	n.s.
Henan	100	88.2^{b}	87.9^{b}	94.0^{ab}	n.s.	*
Shandong	100	92.5^{ab*}	98.3^{a}	87.1^{ab*}	**	n.s.
Xinjiang	100	95.6^{ab}	98.1^{a}	95.1^{ab}	n.s.	n.s.
Yukon	100	100.7^{a}	99.4^{a}	90.8^{ab}	n.s.	n.s.
Sig^{z}	n.s.	**	n.s.	*		
China	100	90.9	92.8	90.1		
N. America	100	94.8	94.7	86.1		
Sig^{z}	n.s.	*	n.s.	n.s.		

Analysis was conducted on square root transformed data
^{z}Linear or quadratic contrasts are significant at $p \leq 0.05$ (*), 0.01 (**), or 0.001 (***)
Values with the same letter within the same column are not different from each other, based on Tukey's Honestly Significantly Difference Test
Values with * within the same row are significantly different from the control (0 mM) based on Dunnett's test ($p \leq 0.05$)

Table 3. Succulence (% of control) of *T. halophila* ecotypes exposed to 0, 200, 400, or 600 mM NaCl (n=6).

	Salinity (mM)				Contrasts	
Ecotype	0	200	400	600	lin	quad
Colorado	100	101^{ab}	106^{b}	107^{a}	n.s.	n.s.
Cracker Creek	100	92^{b}	99^{ab}	87^{c*}	*	n.s.
Dillibrough	100	90^{b}	91^{c}	86^{c*}	*	n.s.
Hebei	100	95^{ab}	100^{b}	101^{b}	n.s.	n.s.
Henan	100	102^{ab}	103^{b}	108^{a}	n.s.	n.s.
Shandong	100	95^{ab}	106^{b}	99^{b}	n.s.	n.s.
Xinjiang	100	105^{a}	117^{a*}	110^{a}	*	n.s.
Yukon	100	100^{ab}	106^{b}	100^{b}	n.s.	n.s.
Sig^{z}		**	n.s.	**		
China	100	98	107	105		
N. America	100	96	100	93		
Sig^{z}		n.s.	n.s.	**		

^{z}Ecotypes are significantly different from one another at $p \leq 0.05$ (*), 0.01 (**), or 0.001 (***)
Values with the same letter within the same column are not different from each other, based on Tukey's Honestly Significantly Difference Test. Analysis was conducted on log transformed data
Values with * within the same row are significantly different from the control (0 mM) based on Dunnett's test ($p \leq 0.05$)

Water relations: An increasing trend was observed in relative water contents (RWC) at 200 mM NaCl but it decreased slightly at 200, 400, or 600 mM in both NA and Chinese ecotypes (Table 2). CO showed maximum RWC at 600 mM and DB minimum. RWC showed significant difference in CC, DB, HB, or SH but non-significant in CO, HN, XJ, or YK between different salt concentration. North American ecotypes showed significant difference in RWC at 200 mM and non-significant difference at 400 mM and 600 mM NaCl compared to Chinese ecotypes.

An increasing trend was observed in succulence at 400 mM but a decreasing trend at 600 mM was analyzed between different ecotypes. CC, DB, or SH showed a decreasing trend by increasing salt concentration and there was a significant difference in CC, DB, or XJ at different salt levels (Table 3). Shoot, root osmotic potential and shoot osmotic potential at 100% of control decreased with increasing concentration of salt from 0-300 mM and hence there was a significant difference among all the ecotypes and salt concentration. At 300 mM maximum decrease in root and shoot osmotic potential was recorded in CC and on the other hand minimum decrease in root and shoot osmotic potential was observed in DB and HN at 0 mM (Fig. 5).

Overall a significant difference was recorded at all salt levels in case of shoot and root osmotic potential. A decreasing trend was observed by increasing the salt concentration from 0-600 mM and a significant difference was recorded between different ecotypes and salt levels, except 400 and 600 mM. Chinese ecotypes showed less decreasing trend in osmotic potential at full turgor as compared to North American ecotypes. Osmotic adjustment decreases down by increasing the salt concentration and a significant difference was recorded at 600 mM between ecotypes and non-significant at 200 and 400 mM. Similarly a significant difference in CC, DB, HB, or SH and non-significant in CO, HN, XJ, or YK at different salt levels was observed. Chinese ecotypes showed less increase as compared to North American. An increasing trend was observed by increasing concentration of salt in the medium in difference in osmotic potential between root and leaves. Chinese ecotypes showed less sharp but North American showed more sharp increase in difference in osmotic potential between root and leaves.

Discussion

The growth of plant with relationship to physiology under salinity stress was studied by many authors (Rengel, 1992; Munns, 1993; Newmann, 1995) with the common theme that plant resistance to high salt maintained their growth at higher salt concentration without physiological changes (Sajid & Khilji, 2020). Salt limit the growth of plant by restricting the absorption of water from the soil so plant have to develop certain physiological mechanism to maintain its growth under severe salt stress conditions. *Thellungiella halophila* is halophyte plant species and it can tolerate higher level of salt (Savoure *et al.*, 1995). During this investigation different parameters regarding its growth and physiology at different salt concentration was studied. Seed germination process is a crucial step in plant growth and development (Khan *et al.*, 2000). Salinity stress delays this process and higher concentration of salt inhabit completely in several plant species and based on these categorized plants as salt tolerant or sensitive (Grieve *et al.*, 2012). In our study *T. halophila* all ecotypes grew well on 100 mM NaCl except DB, SH, or JS and these results indicated that T. halophila ecotype CC, CO, HB, HN, or YK are more tolerant to salt compared to DB, SH, or JS. Such type of germination behavior might be due halophytic nature of this plant and control accumulation of NaCl as reported in many studies (Bressan *et al.*, 2001; Inan *et al*., 2004; Kent *et al.*, 2006; Ghars *et al*., 2008). At higher level of salt (200, 300, or 400 mM), no germination of seed was observed in any ecotype. It appears from our data of seed germination that *T. halophila* is not tolerant at higher salinity level at germination stage. Seedlings of *T. halophila* showed decreased in root length by increasing salt concentration and time duration in the present study. It may be due to higher accumulation of Na^+ by the roots of seedling which disturb the K^+ and Ca^+ uptake. It is well evident from the literature, salinity adequately reduces the amount of Ca^+ uptake and it adversely affects membrane function and growth of root (Lauchli & Epstein, 1970; Cramer *et al.*, 1988; Volkov & Amtmann, 2006; Rothstein, 2008; XiaoJing *et al*., 2015).

It is evident from the results of our growth data soil medium that shoot, and root dry mass was higher in Chinese ecotypes (SH, XJ, HB, or HN) compared to North Americans (CO, CC, YUK, or DB), at lower level of salt 50 mM all ecotypes grew very well but at higher level (400 mM) of NaCl ecotype CO showed maximum growth. Shoot dry mass was more than 50% recorded in DB and SH and more than 40% in ecotypes XJ, CO, DB, or SH at highest salt concentration. These results showed that *T. halophila* was not affected by salinity and it may be an obligate halophyte since it required salt for its growth and development. These results resemble with the previous studies by Mraha *et al*., (2007), Volkov *et al.*, (2003) and Ghars *et al.*, (2008). Maximum shoot and root dry mass in ecotype CO indicates that it is more tolerant to salt compared to other ecotypes. Specific leaf weight was increased when the plants challenged with higher concentrations of salt which might be due accumulation of salt in the leaves.

In general, salinity reduces water contents in plants as the absorption of water from the soil is inhabited and leads to reduced plant growth (Munns, 2005). In our study in *T. halophila* an increasing trend was observed in RWC and succulence up to 400 mM NaCl. CO plants showed maximum relative water contents compared to all other tested ecotypes. This higher level of RWC also correlated the better growth of CO at higher salt levels. This higher relative water contents was also observed in *T. halophila* compared to *A. thaliana* (Ghars *et al*., 2008). Shoot osmotic potential decreased by increasing salt concentration in our study which might explain the higher plant biomass obtained during this investigation. Higher salt tolerance was expected to correspond with greater ability to maintain water loss at higher salinity level as previously observed in salt tolerant genotypes of

Distichlis spicata (Chrisman *et al*., 2009). The decrease in osmotic potential showed that *T. halophila* does not accumulate salts and hence grow at higher salinity levels. In conclusion, North American ecotypes showed less tolerance to salt as compared to Chinese ecotypes.

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