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Increases of Unsaturated Fatty Acids in Membrane Lipids Protects Photosystem II from Photoinhibition under Salinity in Different Halophytes

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Abstract

For the purpose of testing the function of unsaturated fatty acids in different halophytes in the process of photosynthesis under salt stress, the impact of saline stress on plant development, content of chlorophyll, the PSII photochemistry efficiency, content of membrane lipid and composition of fatty acid were investigated in the three halophytes *Thellungiella halophila, Limonium bicolor* and *Suaeda salsa* and non-halophyte *Arabidopsis thaliana*. Salinity (200 mM NaCl) did not reduce the value of Fv/Fm, ΦPSII, and chlorophyll content in the halophytes. While all of them decreased by 200 mM NaCl treatment in *A. thaliana*. In the non-halophytic, *A. thaliana*, when treated with NaCl, the content of unsaturated fatty acid and the DBI of membrane lipids MGDG, SQDG, PG and PC decreased. While the unsaturated fatty acid content and the DBI of *T. halophila*, *L. bicolor* and *S. salsa* increased. The DBI of total lipids increased in all halophytes but decreased in the non-halophyte, *A. thaliana*. The proportion of PG increased in *T. halophila* and *S. salsa*. It decreased in *L. bicolor* and *A. thaliana*. The DGDG (digalactosyldiacylglycerols)/MGDG (monogalactosyldiacylglycerols) ratio of *S. salsa* increased from 1.20 to 1.35, while it decreased in *T. halophila*, *L. bicolor* and *A. thaliana* under salt stress. These results suggest that unsaturated fatty acid levels increase in the halophytes under salt stress relative to the non-halophyte *A. thaliana*. The proportion of membrane lipids and unsaturated fatty acids is related to different levels of salt tolerance among different halophytes.

Keywords: chlorophyll, *Limonium bicolor*, photosystem, salt stress, *Suaeda salsa*, *Thellungiella halophila*, unsaturated fatty acids

1. Introduction

Worldwide, more than 800 million hectares of land are affected by salt. Although high levels of salt generally reduce plant growth, tolerance to soil salinity differs greatly among plant species (Munns & Tester, 2008). Most species of plants are very sensitive to salt conditions and cannot complete their life cycle under high salinity. However, halophytes adapted to grow in saline environments have substantial potential to be developed into vegetable, forage, and oilseed crops. It is also possible that halophytic properties can be developed in crop plants for “saline agriculture”.

Most ions as well as large molecules in plans are barricaded when transporting among cell membrane. lipid composition and the degree of fatty acid desaturation affect membrance structure and fluidity(Mikami & Murata, 2003); the latter has been considered to influence the permeability of the membrane bilayer (Schuler et al., 1991), the transport was mediated by ATPase activity and carrier (Deuticke & Haest, 1987). The levels of unsaturated fatty acids on membranes define lipid membrane fluidity. Cold, heat and drought, such environmental stresses are less unbearable for plants through variation of unsaturated fatty acids content (Dakhma, Zarrouk, & Cherif, 1995; Liu et al., 2008; Olsson, 1995). The most abundant membrane lipids in higher plants are glycolipids, including digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG), sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG). PG is the only phospholipid in

photosynthetic membranes. Glycolipids has been thoroughly studied in terms of membrane structure and function (Siegenthaler & Eichenberger, 1984).

Typically, the lipid components of living cell membranes adjust to physiological conditions and the environment. Former studies have shown that lipids are engaged in protecting the photosystem to reduce salt stress. Müller and Santarius found that if barley (*Hordeum vulgare* L.) seedlings at the root has a high concentration of NaCl during the adaptation, then galactolipids content in membranes of chloroplast reduced comparatively (Müller & Santarius, 1978). In order to protect the photosynthetic apparatus in the slow phase, the desaturation of fatty acids was completely effective (Allakhverdiev, Kinoshita, Inaba, Suzuki, & Murata, 2001). Although some studies have done some research in the level of fatty acid desaturation during salt stress of halophytes(Ben Hamed, Ben Youssef, Ranieri, Zarrouk, & Abdelly, 2005; Ramani, Zorn, Papenbrock, 2004; Sui, M. Li, K. Li, Song, & Wang, 2010), the relationship between the PSII protection mechanism in halophytes and fatty acid desaturation was still ambiguous in a saline environment.

The German plant ecologist Breckle divided halophytes into three categories according to ion accumulation and transport characteristics: recretohalophytes, euhalophytes and pseudo-halophytes. Recretohalophytes expel excess ions from the plant through special tissues such as a salt gland or salt bladder, allowing them to maintain internal ion balance; euhalophytes compartmentalize ions into vacuoles to prevent high concentration of ions from damaging the protoplast; pseudo-halophytes intercept ions in roots and minimize transport to the shoot parts to protect the main metabolic tissues (Breckle, 1995).

Thellungiella halophila is a typical pseudo-halophyte belonging to Cruciferae and it has a close genetic relationship with *Arabidopsis*. However *T. halophila* (Stepien & Johnson, 2009) can complete its life cycle under more than 300 mM NaCl. *Limonium bicolor* (Bunge) Kuntze, belonging to *Limonium*, *plumbagenaceae*, is a typical exo-recretohalophyte and has a typical salt excretory structure called a salt gland. *L. bicolor* can improve and desalinate saline-alkali soil and maintain high rates of photosynthesis in 200-300 mM NaCl treatments. The Chenopodiaceae *Suaeda salsa* L., a C3 euhalophytic herb, is native to saline soils and shows a high salt stress resistance, and also has a strong resistance to photoinhibition under salt treatment conditions, even treated with 400 mM NaCl and full light irradiation (C. Lu, Qiu, Q. Lu, Wang, & Kuang, 2002).

Different types of halophytes have different strategies to cope with high ionic concentrations, but have similarly efficient photosynthetic function under the treatment of NaCl. The present research is designed to detect whether the protection mechanism of unsaturated fatty acids on PSII under salinity is similar in the pseudo-halophyte *T. halophila*, exo-recretohalophyte *L. bicolor*, euhalophyte *S. salsa* and the non-halophyte *A. thaliana*.

2. Materials and Methods

2.1 Plant Cultivation and Treatment

Seeds of *S. salsa*, *L. bicolor* and *T. halophila* were picked from the Yellow River Delta, Shandong Province, P. R. China (37°25′N; 118°58′E). *A. thaliana* seeds were harvested from laboratory culture in October, 2012. Germination conditions: the *S. salsa* seeds were sterilized in 0.5% $HgCl_2$, and then washed with sterile double distilled water and germinated with the method of sand culture, they were then conducted a three days of darkness treatment at 25 °C, and watered with 1/2 MS nutrient solution; *L. bicolor* seeds were sterilized and germinated as the *S. salsa*, and then kept 5 days in the dark at 25 °C and also watered with 1/2 MS solution; After sterilization with 70% ethanol and 1% NaClO, the seeds of *T. halophila* and *A. thaliana* were placed in 7.5 cm diameter Petri dishes containing filter-paper disks moistened with 1/5 Hoagland solution. Seeds were kept moist by periodic addition of 1/5 Hoagland solution. After germination, all seedlings were cultivated in a stable greenhouse condition, 22 °C day/18 °C night under a 16/8 light/dark cycle (150 μmol m^{-2} s^{-1} and 70% relative humidity). The 3 weeks seedlings were treated with 200 mM NaCl. The concentration of NaCl increased 50 mM every day until it reached a final concentration. Measuring physiological indexes after treated with NaCl for 5 days (Sui et al., 2010).

2.2 Pigment Analysis

For the analysis of leaf Chl content, leaves were extracted in 80% acetone and measured by a spectrophotometer according to Li, Meng, Jiang and Zou (2003).

2.3 Lipid Extraction and Analysis

Leaf blades of the same position were collected and immediately frozen in liquid nitrogen. Lipids were extracted with the method described by Siegenthaler and Eichenberger and separated by two-dimensional thin layer chromatography (TLC). For quantitative analysis, lipids were separated by TLC. The individual lipids was then measured by gas chromatography (GC-9A, Shimadzu, Japan) as described by Sui et al. (2010).

2.4 Measurements of Chlorophyll Fluorescence

The chl fluorescence was determined by the portable fluorometer (FMS2, Hansatech, King's Lynn, UK) (Kooten & Snel, 1990). The data of minimal fluorescence (Fo), variable fluorescence (Fv), maximal fluorescence (Fm), steady-state fluorescence (Fs) was recorded. The maximal photochemical efficiency of PSII: Fv / Fm = (Fm – Fo) / Fm, the actural photochemical efficiency of PSII: ΦPSII = (Fm' – Fs) / Fm' (Sui et al., 2010).

2.5 Analysis of the Fresh and Dry Mass of Leaves

Plant leaves were cleaned twice with double distilled water, then the water on the surface of the leaves was dried with absorbent paper, the fresh mass (FM) of plant leaves were determined immediately by a electronic scales. The plant leaves were dried at 80 °C for 24 h, then the dry mass (DM) was measured by a electronic scales. Water content (WC) was then recorded as the formula: WC = (FM – DM) / FM × 100%.

2.6 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 Unlike Arabidopsis, the Growth of Halophytes Was Not Affected by Salt

Growth of *A. thaliana* was significantly decreased by 200 mM NaCl treatment, however, growth of *S. salsa* was significantly increased under the same treatment. Under a treatment of 200 mM NaCl, the fresh and dry mass per plant of *A. thaliana* decreased 45.0% and 34.1%, respectively; meanwhile, the fresh and dry mass per plant of *S. salsa* under the same treatment increased by 50.6% and 54.2%, respectively. The water content (WC) of *A. thaliana* decreased from 85.4% to 82.5%, which showed that 200 mM NaCl could decrease water absorption in non-halophytes. There was no statistical difference in WC of *S. salsa* under treatments of 0 and 200 mM NaCl, with values of 90.7% and 90.0%, respectively. Both the fresh and dry mass of *T. halophila* and *L. bicolor* increased slightly under 200 mM NaCl treatment, but the change was not significant. There were no significant differences in WC of *T. halophila* and *L. bicolor* between treatments (Table 1).

Table 1. Biomass of fresh and dry mass of *A. thaliana*, *T. halophila*, *L. bicolor* and *S. salsa* under NaCl stress

Species	NaCl (mM)	Fresh mass (g plant^{-1})	Dry mass (g plant^{-1})
A. thaliana	0	2.80±0.35^{a}	0.41±0.03^{a}
	200	1.54±0.13^{b}	0.27±0.01^{b}
T. halophila	0	2.32±0.18^{a}	0.41±0.03^{a}
	200	2.54±0.11^{a}	0.39±0.02^{a}
L. bicolor	0	4.66±0.25^{a}	0.64±0.05^{a}
	200	4.94±0.25^{a}	0.67±0.02^{a}
S. salsa	0	2.36±0.11^{b}	0.22±0.02^{b}
	200	4.78±0.13^{a}	0.48±0.03^{a}

Data is represented as the mean of 5 replicates ± SD. Different letters indicate significant differences at P = 0.05.

3.2 PSII of Halophytes Has a Stronger Tolerance to NaCl

To test the light harvesting system of photosynthesis under salt stress, Fv/Fm and ΦPSII were measured. As illustrated in Figure 1, in different halophytes, NaCl treatment has nearly no effect on Fv/Fm. From the result we can infer, PSII of halophytes has a stronger tolerance in NaCl treatment condition and photochemistry of PSII of halophytes dark-adapted leaves was not affected by salt stress. The Fv/Fm decreased by 47.3% in *A. thaliana* under the same NaCl treatment, suggesting that photoinhibition was severe in non-halophytic plants under salt stress. ΦPSII in *A. thaliana* decreased by 33.0% when treated with 200 mM NaCl. This suggested that salt stress decrease photosynthetic electron transport of non-halophyte. Salt stress had very little effect on ΦPSII in *T. halophila* and *L. bicolor*. In contrast, ΦPSII in *S. salsa* increased by 16.8% under the 200 mM NaCl treatment, which indicated an increase in *S. salsa* photosynthetic electron transport under salt stress.

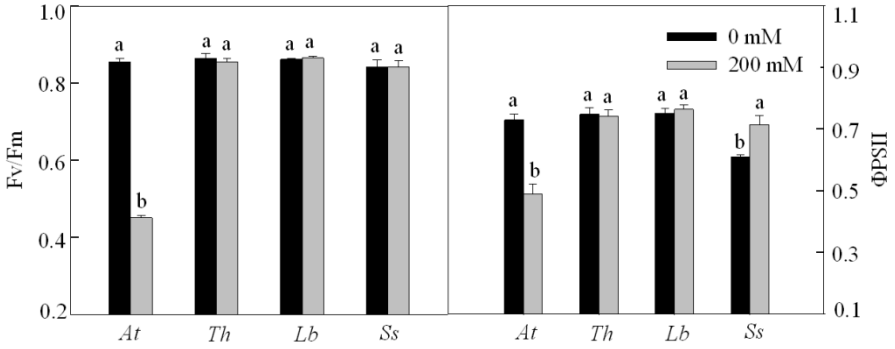

Figure 1. Effect of NaCl stress on Fv/Fm and ΦPSII in *A. thaliana*, *T. halophila*, *L. bicolor* and *S. salsa*

Data are represented as means of 5 replicates ± SD. For each column, different letters indicate significant differences at P = 0.05.

3.3 Chlorophylll Content of Halophytes Did Not Decrease under NaCl Treatmentt

Application of the 200 mM NaCl treatment increased Chl *a* content per DM by 20.2% and the Chl *a/b* ratio by 18.8% in *S. salsa*, but the Chl *a* content and chl *a/b* ratio in *A. thaliana* decreased by 17.9% and 13.4%, respectively. The Chl *a*/*b* ratio increased from 2.87 to 3.41 in *S. salsa* and decreased from 3.65 to 3.16 in *A. thaliana* at 200 mM NaCl treatment. No significant differences in contents of Chl *a* and *b* and Chl *a*/*b* ratios were found in *T. halophila* and *L. bicolor* (Table 2).

Table 2. Comparison of chlorophyll content and Chl *a*/*b* of *A. thaliana*, *T. halophila*, *L. bicolor* and *S. salsa* under NaCl stress

Species	NaCl (mM)	Chl *a* content (mg g^{-1} (DM))	Chl *b* content (mg g^{-1} (DM))	Chl *a*/*b*
A. thaliana	0	10.95±0.34^{a}	3.00±0.14^{a}	3.65±0.29^{a}
	200	8.99±0.21^{c}	2.85±0.06^{a}	3.16±0.14^{b}
T. halophila	0	10.02±0.31^{a}	3.07±0.12^{a}	3.26±0.23^{a}
	200	10.19±0.61^{a}	3.15±0.17^{a}	3.23±0.37^{a}
L. bicolor	0	11.08±0.31^{a}	3.30±0.18^{a}	3.36±0.28^{a}
	200	11.08±0.50^{a}	3.45±0.13^{a}	3.21±0.27^{a}
S. salsa	0	7.03±0.22^{b}	2.45±0.24^{a}	2.87±0.37^{b}
	200	8.45±0.13^{a}	2.48±0.09^{a}	3.41±0.18^{a}

Data are represented as means of 5 replicates ± SD. Different letters indicate significant differences at P = 0.05.

3.4 Comparison of Lipid Content and Fatty Acids Composition

The DBI of total lipids increased in all halophytes but decreased in the non-halophyte *A. thaliana* (Table 3). It increased 69.0% in pseudo-halophyte *T. halophila*, 44.6% in exo-recretohalophyte *L. bicolor* and 30.8% in euhalophyte *S. salsa*, whereas it decreased 50.9% in the non-halophyte *A. thaliana*. In particular, the 18:3 unsaturated fatty acids increased markably in all halophytes, while the 18:1, 18:2 and 18:3 fatty acids all decreased significantly in the non-halophyte *A. thaliana*. These results showed that the unsaturated fatty acid contents increased in the NaCl treatment condition in halophyte plants, while correspondingly, the contents of saturated fatty acids decreased.

Table 3. Comparison of constituent fatty acids of total lipids in *A. thaliana*, *T. halophila*, *L.bicolor* and *S. salsa* leaves under NaCl stress

Species	Fatty acid	Fatty acid composition [mol %]	
		0 mM NaCl	200 mM NaCl
A. thaliana	16:0	22.30±1.31^{b}	38.70±1.51^{a}
	16:1	2.49±0.62^{b}	1.70±1.32^{a}
	18:0	22.78±1.30^{a}	32.12±1.42^{a}
	18:1	22.73±1.12^{a}	14.81±0.72^{b}
	18:2	7.80±0.49^{a}	1.75±0.08^{b}
	18:3	21.90±1.23^{a}	10.92±0.41^{a}
	DBI	**104.03**	**51.06**
T. halophila	16:0	41.01±2.20^{a}	34.75±2.15^{ab}
	16:1	6.01±1.30^{a}	0.94±0.31^{c}
	18:0	22.99±1.21^{a}	17.55±1.12^{ab}
	18:1	7.71±0.36^{a}	7.20±0.55^{a}
	18:2	0.93±0.04^{b}	1.51±0.10^{a}
	18:3	21.34±1.24^{b}	38.05±2.35^{a}
	DBI	**73.59**	**124.38**
L. bicolor	16:0	46.79±1.94^{a}	34.84±1.22^{b}
	16:1	1.47±0.73^{a}	0.16±0.53^{b}
	18:0	21.17±0.88^{a}	18.23±0.72^{b}
	18:1	12.10±0.48^{b}	22.05±0.82^{a}
	18:2	0.96±0.03^{b}	--
	18:3	17.52±0.66^{c}	24.73±0.93^{b}
	DBI	**66.56**	**96.23**
S. salsa	16:0	28.32±0.88^{a}	16.64±0.71^{b}
	16:1	1.22±0.05^{d}	3.74±0.14^{b}
	18:0	20.19±0.73^{a}	13.77±0.51^{b}
	18:1	1.56±0.07^{c}	2.44±0.14^{a}
	18:2	11.57±0.47^{b}	14.58±0.57^{a}
	18:3	37.15±1.58^{b}	48.83±1.77^{a}
	DBI	**136.15**	**178.09**

Data are represented as means of 5 replicates ± SD. Different letters indicate significant differences at P = 0.05.

Different changes of membrane fatty acid content under salt stress in these plants were analyzed. In the non-halophytic, *A. thaliana,* the unsaturated fatty acid content and the double bond index (DBI = 18:1 × 1 + 18:2 × 2 + 18:3 × 3) of membrane lipids MGDG, SQDG, PG and PC (Table 4a) decreased under salt stress. Decreases were also noted in contents of the unsaturated fatty acids linoleic acid (18:2) and linolenic acid (18:3) of MGDG, 18:2 of DGDG, 18:3 of SQDG, oleic acid (18:1), 18:2 and 18:3 of PG, and 18:1 and 18:3 of PC. The palmitic acid (16:0) content of MGDG and PC under NaCl treatment was more than that in other membrane lipids. The content of 16:1 in PG also decreased under salt stress.

Table 4a. Fatty acid composition of membrane liqids in *A. thaliana* leaves under NaCl treatment

Membrane lipid	Fatty acid	Fatty acid composition [mol%]	
		0 mM NaCl	200 mM NaCl
MGDG	16:0	31.59±1.91^{c}	69.32±2.16^{a}
	16:1	--	--
	18:0	16.71±1.01^{a}	10.35±0.32^{b}
	18:1	9.87±0.59^{a}	10.72±0.33^{a}
	18:2	19.97±1.20^{a}	2.37±0.07^{b}
	18:3	21.87±1.32^{a}	7.24±0.23^{b}
	DBI	**115.42**	**37.18**
DGDG	16:0	34.82±1.63^{a}	23.66±0.86^{b}
	16:1	--	--
	18:0	16.23±0.76^{c}	21.40±0.77^{b}
	18:1	8.25±0.39^{c}	15.72±0.57^{b}
	18:2	23.91±1.12^{a}	1.61±0.06^{b}
	18:3	16.79±0.79^{c}	37.68±1.37^{a}
	DBI	**106.44**	**131.98**
SQDG	16:0	24.52±1.33^{b}	20.24±0.88^{c}
	16:1	--	--
	18:0	38.51±2.09^{a}	49.98±2.18^{a}
	18:1	7.16±0.39^{b}	23.51±1.03^{a}
	18:2	1.62±0.09^{b}	1.74±0.08^{b}
	18:3	28.20±1.53^{a}	4.55±0.20^{b}
	DBI	**95.00**	**40.64**
PG	16:0	20.01±0.70^{a}	36.26±0.56^{a}
	16:1	19.45±0.68^{b}	6.61±0.10^{c}
	18:0	21.89±0.77^{b}	36.00±0.56^{a}
	18:1	7.79±0.27^{b}	7.02±0.11^{b}
	18:2	2.03±0.07^{b}	0.60±0.01^{c}
	18:3	28.83±1.01^{a}	13.52±0.21^{b}
	DBI	**98.34**	**48.78**
PC	16:0	8.15±0.35^{c}	65.47±1.95^{a}
	16:1	--	--
	18:0	14.70±0.62^{b}	16.30±0.48^{c}
	18:1	62.10±2.63^{a}	12.25±0.36^{c}
	18:2	--	4.80±0.14^{a}
	18:3	15.05±0.64^{a}	1.18±0.11^{c}
	DBI	**107.25**	**25.39**

Data are represented as means of 5 replicates ± SD. Different letters a, b, c, d, e, f, g, h, indicate significant differences at P = 0.05.

In contrast, the pseudo-halophyte *T. halophila* under salt stress (Table 4b) showed increases in the DBI of MGDG, DGDG, SQDG, PG and PC. Unsaturated fatty acid contents, 18:1, 18:2 and 18:3 of MGDG, 18:2 and

18:3 of DGDG, 18:3 of SQDG, 18:1, 18:2 and 18:3 of PG, and 18:3 of PC increased under 200 mM NaCl treatment. However, the 16:1 of PG decreased under salt stress.

Table 4b. Fatty acid composition of membrane liqids in *T. halophila* leaves under NaCl treatment

Membrane lipid	Fatty acid	Fatty acid composition [mol%]	
		0 mM NaCl	200 mM NaCl
MGDG	16:0	40.82±2.26^{a}	29.68±1.82^{b}
	16:1	--	--
	18:0	27.22±1.51^{a}	21.71±1.33^{a}
	18:1	4.38±0.24^{c}	4.82±0.30^{b}
	18:2	0.61±0.03^{b}	2.23±0.14^{a}
	18:3	26.97±1.49^{b}	41.56±2.55^{a}
	DBI	**86.51**	**133.96**
DGDG	16:0	47.72±1.79^{a}	35.14±1.51b
	16:1	--	--
	18:0	19.08±0.71^{b}	18.87±0.81^{b}
	18:1	10.36±0.39^{a}	9.52±0.41^{a}
	18:2	1.55±0.06^{b}	1.69±0.07^{a}
	18:3	21.28±0.80^{b}	34.78±1.50^{a}
	DBI	**77.30**	**117.24**
SQDG	16:0	38.64±1.55^{b}	40.05±1.42^{a}
	16:1	--	--
	18:0	34.82±1.40^{a}	15.65±0.55^{c}
	18:1	11.79±0.47^{a}	2.35±0.08^{c}
	18:2	1.27±0.05^{b}	1.09±0.04^{b}
	18:3	13.50±0.54^{c}	40.87±1.45^{a}
	DBI	**54.83**	**127.14**
PG	16:0	31.27±3.88^{a}	32.76±4.06^{a}
	16:1	27.92±3.46^{a}	3.98±0.49^{d}
	18:0	16.46±2.04^{b}	16.96±2.10^{b}
	18:1	3.55±0.44^{c}	12.09±1.50^{a}
	18:2	--	1.35±0.17^{a}
	18:3	20.81±2.58^{b}	32.86±4.08^{a}
	DBI	**65.98**	**113.37**
PC	16:0	48.82±2.55^{a}	36.46±1.90^{b}
	16:1	--	--
	18:0	--	--
	18:1	18.25±0.95^{a}	11.09±0.58^{bc}
	18:2	--	--
	18:3	32.03±1.67^{c}	52.45±2.74^{a}
	DBI	**114.34**	**168.44**

Data are represented as means of 5 replicates ± SD. Different letters indicate significant differences at P = 0.05.

Table 4c. Fatty acid composition of membrane liqids in *L. bicolor* leaves under NaCl treatment

Membrane lipid	Fatty acid	Fatty acid composition [mol%]	
		0 mM NaCl	200 mM NaCl
MGDG	16:0	23.94±0.69^{c}	36.43±1.04^{b}
	16:1	--	--
	18:0	32.69±0.94^{a}	18.34±0.52^{b}
	18:1	19.40±0.56^{b}	22.10±0.63^{b}
	18:2	--	--
	18:3	23.94±0.69^{b}	23.13±0.66^{b}
	DBI	**91.22**	**91.49**
DGDG	16:0	65.28±2.76^{b}	43.10±1.59^{c}
	16:1	--	--
	18:0	13.82±0.58^{c}	23.75±0.88^{a}
	18:1	6.37±0.27^{c}	29.51±1.09^{a}
	18:2	0.69±0.03^{b}	--
	18:3	13.82±0.58^{c}	26.49±0.98^{a}
	DBI	**49.21**	**108.98**
SQDG	16:0	28.78±0.57^{c}	43.03±1.00^{b}
	16:1	--	--
	18:0	15.11±0.30^{a}	--
	18:1	23.02±0.46^{a}	19.76±0.46^{a}
	18:2	4.33±0.09^{a}	--
	18:3	28.76±0.57^{c}	37.21±0.87^{a}
	DBI	**117.96**	**131.39**
PG	16:0	20.74±1.31^{c}	39.80±2.51^{a}
	16:1	10.47±0.66^{b}	5.64±0.36^{c}
	18:0	40.06±2.53^{a}	21.16±1.33^{c}
	18:1	18.26±1.15^{a}	--
	18:2	--	--
	18:3	10.47±0.66^{c}	33.40±2.11^{a}
	DBI	**49.67**	**100.20**
PC	16:0	29.49±2.44^{b}	16.78±1.30^{c}
	16:1	--	--
	18:0	27.52±2.28^{a}	27.33±2.12^{a}
	18:1	15.48±1.28^{b}	25.08±1.95^{a}
	18:2	--	--
	18:3	27.52±2.28^{a}	30.83±2.39^{a}
	DBI	**98.04**	**117.57**

Data are represented as means of 5 replicates ± SD. Different letters indicate significant differences at P = 0.05.

In exo-recretohalophyte *L. bicolor* (Table 4c), the DBI of DGDG, SQDG, PG and PC increased under NaCl treatment. Increases were noted in contents of unsaturated fatty acids 18:1 and 18:3 of DGDG, 18:3 of SQDG and PG, 18:1 and 18:3 of PC. However, 16:1 of PG also decreased under the 200 mM NaCl treatment.

Table 4d. Fatty acid composition of membrane liqids in *S. salsa* leaves under NaCl treatment

Membrane lipid	Fatty acid	Fatty acid composition [mol%]	
		0 mM NaCl	200 mM NaCl
MGDG	16:0	11.01±0.47^{a}	1.31±0.06^{b}
	16:1	--	--
	18:0	13.81±0.59^{b}	14.67±0.62^{ab}
	18:1	1.50±0.06^{c}	4.02±0.17^{b}
	18:2	4.40±0.19^{a}	--
	18:3	69.27±2.94^{b}	80.00±3.39^{a}
	DBI	**218.11**	**244.02**
DGDG	16:0	20.32±0.75^{a}	18.26±0.68^{b}
	16:1	--	--
	18:0	18.85±0.70^{a}	11.72±0.43^{c}
	18:1		1.46±0.05^{a}
	18:2	1.90±0.07^{d}	6.64±0.25^{b}
	18:3	58.94±2.19^{b}	61.91±2.30^{ab}
	DBI	**180.62**	**200.47**
SQDG	16:0	31.10±1.30^{ab}	29.26±1.22^{b}
	16:1	--	--
	18:0	21.80±0.91^{a}	9.06±0.38^{c}
	18:1	5.60±0.23^{b}	8.63±0.36^{a}
	18:2	30.20±1.26^{b}	35.03±1.46^{b}
	18:3	11.30±0.47^{c}	18.01±0.75^{a}
	DBI	**99.90**	**132.72**
PG	16:0	27.36±1.07^{a}	24.54±0.96^{b}
	16:1	10.08±0.39^{c}	14.15±0.55^{a}
	18:0	20.17±0.79^{a}	14.26±0.56^{b}
	18:1	--	2.55±0.10^{a}
	18:2	28.20±1.10^{a}	27.39±1.07^{a}
	18:3	14.19±0.55^{c}	17.11±0.67^{b}
	DBI	**98.97**	**108.66**
PC	16:0	43.34±1.00^{a}	43.98±1.01^{a}
	16:1	--	--
	18:0	32.13±0.74^{a}	25.61±0.59^{b}
	18:1	--	--
	18:2	--	--
	18:3	24.52±0.56^{b}	30.41±0.70^{a}
	DBI	**73.56**	**91.23**

Data are represented as means of 5 replicates ± SD. Different letters indicate significant differences at P = 0.05.

In euhalophyte *S. salsa* (Table 4d), 18:3 unsaturated fatty acids were mostly in the forms of MGDG (80.0%) and DGDG (61.9%). Like the other two halophytes, the DBI of MGDG, DGDG, SQDG, PG and PC increased under

NaCl treatment. Under salt stress, the content of 16:1 of PG, 18:1 and 18:3 of MGDG, 18:1, 18:2 and 18:3 of DGDG and SQDG, 18:1 and 18:3 of PG, and 18:3 of PC increased.

It is interesting that the DBI in non-halophyte *A. thaliana* decreased under salt stress, while the DBI increased in all halophytes tested in this section.

As shown in Table 5, the level of PG in *A. thaliana* decreased from 11.8% to 6.5% and the ratio of DGDG/MGDG decreased from 0.8 to 0.5 under the 200 mM NaCl treatment. In *T. halophila*, the level of PG increased from 18.7% to 22.6% and the ratio of DGDG/MGDG decreased from 1.6 to 1.1. In *L. bicolor*, the PG level decreased from 11.8% to 2.4% and the ratio of DGDG/MGDG decreased from 3.6 to 0.4. In *S. salsa*, the level of PG increased from 12.1% to 27.2% and the ratio of DGDG/MGDG increased from 1.2 to 1.4 under the 200 mM NaCl treatment.

Table 5. Composition of lipid classes in *A. thaliana*, *T. halophila*, *L.bicolor* and *S. salsa* leaves under NaCl stress.

Species	**Lipid class**	**Lipid content [mol %]**	
		0 mM NaCl	**200 mM NaCl**
A. thaliana	MGDG	18.72±1.48^{b}	24.85±1.31^{a}
	DGDG	15.74±0.95^{b}	12.41±0.56^{c}
	SQDG	27.98±0.47^{c}	37.54±0.40^{a}
	PG	**11.81±1.89^{b}**	**6.51±2.82^{a}**
	PC	25.76±1.09^{a}	18.69±0.38^{c}
	DGDG/ MGDG	**0.84**	**0.50**
T. halophila	MGDG	24.61±0.00^{b}	22.17±1.43^{b}
	DGDG	39.37±0.00^{a}	24.50±1.12^{b}
	SQDG	16.87±0.00^{c}	27.07±1.00^{a}
	PG	**18.65±0.00^{c}**	**22.64±2.81^{b}**
	PC	0.51±0.00^{d}	3.63±0.22^{b}
	DGDG/ MGDG	**1.60**	**1.11**
L.bicolor	MGDG	15.39±0.52^{c}	60.28±0.01^{a}
	DGDG	55.06±2.82^{a}	21.99±0.01^{d}
	SQDG	14.65±0.38^{a}	6.61±0.00^{c}
	PG	**11.80±0.74^{a}**	**2.40±0.00^{d}**
	PC	3.10±0.26^{c}	8.71±0.00^{a}
	DGDG/ MGDG	**3.58**	**0.36**
S.salsa	MGDG	22.49±0.95^{b}	21.40±0.99^{b}
	DGDG	27.07±1.15^{a}	28.87±1.03^{b}
	SQDG	25.83±0.91^{a}	15.51±0.65^{b}
	PG	**12.06±0.47^{c}**	**27.15±1.02^{a}**
	PC	12.55±0.29^{a}	7.07±0.16^{b}
	DGDG/ MGDG	**1.20**	**1.35**

Data are represented as means of 5 replicates ± SD. Different letters indicate significant differences at P=0.05.

4. Discussion

In this study, we discuss the comparative analysis of the unsaturated fatty acids in membrane lipid between different types of halophytes and the non-halophyte *A. thaliana* to reveal the regulatory mechanism of unsaturated fatty acids under salt stress.

We have observed that, unlike *A. thaliana*, the growth of halophytes was not impacted by concentrations of 200 mM NaCl. The growth of the *S. salsa* increased significantly under salt treatment; the marked induction of leaf succulence in this species can be attributed to the accumulation of Na^+ and Cl^-. The respective salt-exclusion and secretion properties of *T. halophila* and *L. bicolor* help the plants maintain a normal growth cycle. The results reflect that different halophytes have their own unique mechanisms to cope with NaCl stress.

Photosystem II is thought to have significant function in plant photosynthesis under abiotic stress (Baker, 1991). Many abiotic stresses, especially high light and heat stress, have been shown to target primarily the PSII complex (Aro, Virgin, & Andersson, 1993). Salt stress did not affect the PSII activity of halophytes, as shown by more stable Fv/Fm and ΦPSII values when compared to that in *A. thaliana* (Figure 1). ΦPSII of *S. salsa* untreated with NaCl was lower than those of *A. thaliana*, *T. halophila* and *L. bicolor*, which showed that proper growth of *S. salsa* requires some salt. However, treatment with 200 mM NaCl increased ΦPSII by 16.8% in *S. salsa*, suggesting that PSII of *S. salsa* have salinity resistance to some extent and halophytes have better mechanisms for the protection of photosystem II under salinity.

Chl acts as an antenna which is the key component of light harvesting and electron transferring complex (LHCII). In general, the chlorophyll contents of leaves decrease under salt stress(Aro et al., 1993); However, Wang and Nii (2000) have reported that chlorophyll content increases under saline conditions in *Amaranthus*. The 200 mM NaCl treatment of *A. thaliana* decreased Chl *a* levels and the Chl *a*/*b* ratio but there was no significant difference between salt treatment and the untreated control in *T. halophila* or *L. bicolor*. When treated with 200 mM NaCl, the Chl *a* content and Chl *a*/*b* ratio increased in *S. salsa* (Table 2). Higher chlorophyll content will inevitably lead to higher Fv/Fm and ΦPSII (Figure 1), and also inevitably lead to higher yields of plants (Table 1). Light energy is absorbed and efficient used by LHCII, thus reducing the appearance of photoinhibition. On the other hand, in the non-halophyte *A. thaliana*, a lower chl *a* content leads to less light absorption, less transition and less distribution, so photoinhibition is likely to occur more frequently. This can be demonstrated by the 47.3% decrease in Fv/Fm and 33.0% decrease in ΦPSII of *A. thaliana* under salt stress (Figure 1). The results of this study indicated that a saline environment could damage the photosynthetic apparatus and inhibit photosynthesis in the non-halophyte *A. thaliana*. The halophytic *T. halophila* and *L. bicolor* can maintain constant levels of photosynthesis, which is reflected by stability of Fv/Fm, ΦPSII and Chl content among treatments.

Many ions and large molecules are blocked by the hydrophobic lipid in the membrane (Upchurch, 2008). Studies had found that by reducing the photoinhibition of PSII and PSI, the unsaturated fatty acids existing in membrane protect photosynthesis system of plants at low temperature and low light conditions (Sui et al., 2007). Recent researches have found that high salt levels increase membrane lipids' unsaturated fatty acid contents in halophytes but not in *A. thaliana* (Table 4).As known o all, among halophytes, 18:3 is the predominant unsaturated fatty acid during NaCl stress. The x-3 desaturases transgenic tobacco improved resistance ability to salt and drought stress (Zhang et al., 2005), which implies the salt and drought tolerance of plant depends on the levels of unsaturated fatty acids (Berberich et al., 1998; Mikami & Murata, 2003). *Synechocystis* mutants, which lacked the activity of x-6 and x-3 desaturase, reduced the resistant ability to salt stress (Allakhverdiev et al., 2001). Study found that yeast cells transformed x-6 desaturase gene of sunflower, increased the tolerance to salt and low temperature stress. The present study acknowledged three types of halophytes which increase their tolerance to salt stress through maintaining or increasing their unsaturated fatty acids contents. One of the possible explanation is that the unsaturated fatty acids content in membrane phospholipid determine the membrane (Na^+ or K^+) ion channels and Na^+/H^+ antiporter systems to some extent .The more unsaturated fatty acids contain in membrane phospholipids, the stronger membrane fluidity, so that active the activity of Na^+/H^+ antiporter and H^+-ATPase to protect the photosynthesis system effectively(Allakhverdiev et al., 2001; Allakhverdiev, Los, & Murata, 2010). Kamada's research also indicates that the activities of certain membrane bound enzymes can change with changes in membrane fluidity(Kamada, Jung, Piotrowski, & Levin, 1995). It would be expected that euhalophytes such as *S. salsa* need more unsaturated fatty acids to improve membrane fluidity for the ion compartments.

Two groups, those of Somerville in the USA and Murata in Japan, used genetic approaches to demonstrate that the unsaturation of fatty acids in thylakoid lipids plays important roles in the acclimation of the photosynthetic machinery to changes in various forms of environmental stress (Allakhverdiev et al., 2010). A comparison of the turnover of the Dl protein, which is an important component of the photochemical reaction center of PSII, in wild type and $desA^-/desD^-$ cells of *Synechocystis* revealed that posttranslational carboxy-terminal processing of the precursor to the D1 protein was dependent on the extent of unsaturation of fatty acids in the lipids of thylakoid membranes(Kanervo, Aro, & Murata, 1995; Kanervo, Tasaka, Murata, & Aro, 1997). The oxygen-evolving machinery in thylakoid membranes isolated from $desA^-/desD^-$ cells was more sensitive to

NaCl than that from wild-type cells. This finding suggests that the unsaturation of fatty acids in membrane lipids might act directly to protect the oxygen-evolving machinery against saltinduced inactivation (Allakhverdiev et al., 2010). The inverse hexagonal forming lipid (MGDG) was formed by the proportion variaty of a bilayer lipid (DGDG), the membranes structure and the phospholipids accumulation in leaves can be impacted by DGDG/MGDG ratio; it also can disrupt the resistance of plants to abiotic stress, such as salinity. Several experiments have been proved that, the higher DGDG / MGDG ratio, and the more polyunsaturated fatty acids would improve the resistance of plant to abiotic stress. (Gigon, Matos, Laffray, Zuily-Fodil, & Pham-Thi, 2004) and more PSII stability. However, the DGDG/MGDG ratio increased only in *S. salsa* under salt stress, whereas the PG contents increased in *T. halophila* and *S. salsa*. PG is very important for the plant photosynthesis organ in development and function(Domonkos, Laczkó-Dobos, & Gombos, 2008). PG is very important for chloroplast development in higher plants (Hagio et al., 2002). Therefore, the increase of PG content is very important for T. halophila and S. salsa's PSII in resistance to saline stress. The extent of unsaturation of fatty acids is clearly important in the protection of the oxygen-evolving machinery of the PSII complex against salt induced inactivation. Present results reveal that pseudo-halophyte *T. halophila* improves the tolerance to salt stress by increasing the PG content, euhalophyte *S. salsa* improves the tolerance to salt stress by increasing the DGDG/MGDG ratio and the PG content, while the exo-recretohalophyte *L. bicolor* improves the tolerance to salt stress only by increasing the unsaturated fatty acid content of membrane lipids.

Together, our results show that all halophytes display a strong capacity to withstand 200 mM NaCl. Indications for this are: (a) the halophytes do not lose chl under salt stress; (b) PSII is not damaged in halophytes under salt stress; (c) all halophytes increase levels of unsaturated fatty acids under salt stress; (d) PG concentration increases in *T. halophila* and *S. salsa*; (e) DGDG/MGDG ratio increases only in *S. salsa*. These results show that pseudo-halophyte *T. halophila*, exo-recretohalophyte *L. bicolor* and euhalophyte *S. salsa* have their own regulatory mechanism to adapt to salt stress; further understanding of the mechanisms in these species will require further study.

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