**Suaeda salsa** is Adaptive to Chilling Stress under Salinity at Stages of Seed Germination and Seedling Establishment

Chen Meng¹,†, Mingjie Wang²,†, Min Chen² & Na Sui²

¹ Key Laboratory of Plant Cell Engineering and Germplasm Innovation, Ministry of Education, School of Life Sciences, Shandong University, Jinan, China
² Key Laboratory of Plant Stress Research, College of Life Science, Shandong Normal University, Jinan, China

Correspondence: Na Sui, Key Laboratory of Plant Stress Research, College of Life Science, Shandong Normal University, Jinan 250014, China. E-mail: suina800101@163.com

† These authors equally contributed to this work.

Received: March 4, 2015   Accepted: April 17, 2015   Online Published: May 15, 2015
doi:10.5539/jas.v7n6p217          URL: http://dx.doi.org/10.5539/jas.v7n6p217

**Abstract**

*Suaeda salsa* L., a C3 euhalophytic herb, is a native of saline soils. This herb displays high resistance to salinity stress. In the present study, we investigated the interaction between low temperature and salinity on seed germination and seedling establishment. Low temperature (4°C) conditions severely inhibited seed germination at 0 and 200 mM NaCl. The percent germination at 200 mM NaCl was higher than that at 0 mM NaCl. After 24 h of chilling stress, the fresh weight and dry weight of *S. salsa* seedlings at 0 mM NaCl was reduced, but the fresh weight and dry weight of *S. salsa* seedlings at 200 mM NaCl increased. APX and CAT activities decreased during chilling stress at both 0 and 200 mM NaCl, yet the activity of both enzymes was not affected as strongly at 200 mM NaCl compared to 0 mM NaCl. After a 12 h chilling treatment, the unsaturated fatty acid content and the double bond index (DBI) of DGDG, SQDG and PG major membrane lipids was significantly increased at 200 mM NaCl. The level of PG and the ratio of DGDG/MGDG in seedling leaves were also increased in the presence of 200 mM NaCl. These results suggest that *S. salsa* is more adaptive to chilling stress under 200 mM NaCl conditions at stage of seed germination and during seedling establishment.

**Keywords:** chilling stress, germination, halophyte, seedling establishment, *Suaeda salsa*, unsaturated fatty acids

1. **Introduction**

Salt stress is a major environmental factor that limits crop production. The occurrence of halophytes in inland salt marshes may depend on their tolerance to salt stress at different stages of their plant development (Ungar, 1996). Germination is a key stage in the life cycle of plants existing in saline environments as it determines whether or not plants can successfully mature in certain areas (Guo et al., 2012; Khan et al., 2004; Ungar, 1978). Salinity either completely inhibits seed germination or induces a state of seed dormancy (Iqbal et al., 2006). The effect of salinity on seed germination can be attributed to an osmotic effect and/or toxicity induced by specific ions, although the strength of these effects is dependent on the plant species (Song et al., 2005, 2006).

Low temperature and low irradiance are major factors that limit productivity. Chilling stress inhibits photosynthesis via the process of photoinhibition (Aro et al., 1993). Chloroplast membranes are the first victims of damage from chilling stress (Kratsch & Wise, 2000). Lyons and Raison (Lyons & Raison, 1970) suggested that chilling stress impairs membrane permeability by transforming membrane lipids from a liquid-crystalline phase to a gel phase, and since then, other groups have suggested that chilling tolerance is related to the composition and structure of plant membrane lipids (Murata & Los, 1997; Nishida & Murata, 1996; Somerville, 1995). In higher plants, the most abundant lipids in thylakoid membranes are glycolipids, including monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG). Tolerance to chilling stress is closely connected with the fatty acid unsaturation content of plant membrane lipids (Somerville, 1991; Szalontai et al., 2003). The chilling resistance of higher plants is closely correlated with the amounts of cis-unsaturated fatty acids in phosphatidylglycerol (PG) of chloroplast membranes (Murata et al., 1992; Nishida & Murata, 1996). PG molecules are important for both
the formation and function of the photosynthetic apparatus (Domonkos et al., 2004; Hagio et al., 2002). The majority of PG molecules are localized within thylakoid membranes, the sites of oxygenic electron transport (Wada & Murata, 1998). Changes in the fatty acid species of PG can influence the photosynthetic function of PSI and PSII and the activities of chloroplast antioxidant enzymes.

Temperature and salinity are two important factors that determine where and when seeds can germinate (Ajmal Khan & Ungar, 1998). For example, the seed germination rate of Haloxylon ammodendron is relatively high at 10-20 °C, but decreases severely when temperatures are over 20 °C (Huang et al., 2003; Tobe et al., 2000), indicating that seeds of these species likely germinate in early spring rather than late spring or summer. The Chenopodiaceae Suaeda salsa L., a C3 euhalophytic herb, is native to saline soils and has adapted high resistance towards salinity stress. When germinates outside in saline soils, S. salsa often suffers from both salinity and chilling stress. But there is little information on the seed germination responses of S. salsa under both salt and chilling stress conditions. Therefore, the aim of the present study was to investigate how temperature and salinity interact to affect seed germination and membrane lipid composition during seedling establishment.

2. Materials and Methods

2.1 Seed Germination

Seeds of S. salsa were collected during November 2011 in the saline inland of the Yellow River Delta (37°25′N, 118°58′E) located in the Shandong province of China. Dry seeds were stored at −4 °C before use. S. salsa seeds from the saline inland populations were sown in Petri dishes (6 cm diameter) on two filter paper layers moistened with 3 mL of NaCl solutions under a tight-fitting lid to prevent evaporation, under 14 h of light/10 h of dark with light condition of 100 μmol m−2 s−1. The solution used for the study consisted of 1/5 Hoagland (Hoagland & Arnon, 1950) with 0 and 200 mM NaCl. For each plant species, 100 seeds were used. Petri dishes were maintained at 25 °C and at 4 °C under a 14 h light/10 h dark period for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24 h. To maintain adequate moisture, 2 mL of each solution was added to each Petri dish. Germinated seeds (seeds were considered to be germinated with the emergence of the radical) were counted (Song et al., 2006).

2.2 Plant Culture and Treatments

S. salsa seeds from the saline inland populations were sown in Petri dishes (6 cm diameter) under the conditions mentioned above. The solutions used for the study consisted of 1/5 Hoagland with 0 or 200 mM NaCl. For each plant species, 100 seeds were used. Petri dishes were maintained in a glasshouse under a 14 h light/10 h dark period. The temperature in the glasshouse was 28±5 °C during the day and 20±3 °C at night. After germination for 4 days, seedlings were kept at 4 °C and at 4 °C under a 14 h light/10 h dark period for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24 h. The treated seedling leaves were then frozen in liquid nitrogen and stored at −80 °C until further use for the determination of antioxidant enzymatic activities and lipid content.

2.3 Activities of Antioxidant Enzymes

APX activity was determined by following the decrease in absorbance at 290 nm. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.2 mM H2O2 and suitable volume of enzyme extract (Jimenez et al., 1997). Catalase activity was determined by Aebi’s method (D. H. Lee & C. Lee, 2000). The reaction mixture were aspirated via a peristaltic pump (4 ml min−1) contained 50 mM potassium phosphate buffer (pH 7.0) and plant extract in a 3 mL volume. The reaction was initiated by adding 10 mM H2O2 at 240 nm using the spectrophotometric method.

2.4 Lipid Extraction and Analysis

S. salsa leaf tissues were harvested and frozen immediately in liquid nitrogen. Lipids were extracted as described by Siegenthaler and Eichenberger (1984) and separated by two-dimensional thin layer chromatography (TLC) (Xu & Siegenthaler, 1997). For quantitative analysis, lipids were separated by TLC, scraped from the plates, and used to prepare fatty acid methyl esters. The fatty acid composition of individual lipids was determined using gas chromatography (GC-9A, Shimadzu, Japan) as described by Chen et al. (1994).

2.5 Statistical Analysis

Each graphical plot represents the results from multiple independent experiments, and the values are means ±SD. Statistical significance was determined by Duncan’s tests, and p values = 0.05 were considered statistically significant.
3. Results

3.1 Effects of Temperature and Salinity on Seed Germination

Seed germination was strongly inhibited by 200 mM NaCl at normal temperature (25 °C) (Figure 1A). After 24 h, the seed germination percentage was 76% at 0 mM NaCl conditions, while the germination percentage was 38% at 200 mM NaCl conditions. Low temperature (4 °C) severely inhibited the percentage of seed germination at both 0 and 200 mM NaCl concentrations (Figure 1B). It was apparent, however, that at 200 mM NaCl, inhibition via chilling stress was alleviated. Seeds began to germinate after 6 h of chilling stress both at 0 and 200 mM NaCl concentrations. Interestingly, the percentage of germination at 200 mM NaCl was highest at 12% after 8 h of chilling stress, while the germination percentage of 0 mM NaCl was highest at 10% after 9 h of chilling stress (less than that of 200 mM NaCl after 8 h).

Figure 1. Seed germination of *S. salsa* after treated with 0 and 200 mM NaCl at normal condition (25 °C) (A) and 4 °C (B) for 0-24 h under low irradiance (100 μmol m⁻² s⁻¹). Data are means of 5 replicates (n = 5) ± SD. Different letters indicate significant differences at P = 0.05

3.2 Effects of Temperature and Salinity on Fresh Weight and Dry Weight

To compare the chilling tolerance of *S. salsa* during seedling establishment, the fresh weight and dry weight of the seedlings were examined. After 24 h of chilling stress, the fresh weight and dry weight of *S. salsa* seedlings at 0 mM NaCl were reduced to 1.6% and 4.2%, respectively. While the fresh weight and dry weight of *S. salsa* seedlings at 200 mM NaCl increased to 16.5% and 18.6%, respectively (Figure 2).

Figure 2. Fresh weight and dry weight of *S. salsa* seedling after 4 days of germination at 0 and 200 mM NaCl at 4 °C for 24 h. The data of each column represent mean (n = 5) ± SD. Different letters indicate significant differences at P = 0.05
3.3 Activity of APX and CAT at Chilling and High Salt Stress Conditions

APX and CAT activities decreased during chilling stress at both 0 and 200 mM NaCl concentrations, however the activities of both enzymes decreased less at 200 mM NaCl compared to 0 mM NaCl. At the end of the chilling stress period, APX and CAT activities at the 0 mM NaCl concentration decreased by 51.1% and 52.8%, respectively. The APX and CAT activities at 200 mM NaCl decreased by 30.8% and 36.5%, respectively (Figure 3).

![Figure 3. Changes in the activities of APX and CAT of *S. salsa* seedling after 4 days of germination at 0 and 200 mM NaCl at 4 °C for 0, 3, 6, 9, 12 and 24 h. Data are means of 5 replicates (n = 5) ± SD. Different letters indicate significant differences at P = 0.05](image-url)

3.4 Lipid Content and Fatty Acid Composition

After chilling treatment at 4 °C and low irradiance for 12 h, the unsaturated fatty acid content and the DBI (DBI = 18:1×1 + 18:2×2 + 18:3×3) of the major membrane lipids of DGDG, SQDG and PG (Table 1) significantly increased with the 200 mM NaCl treatment. The unsaturated fatty acid contents of linoleic acid (18:2) in MGDG, DGDG, SQDG and PG, and linolenic acid (18:3) in DQDG and SQDG increased with the 200 mM NaCl treatment.
Table 1. Fatty acid composition of membrane lipids in *S. salsa* seedling during chilling stress under NaCl treatment. Data are means of 5 replicates (n=5) ± SD. Different letters indicate significant differences at P = 0.05

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>Fatty acid composition (mol %)</th>
<th>0 mM NaCl</th>
<th>200 mM NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16:0</td>
<td>16:1</td>
<td>18:0</td>
</tr>
<tr>
<td>MGDG 0 mM</td>
<td>3.7±0.11*</td>
<td>0±0.01*</td>
<td>0.6±0.01*</td>
</tr>
<tr>
<td></td>
<td>0 h</td>
<td>11.0±0.11</td>
<td>1.1±0.03*</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>7.4±0.33a</td>
<td>2.2±0.13*</td>
</tr>
<tr>
<td>DGDG 200 Mm</td>
<td>5.1±0.25b</td>
<td>0.9±0.02a</td>
<td>1.2±0.06a</td>
</tr>
<tr>
<td></td>
<td>5.0±0.25a</td>
<td>7.4±0.39d</td>
<td>3.7±0.11*</td>
</tr>
<tr>
<td>SQDG 0 mM</td>
<td>13.7±0.51b</td>
<td>3.1±0.12c</td>
<td>1.1±0.07c</td>
</tr>
<tr>
<td></td>
<td>200 mM NaCl 0 h</td>
<td>24.0±1.23</td>
<td>7.2±0.31b</td>
</tr>
<tr>
<td>PG 0 mM NaCl</td>
<td>43.6±1.89b</td>
<td>4.2±0.27a</td>
<td>3.1±0.21cd</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>43.9±1.67b</td>
<td>8.3±0.35b</td>
</tr>
<tr>
<td></td>
<td>200 mM NaCl 0 h</td>
<td>47.9±1.79b</td>
<td>10.9±0.44b</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>35.9±1.63b</td>
<td>6.0±0.37</td>
</tr>
</tbody>
</table>

At 200 mM NaCl, the unsaturated fatty acid content of 18:2 increased significantly by 133.7% and the DBI also increased from 195.1 to 198.0 after 12 h of chilling stress (Table 2). Oleic acid (18:1), 18:2 and 18:3 all decreased significantly at 0 mM NaCl, and the DBI decreased by 11.6% after 12 h of chilling stress. These results indicated that the content of unsaturated fatty acids in *S. salsa* seedlings increased at a concentration of 200 mM NaCl during chilling stress.

Table 2. Constituent fatty acids of total lipids in *S. salsa* seedling during chilling stress under NaCl treatment. Data are means of 5 replicates (n=5) ± SD. Different letters indicate significant differences at P=0.05.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>0 mM NaCl</th>
<th>200 mM NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>12 h</td>
</tr>
<tr>
<td>16:0</td>
<td>18.5±0.56b</td>
<td>25.5±1.07a</td>
</tr>
<tr>
<td>16:1</td>
<td>1.1±0.11a</td>
<td>0.2±0.02c</td>
</tr>
<tr>
<td>18:0</td>
<td>1.9±0.78c</td>
<td>5±0.32a</td>
</tr>
<tr>
<td>18:1</td>
<td>1.3±0.01c</td>
<td>2.5±0.12a</td>
</tr>
<tr>
<td>18:2</td>
<td>20.5±1.17b</td>
<td>14.8±0.46c</td>
</tr>
<tr>
<td>18:3</td>
<td>56.8±2.56a</td>
<td>52±1.83b</td>
</tr>
<tr>
<td>DBI</td>
<td>212.7</td>
<td>188.1</td>
</tr>
</tbody>
</table>

After chilling treatment at 4 °C and low irradiance for 12 h, PG content and the ratio of DGDG/MGDG in leaves at 0 mM NaCl decreased by 22.9% and 6.7%, respectively (Table 3), while they increased 75.0% and 17.0%, respectively, at 200 mM NaCl.
Table 3. Composition of lipid classes in *S. salsa* seedling during chilling stress under NaCl treatment. Data are means of 5 replicates (n = 5) ± SD. Different letters indicate significant differences at P = 0.05

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>Lipid content (mol %)</th>
<th>0 mM</th>
<th>200 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>12 h</td>
<td>0 h</td>
</tr>
<tr>
<td>Monogalactosyldiacylglycerols (MGDG)</td>
<td>43.7±2.28b</td>
<td>42.8±2.17b</td>
<td>47.5±2.25a</td>
</tr>
<tr>
<td>Digalactosyldiacylglycerols (DGDG)</td>
<td>19.8±1.17c</td>
<td>17.8±1.09d</td>
<td>25.4±2.84a</td>
</tr>
<tr>
<td>Sulphoquinovosyldiacylglycerols (SQDG)</td>
<td>10.7±0.07d</td>
<td>19.5±0.75a</td>
<td>13.1±0.56c</td>
</tr>
<tr>
<td>Phosphatidylglycerols (PG)</td>
<td>25.8±1.45a</td>
<td>19.9±0.55b</td>
<td>14.0±0.89c</td>
</tr>
<tr>
<td>DGDG/MGDG</td>
<td>0.45</td>
<td>0.42</td>
<td>0.53</td>
</tr>
</tbody>
</table>

4. Discussion

Interaction between temperature and salinity levels appeared to have an effect on halophyte seed germination (Ungar, 1978). A similar observation was shown in the present study. Seed germination was strongly inhibited by chilling and NaCl stress. The seed germination percentage decreased 38% at 25 °C in the presence of 200 mM NaCl. Increased salt conditions were shown to increase chilling tolerance during the seed germination stage. Results suggested that after 24 h of 4 °C chilling stress, the seed germination percentage at 200 mM NaCl was higher than that at 0 mM NaCl. Therefore, the interaction of low temperature and salinity increases seed germination in *S. salsa*, and this may be an important strategy for *S. salsa* to survive and germinate in early spring (conditions of low temperature and salt stress).

Chilling stress inhibited seedling growth (the fresh weight and dry weight) of plants. This trend was more prominent in *S. salsa* seedlings at 0 mM NaCl. Interestingly, the fresh weight and dry weight of *S. salsa* seedlings at 200 mM NaCl increased after 24 h of chilling stress. The present results indicates that the inhibitory effect of chilling stress in seedling growth can be alleviated by salinity.

During salt and chilling stresses, reactive oxygen species (ROS) are produced. ROS, including superoxide anions (O$_2$·$^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radicals (OH ·) are formed in all aerobic cells as by-products of normal metabolic processes (Asada, 1992). Production of these molecules, especially under environmental stress, results in oxidative damage at the cellular level. Accumulation of ROS is the main cause of oxidative damage. Meanwhile, plants have a series of antioxidant enzymes which can scavenge toxic ROS and protect the plants from ROS damage. APX reduces H$_2$O$_2$ to water using ascorbic acid (AsA) as a specific electron donor and is thus the most important peroxidase in H$_2$O$_2$ detoxification in plants (Foyer & Halliwell, 1976; Noctor & Foyer, 1998). In the present study, though APX and CAT activities decreased during chilling stress, the activities of both enzymes decreased more extensively at 0 mM NaCl than at 200 mM NaCl.

Many experiments suggest that changes in unsaturated fatty acid content can enhance the plant tolerance to environmental stresses such as cold, heat and drought (Dakhma et al., 1995; Matos et al., 2002; Sui et al., 2007a, 2007b, Sui & Han, 2014). Our former study suggested that *S. salsa* displays high resistance to photoinhibition under salt stress conditions and that increased concentration of unsaturated fatty acids in membrane lipids of *S. salsa* enhances the tolerance of photosystem II to salt stress (Sui et al., 2010; Sui et al., 2007b). Whether the interaction of low temperature and salinity can also affect unsaturated fatty acid content of *S. salsa* seedlings is still unknown. In this study, after treatment at 4 °C for 12 h, the unsaturated fatty acid content and the DBI of the major membrane lipids of DGDG, SQDG and PG (Table 1) significantly increased with the 200 mM NaCl solution. At 200 mM NaCl, unsaturated fatty acids of 18:2 and the DBI increased after 12 h of chilling stress. While 18:1, 18:2, 18:3 and the DBI all decreased significantly at 0 mM NaCl (Table 2). These results indicated that the interaction of low temperature and salinity can increase the content of unsaturated fatty acids in *S. salsa* seedlings.

A change in the ratio of a bilayer-forming lipid (DGDG) to an inverse hexagonal-forming lipid (MGDG) (DGDG/MGDG ratio) can affect the structure and microviscosity of membranes, determine the accumulation of phospholipids in leaves and also perturb resistance of organisms to environmental stresses. After 12 h of chilling stress, the ratio of DGDG/MGDG at 200 mM NaCl treatment leaves increased, while it decreased at the 0 mM NaCl treatment. The increased ratio of DGDG/MGDG may protect the photosynthetic apparatus and stabilize photosynthetic processes. These results indicate that salinity may counteract chilling stress.
PG is the only phospholipid in thylakoid membranes, the sites of oxygenic electron transport in PSII (Wada & Murata, 1998). The content of fatty acid species in PG affects the function of PSII. The content of saturated fatty acids of PG in the thylakoid membrane was related to plant sensitivity to chilling stress, which closely correlated with membrane fluidity. The importance of membrane fluidity in temperature tolerance has been widely discussed in various mutational analyses, TG studies, and physiological studies (Örvar et al., 2000; Sung et al., 2003). Increasing cis-unsaturated fatty acid contents of PG can increase tolerance of plants to chilling stress (Nishida & Murata, 1996). In this study, we have found that 18:2 of PG increased (Table 1). In the previous study, PG depletion was involved in the degradation of PSI trimers and accumulation of PSI monomers. In vitro studies suggest that trimer formation is enhanced by the lipid environment (Kruip et al., 1999). Three PG molecules are bound to the reaction center of the PSI core complex, which suggests that PG has an important function in PSI, presumably in the assembly of the PSI core complex (Jordan et al., 2001; Sakurai et al., 2003).

After treatment at 4 °C for 12 h, the level of PG in 0 mM NaCl-treated leaves decreased 22.9%, while it increased 75.0% in 200 mM NaCl-treated leaves (Table 3). The increased PG content might protect PSII and PSI during chilling and salinity stress. This result also revealed that salinity could alleviate chilling stress at several stages of seed germination and during seedling establishment.

In conclusion, chilling stress decreased seed germination, the fresh weight and dry weight, and also the activities of APX and CAT enzymes. However, seed germination, the fresh weight and dry weight, APX and CAT activities were higher at 200 mM NaCl compared to 0 mM NaCl. After 12 h of chilling stress, the unsaturated fatty acid content and the DBI of the major membrane lipids of DGDG, SQDG and PG significantly increased in the 200 mM NaCl treatment. The level of PG and the ratio of DGDG/MGDG in seedling leaves were also increased in the presence of 200 mM NaCl. These results suggest that seed germination and seedling establishment of S. salsa can be ensured at chilling and salinity conditions and S. salsa seedlings at 200 mM NaCl are rather tolerant to chilling stress. This process is most likely an important adaptive strategy for S. salsa to survive in a harsh saline environment and low temperature.

Acknowledgements

We are grateful for financial support from the NSFC (National Natural Science Research Foundation of China (31300205), Natural Science Research Foundation of Shandong (ZR2013CQ009), the Science and Technology Development Projects of Shandong Province (2014GNC113005), the Doctoral Foundation of Shandong Province (BS2011SW048) and the Program for Scientific research innovation team in colleges and universities of Shandong Province.

References


**Abbreviations**

DBI – double bond index; DGDG – digalactosyldiacylglycerols; MGDG – monogalactosyldiacylglycerols; PG – phosphatidylglycerols; SQDG – sulphoquinovosyldiacylglycerols.
Copyrights
Copyright for this article is retained by the author(s), with first publication rights granted to the journal.
This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).