Journal of Agricultural Science; Vol. 8, No. 9; 2016
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Responses of Unsaturated Fatty Acid in Membrane Lipid and Antioxidant Enzymes to Chilling Stress in Sweet Sorghum (*Sorghum bicolor* (L.) Moench) Seedling

Yuanyuan Guo[1], Shanshan Liu[1], Zhen Yang[1], Shanshan Tian[1] & Na Sui[1]

[1] 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

Received: May 30, 2016 Accepted: July 1, 2016 Online Published: August 15, 2016

doi:10.5539/jas.v8n9p71 URL: http://dx.doi.org/10.5539/jas.v8n9p71

Abstract

Low temperature is a major factor limiting the productivity and geographical distribution of many plant species. In this study, we investigated the effect of chilling stress (10 °C) on seedling growth in two sweet sorghum (*Sorghum bicolor* (L.) Moench) inbred lines (M-81E and Roma). Results showed that the chilling resistance of M-81E was higher than that of Roma. The Fv/Fm in leaves of M-81E decreased less than that of Roma during chilling stress. After 24 h of chilling stress, the Fv/Fm of M-81E and Roma decreased by 24.3 and 45.8%, respectively. Fo was also affected significantly during chilling stress. Malondialdehyde (MDA), an indicator of lipid peroxidation caused by ROS, increased during chilling stress. The contents of MDA increased less in leaves of M-81E than that in Roma under chilling stress. The antioxidant enzymes (SOD and APX) activity of M-81E was higher than those of Roma during chilling stress. The unsaturated fatty acid content and the double bond index (DBI) of major membrane lipids of MGDG, DGDG, SQDG, PC, PE and PG of M-81E significantly increased after 24 h of chilling treatment (10 °C). The DBI of MGDG, DGDG, SQDG, PC and PG of Roma significantly decreased. These results showed that the chilling tolerance of M-81E was higher than that of Roma by increasing of unsaturated fatty acid in membrane lipid and powerful protective enzyme system at seedling stage.

Keywords: chilling stress, sweet sorghum, photoinhibition, antioxidant enzymes, unsaturated fatty acids

1. Introduction

Sweet sorghum (*Sorghum bicolor* (L.) Moench) is known to be an annual C4 plant of African (tropical) origin and is well adapted to semiarid and arid tropic regions, being highly biomass productive (Jordan et al., 1983). Amongst widely grown crop species, sorghum has one of the greatest degrees of stress tolerance (Paterson et al., 2009). However, Sorghum is a species sensitive to chilling temperatures, meanwhile, the life cycle of sweet sorghum will inevitably meet a period of low temperature (Ercoli et al., 2004; Hetherington et al., 1989). Chilling stress is a major factor limiting the plants productivity, including vegetables such as cucumber, tomato and crops such as sweet sorghum (Kong et al., 2013; Kratsch & Wise, 2000). It is a time dependent and changes in physiological activity preced the development of visual symptoms of injury (Ercoli et al., 2004). Photosynthetic activity is sensitive to chilling stress because low temperature causes impaired photochemical activity due to membrane injury (Hu et al., 2010). Also, low temperature can lead to excessive excitation energy, inhibiting PSII activity (Duan et al., 2012; Pinheiro & Chaves, 2011). Plants have evolved various mechanisms to against light-induced damage (Cushman & Bohnert, 2000; Vij & Tyagi, 2007), such as regulation of PSII light-capture efficiency. PSII is considered to play an important role in the adaptation of photosynthesis to stress (Baker, 1991); by increases of unsaturated fatty acids in membrane lipids and antioxidant enzymes (Sui, 2015; Sui & Han, 2014); state transition between PSI and PSII; which coordinate the balance of the electron transport chain between PSI and PSII (Havaux & Kloppstech, 2001; Ivanov et al., 2008).

The utilization of photochemical and non-photochemical process for absorbed energy is unbalanced under low temperature stress, and it can result in an excess light energy (Hu et al., 2010; Sonoike, 1999). The excessive light energy in the photosynthetic apparatus cannot be used to photosynthesis timely, and it gives rise to the accumulation of ROS in plant tissue (Sala & Lafuente, 2004; Shigeoka et al., 2002). The overproduction of ROS

damages plant proteins, lipids and carbohydrates, meanwhile, it also forming toxic products of malondialdehyde (MDA), which ultimately results in cell death (Hu et al., 2010; Ogweno et al., 2008; Posmyk et al., 2005; Wang et al., 2013). In plant, there are many defense mechanisms against low temperature, such as antioxidant enzymes and non enzymatic antioxidants. Superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) (Xi et al., 2013) are important antioxidant enzymes, while ascorbate (AsA) and glutathione (GSH) are common non enzymatic antioxidants (Namdjoyan et al., 2011). Their function is to scavenge properly the toxic ROS and MDA, and dissipate excess energy from chilling stress (Noctor & Foyer, 1998; Song et al., 2005; Sui, 2015).

Chilling-sensitive plants also have a critical element to relate to the cellular membrane integrity. Some reports suggested that the changes in the content of unsaturated fatty acids can improve plant tolerance to environmental stresses such as cold, heat and drought (Dakhma et al., 1995; Liu et al., 2008; Matos et al., 2002; Sui et al., 2007). Under chilling stress, lipids in cell membranes change from a lipid-crystalline condition to a solid state at a critical temperature that is determined by the ratio of saturated to unsaturated fatty acids (Quinn, 1988). Tolerance to chilling stress is closely connected with the unsaturated fatty acid content of plant membrane lipids (Cheng et al., 2014; Szalontai et al., 2003). Previous evidences have reported that over expression of unsaturated fatty acids gene improves chilling tolerance in tomato (Sui et al., 2007). Also, over expression of tomato chloroplast omega-3 fatty acid desaturase gene alleviates the photoinhibition of photosystems II and I under chilling stress (Liu et al., 2008). In higher plants, the most abundant lipids of thylakoid membranes are glycolipids, including mono-galactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG). Among them, PG is a phospholipid containing trans sixteen carbon triple acid, and is widely present in the biological membranes. The majority of PG molecules are localized in the thylakoid membranes and functions as the site of oxygenic electron transport in PSII (Domonkos et al., 2004; Murata, 1983; Wada & Murata, 1998; Yu & Benning, 2003). An increase in the proportion of unsaturated fatty acids results in the membrane remaining functional at lower temperatures (O'Kane et al., 1996). Graham and Patterson (1982) reports indicated that chilling stress induce changes in fatty acid composition, mainly in the content of linolenic acid (18:3), the increased production of 18:3 was found to accompany cold acclimation in plants, and a positive relationship was found between a higher degree of fatty acid desaturation and cold tolerance (Xu & Siegenthaler, 1997).

Fewer studies have examined the effects of chilling on membrane lipid composition and antioxidant enzyme in this plant. Here, we describe an investigation into the effects of chilling stress on the unsaturated fatty acid contents of membrane lipids and antioxidant enzyme in two sweet sorghum inbred lines. We show that M-81E and Roma respond to chilling stress in highly contrasting ways.

2. Material and Methods

2.1 Plant Culture and Treatments

Seeds of two sweet sorghum lines M-81E and Roma were used as material. Dry seeds were stored in a refrigerator at 4 ^{o}C before used.

After being washed with tap water for 10 h, 5 seeds were sowed in each plastic pots filled with river sand and irrigated with 1/2 Hoagland solution after emergence. Seedlings grow to 3 leaves, thinning, 3 trees per pot. 5 replicates were used for each treatment. The plants were cultured in a glasshouse in light cycle, under 14 h of light/10h of dark with natural light. The temperature in the glasshouse was 28 ± 5 ^{o}C during the day and 20 ± 3 ^{o}C at night. Chilling treatment was performed at the four-leaf stage. The treated plants were kept at 10 ^{o}C for 0, 12, 24, 36 and 48 h. All plants were submitted to these treatments successively. The treated leaves were then frozen in liquid nitrogen and stored at -80 °C until further use for the determinations of antioxidant enzyme activities and Lipid content.

2.2 Measurements of Chlorophyll Fluorescence

Chl fluorescence was measured using a portable fluorometer (FMS2, Hansatech, King's Lynn, UK) according to the protocol described by Kooten et al. (1990). Minimal fluorescence (Fo) with all PSII reaction centers open was determined with modulated light which was low enough not to induce any significant variable fluorescence (Fv). Maximal fluorescence (Fm) with all reaction centers closed was determined by irradiating for 0.8 s with saturating light of 8000 μmol m^{-2} s^{-1} on a dark-adapted leaf (adapted 15 min in darkness). Maximal photochemical efficiency (Fv/Fm) of PSII was expressed as Fv/Fm = (Fm – Fo)/Fm.

2.3 Measurement of MDA Content

Lipid peroxidation was histochemically detected by SchiV's reagent (Yamamoto et al., 2001), which detected aldehydes originating from lipid peroxides. The amount of MDA, the end product of lipid peroxidation, was

assessed by the Lin method (Lin et al., 1984). The leaves (same position 0.4 g) treated with different chilling stress were homogenized by mortar and pestle in 5 mL of 0.1% trichloroacetic acid (TCA). Added 5 mL of 0.5% trichloroacetic acid (TCA) into homogenate then put the tube into the boiling water for 10 minutes, took out and placed in cold water immediately. After the tube cooling the homogenate was centrifuged at 3000 rpm for 15 min. The supernatant was used for MDA assay and MDA content was calculated using 155 mM^{-1} cm^{-1} as extinction coefficient.

2.4 The activity of Antioxidant Enzymes Assay

SOD was prepared by first freezing 0.2 g of leaves sample in liquid nitrogen to prevent proteolytic activity, followed by grinding with 5 ml extraction buffer (0.1 M phosphate buffer, pH 7.5, containing 0.5 mM EDTA, and 1 mM ascorbic acid). The homogenate was centrifuged for 20 min at 15000 g and the supernatant was used as an enzyme. The soluble proteins concentration in the supernatant was determined using the method of Bradford with bovine serum albumin (BSA) as standard (Bradford, 1976). The activity Per unit of SOD was estimated by recording the decrease in optical density of nitro blue tetrazolium (NBT) induced by the enzyme. The reaction mixture contained 13 mM methionine, 75 uM nitrobluetetrazolium chloride, 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), 50 mM sodium carbonate, and 0.1 ml enzyme solution. The reaction was started by adding 2 uM riboflavin. The reaction mixtures were illuminated for 20 min at 90 umol m^{-2} s^{-1} (placing the test tubes under two 15 W fluorescent lamps). A complete reaction mixture without enzyme, which gave the maximal colour, was served as the control. The reaction was stopped by switching off the light and putting the tubes into dark. A non-irradiated complete reaction mixture was served as a blank.

The activity of APX was determined by following the decrease of absorbance at 290 nm. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mMascorbate, 0.2 mM H_2O_2 and the suitable volume of enzyme extract (Jimenez et al., 1997). Enzyme activity was calculated per mg of total protein in U which represents the amount of enzyme needed to oxide 1 umol of AsA within 1 min at room temperature.

2.5 Lipid Extraction and Fatty Acid Composition Analysis

Lipids were extracted as described by Siegenthaler et al. (1984). The pre-cooled mortar and placed on ice for 10 min, in the fight to take five sweet sorghum leaves leaf discs were placed in a mortar, add 9 ml of chloroform:methanol (1:2), the blade ground into homogenate; The homogenate is transferred to 50 ml centrifuge tube and large, on a shaker and mix stand for 20 min; and to the centrifuge tube was added 3 ml of chloroform and 5.4 ml 0.9 M KCL, 1500 g centrifugation after mixing 15 min; while centrifugal silica gel plate and marked at the point of the sample and placed in an oven at 105 °C activated 1 h; After centrifugation the lower draw liquid into a glass tube and the bottom tip dry with N_2; The bottom of the tube powder with 0.2 ml of chloroform:methanol (2:1) was dissolved, set the volume, followed by separation with two-dimensional thin layer chromatography (TLC). 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. (1993). The chromatographic column was 3 mm × 2 m glass column packed with the carrier body Chromosorb W AW DWCS 80-100 mesh, 15% fixative DEGS (polyethylene diethylene glycol butyl acid fat), chromatograph equipped CR-2AX data processor.

2.6 Statistical Analysis

For all data we used SPSS Version 16.0 for Windows (SPSS, Chicago. IL, USA). Multiple comparisons were performed between different environmental conditions using Duncan's test at the 0.05 significance level. Figures were drawn by origin data analysis and graphing software, Sigma Plot 10.0.

3. Results

3.1 Effect of Chilling Stress on PSII Photochemical Efficiency

Previous studies have shown that PSII plays an important role in the response of leaf photosynthesis to environmental stresses (Baker, 1991). Photoinhibition of PSII was estimated by measuring Fv/Fm. As shown in Figure 1A, Fv/Fm decreased in leaves of the two inbred sweet sorghum under chilling stress. But the Fv/Fm of Roma decreased significantly than that of M-81E (Figure 1A). Simultaneously, after a 24 h of chilling stress, the Fv/Fm of M-81E and Roma rapidly decreased by 24.3 and 45.8%, respectively. The Fv/Fm of M-81E and Roma decreased slowly at 36 h and 48 h, respectively. The Fv/Fm of M-81E decreased by 27.8 and 33.3%, after 36 h and 48 h of chilling stress, respectively; and the Fv/Fm of Roma decreased by 51.6 and 58.8%, after 36 h and 48 h of chilling treatment, respectively.

Fo was also affected by chilling stress. As shown in Figure 1B, after 24 h of chilling stress, the Fo of M-81E and Roma rapidly increased by 11.8 and 17.4%, respectively. After 36 h and 48 h of chilling stress, the Fo of M-81E and Roma increased slowly (Figure 1B). The Fo of M-81E decreased by 13.9 and 16.1%, after 36 h and 48 h of chilling stress, respectively; and the Fo of Roma decreased by 22.0 and 24.2 %, after 36 h and 48 h of chilling treatment, respectively.

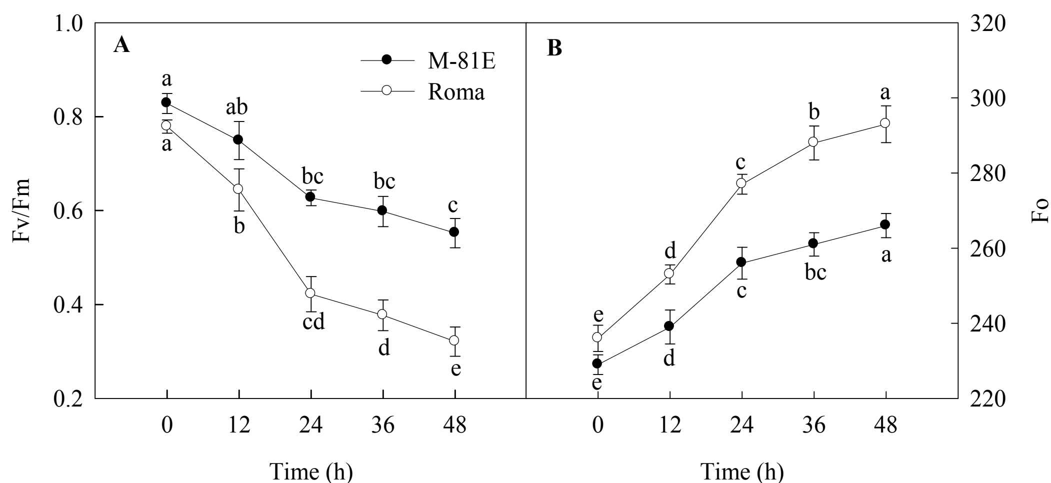

Figure 1. Changes of the maximal photochemical efficiency of PSII (Fv/Fm) and the minimum fluorescence (Fo) in two sweet sorghum lines under chilling stress

Note. Values are means ± SD of five measurements for each of five plants (n = 5). Bars with different lowercase letters show significant differences at the $P < 0.05$ level.

3.2 The Effect of Chilling Stress on MDA Content

MDA is generally considered to be one of the main products of reactive oxygen species, and its content can be used to measure the extent of reactive oxygen species damage. The accumulation of MDA also usually leads to the damage of cell membrane in plant and animal. As showed in Figure 2, MDA content of the two inbred of sweet sorghum showed an increasing trend during the whole chilling period. The MDA level in M-81E under chilling stress was lower than that in Roma. The MDA content of M-81E and Roma rapidly increased by 98.1 and 198.9%, after 24 h of chilling stress, respectively. The MDA content of M-81E and Roma increased slowly after 36 h and 48 h of chilling stress (Figure 2). The MDA content of M-81E increased by 129.9 and 143.9, after 36 h and 48 h of chilling treatment, respectively. The MDA content of Roma increased by 249.8 and 266.1%, after 36 h and 48 h of chilling stress, respectively.

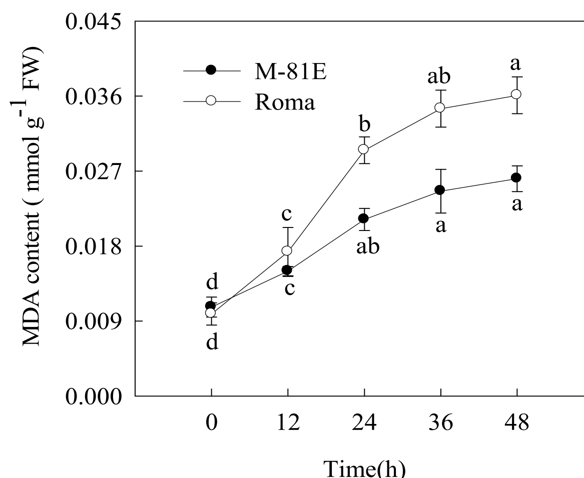

Figure 2. Changes of MDA content in two sweet sorghum lines under chilling stress

Note. Values are means ± SD of five measurements for each of five plants (n = 5). Bars with different lowercase letters show significant differences at the P < 0.05 level.

3.3 The Effect of Chilling Stress on Antioxidant Enzymes Activity

SOD is one of the key enzymes to scavenge ROS produced in active cells. The activities of SOD and APX were increased before 24 h of chilling stress and then decreased (Figure 3). After 24 h of chilling stress, SOD activity of M-81E and Roma increased by 63.8 and 58.3%, respectively, compared to control group (0 h of chilling stress). After 48 h of chilling stress, the SOD activity of M-81E and Roma decreased by 10.7 and 21.4%, respectively, compared to that at 24 h of chilling stress (Figure 3A). APX is an active oxygen scavenger in plant. The APX activity of M-81E and Roma increased by 96.5 and 86.9% after 24 h of chilling stress, respectively, relative to the original value. After 48 h of chilling stress, the APX activity of M-81E and Roma decreased by 8.9 and 24.6%, respectively, relative to the 24 h of chilling stress (Figure 3B). During the whole chilling stress, the activities of both enzymes in leaves of M-81E were higher than that of Roma.

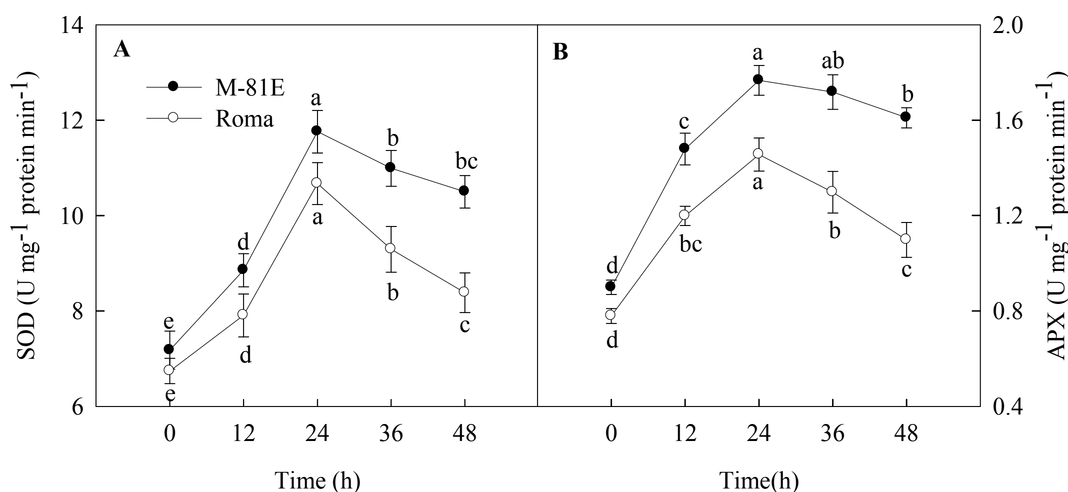

Figure 3. Expressions of ROS scavenging systems related SOD (A) and APX (B) in two sweet sorghum lines under chilling treatment

Note. Values are means ± SD of five measurements for each of five plants (n = 5). Bars with different uppercase letters show significant differences at the P < 0.05 level.

3.4 Lipid Content and Fatty Acid Composition

After 24h of chilling treatment, the unsaturated fatty acid content and the double bond index (DBI = 18:1 × 1 + 18:2 × + 18:3 × 3) of the major membrane lipids of MGDG, DGDG, SQDG, PC, PE and PG of M-81E significantly increased (Table 1). However, DBI of MGDG, DGDG, SQDG, PC and PG of Roma significantly decreased (Table 1). Unsaturated fatty acid contents, linoleic acid (18:2) of MGDG, DGDG, SQDG, PC, PE and PG, linolenic acid (18:3) of DGDG, SQDG PC and PE of M-81E increased after 24 h of chilling treatment (Table 1). However, after 24 h of chilling stress, linoleic acid (18:2) of MGDG, DGDG, PC and PG, 18:3 of MGDG, SQDG, PC and PG of Roma decreased compared to the control group (Table 1).

As showed in Table 2, after 24 h of chilling stress, the 18:1 and 18:2 unsaturated fatty acid content and DBI of total lipids increased by 61.0, 350.7 and 94.2% in M-81E, respectively, and the saturated fatty acids of 16:0 and 18:0 decreased 7.7 and 68.2%, respectively. Oleic acids 18:1, 18:2 and DBI of total lipids all decreased significantly in Roma, after 24 h chilling stress. 18:1, 18:2, and DBI decreased by 59.6, 30.0 and 30.0% in Roma, respectively. Also, the stearic acid (18:0) increased 440.9%. The 18:3 unsaturated fatty acid content increased in two inbred of sweet sorghum lines, but the M-81E increased more than Roma. It increased by 20.8 and 5.2% in M-81E and Roma, respectively. These results showed that the content of unsaturated fatty acids increased in M-81E during chilling stress (Table 2).

After the 10 ^{o}C chilling treatment for 24 h, the level of MGDG and PC in leaves of the two inbred sweet sorghum increased significantly. The level of MGDG and PC increased by 62.9 and 115.2% in M-81E, respectively, and they increased by 55.5 and 54.2% in Roma, respectively. The level of DGDG and PE both decreased in M-81E and Roma. The level of DGDG and PE decreased by 36.0 and 34.7% in M-81E, respectively, and they decreased by 28.9 and 76.5% in Roma. The level of SQDG decreased by 47.6% in M-81E and increased by 239.5% in Roma, while the level of PG increased by 65.6% in M-81E and decreased by 49.4% in Roma compared to the untreatment group (Table 3).

Table 1. Fatty acid composition of membrane lipids in leaves of the two inbred sweet sorghum during chilling treatment

Lipid class		16:0	16:1	18:0	18:1	18:2	18:3	DBI
MGDG	MCK	68.46±3.36[a]	-	5.12±0.28[b]	13.44±0.61[a]	4.74±0.41[b]	8.24±0.47[a]	47.64
	ML24	60.89±3.12[b]	-	5.64±0.31a	12.34±0.57[b]	**19.70±0.75[a]**	2.06±0.16[b]	**57.92**
	RCK	71.73±3.53[a]	-	7.09±0.45[b]	11.58±0.47[a]	3.63±0.16[a]	5.97±0.36[a]	36.75
	RL-24	65.84±3.21[b]	-	24.73±1.23[a]	4.33±0.26[b]	0.88±0.04[b]	4.22±0.31[b]	18.75
DGDG	MCK	76.29±2.57[b]	-	16.63±0.76[a]	5.25±0.29[b]	0.84±0.03[b]	0.99±0.11[b]	9.9
	ML24	83.72±4.40[a]	-	2.47±0.09[b]	9.40±0.41[a]	**3.35±0.23[a]**	**1.06±0.15[a]**	**19.28**
	RCK	83.32±4.26[a]	-	4.20±0.22[b]	8.61±0.33[a]	1.46±0.12[a]	2.41±0.21[b]	18.76
	RL-24	62.55±3.08[b]	-	29.73±1.23[a]	4.15±0.23[b]	0.54±0.02[b]	3.03±0.28[a]	14.32
SQDG	MCK	63.79±2.05[b]	-	27.35±1.03[a]	6.31±0.38[b]	1.25±0.11[b]	1.30±0.19[b]	12.71
	ML24	68.54±2.45[a]	-	7.11±0.48[b]	15.96±0.81[a]	**5.52±0.49[a]**	**2.86±0.26[a]**	**35.58**
	RCK	78.39±3.62[a]	-	4.90±0.26[b]	12.42±0.56[a]	1.85±0.08[b]	2.45±0.21[a]	23.47
	RL-24	73.15±3.28[b]	-	16.57±0.72[a]	5.43±0.34[b]	3.02±0.21[a]	1.84±0.21[b]	16.99
PC	MCK	77.43±3.32[a]	-	13.67±0.61[a]	5.93±0.39[b]	1.15±0.09[b]	1.82±0.18[b]	13.69
	ML24	77.34±2.67[a]	-	6.25±0.39[b]	7.74±0.51[a]	**4.86±0.43[a]**	**3.82±0.31[a]**	**28.92**
	RCK	82.36±3.17[a]	-	3.44±0.16[b]	8.10±0.58[a]	2.04±0.14[a]	4.07±0.31[a]	24.39
	RL-24	71.59±2.01[b]	-	21.89±1.11[a]	2.61±0.14[b]	0.89±0.06[b]	3.02±0.28[b]	13.45
PE	MCK	77.19±2.56[a]	-	12.74±0.52[a]	6.70±0.37[b]	1.05±0.08[b]	2.33±0.20[b]	15.79
	ML24	48.71±1.64[b]	-	4.49±0.23[b]	12.52±0.61[a]	**4.09±0.36[a]**	**3.3±0.29[a]**	**30.6**
	RCK	83.34±3.43[a]	-	3.76±0.18[b]	9.16±0.38[a]	2.01±0.13[b]	1.73±0.16[b]	18.37
	RL-24	76.01±2.48[b]	-	9.68±0.65[a]	6.03±0.34[b]	2.72±0.21[a]	5.56±0.35[a]	28.15
PG	MCK	78.88±3.91[a]	1.40±0.04[b]	10.92±0.71[a]	4.26±0.24[b]	1.43±0.12[b]	3.10±0.27[a]	16.42
	ML24	57.73±1.19[b]	22.89±1.13[a]	4.74±0.27[b]	8.56±0.62[a]	**4.05±0.34[a]**	2.02±0.19[b]	**22.72**
	RCK	70.22±3.11[b]	14.76±0.76[a]	0.69±0.02[b]	10.67±0.49[a]	2.42±0.18[a]	1.24±0.17[a]	19.23
	RL-24	89.09±4.13[a]	5.54±0.33[b]	2.95±0.13[a]	0.87±0.03[b]	0.74±0.04[b]	0.80±0.06[b]	4.75

Note. MCK: 0 h of chilling stress in M-81E; ML24: 24 h of chilling stress in M-81E; RCK: 0 h of chilling stress in Roma; RL24: 24 h of chilling stress in Roma.

Each point represents the mean ± SD of five measurements on each of five plants. Means identified by different letters are significantly different at $P < 0.05$.

Table 2. Constituent fatty acids of total lipids in leaves of the two inbred sweet sorghum during chilling treatment

Fatty acid	Fatty acid composition (mol%)			
	MCK	ML24	RCK	RL24
16:0	73.82±3.86[a]	68.14±3.05[b]	79.18±3.98[a]	71.59±3.36[b]
16:1	1.39±0.08[b]	7.67±0.34[a]	2.77±0.19[b]	0.53±0.05[a]
18:0	16.09±1.13[a]	5.12±0.26[b]	3.64±0.09[b]	19.69±1.36[a]
18:1	6.38±0.34[b]	**10.27±0.52[a]**	9.62±0.47[a]	3.89±0.21[b]
18:2	1.36±0.08[b]	**6.13±0.36[a]**	2.11±0.12[a]	1.48±0.09[b]
18:3	2.21±0.13[b]	**2.67±0.14[a]**	2.68±0.15[a]	2.82±0.16[a]
DBI	15.73	**30.54**	21.88	15.31

Note. Each point represents the mean ± SD of five measurements on each of five plants. Means identified by different letters are significantly different at $P < 0.05$.

Table 3. Composition of lipid classes in leaves of the two inbred sweet sorghum during chilling treatment

Lipid class	Lipid content (mol%)			
	MCK	ML24	RCK	RL24
MGDG	7.35±0.86[b]	11.97±1.19[a]	9.77±1.05[b]	15.19±1.06[a]
DGDG	26.66±1.46[a]	17.05±1.02[b]	22.42±1.12[a]	15.95±0.89[b]
SQDG	20.08±0.84[a]	10.53±0.65[b]	7.80±0.56[b]	26.48±1.23[a]
PC	13.69±1.06[b]	29.46±1.89[a]	17.76±1.26[b]	27.38±1.76[a]
PE	22.31±1.52[a]	14.57±0.52[b]	23.51±1.61[a]	5.52±0.08[b]
PG	9.91±0.48[b]	16.41±1.02[a]	18.75±1.07[a]	9.49±0.45[b]
DGDG/MGDG	3.63	1.42	2.29	1.05

Note. Each point represents the mean ± SD of five measurements on each of five plants. Means identified by different letters are significantly different at $P < 0.05$.

4. Discussion

Changes in unsaturated fatty acid content can enhance tolerance to environmental stresses such as cold, salt, drought and heat (Cheng et al., 2014; Matos et al., 2002; Sui & Han, 2014; Wada et al., 1990). Chilling stress inhibits photosynthesis by the process of photoinhibition (Xu et al., 1999; Zhang et al., 2011). Previous research suggested that plant tolerance to chilling is dependent on unsaturated fatty acid levels (Berberich et al., 1998; Mikami & Murata, 2003). In the present study, we investigated the effects of chilling stress on the photosynthesis, antioxidant enzyme and unsaturated fatty acids in membrane lipids on seedlings growth of two sweet sorghum inbred lines.

Chilling stress is known to inhibit photosynthesis by the process of photoinhibition (Aro et al., 1993). There is a lot of evidences have reported that PSII is believed to play an important role in the response of leaf photosynthesis to environmental stresses. Low temperature treatment can damage the electron transfer machinery of PSII (Baker, 1991; Zhang et al., 2000; Cheng et al., 2014). The maximal efficiency of PSII photochemistry (Fv/Fm) reflects the PSII activity (Figure 1A). Results showed that Fv/Fm of M-81E decreased less than that of Roma, especially in 24h under chilling stress (Figure 1A). These results indicated that the photosynthetic system of M-81E is less damaged. Fo represents minimum fluorescence intensity after dark adaptation. After 24 h of chilling stress, the Fo of Roma rapidly increased more than that of M-81E (Figure 1B). The present study revealed that M-81E had more powerful photosynthetic system than Roma under chilling stress.

Changes of cell membrane permeability in plants under stress condition can reflect the damage extent (Agarie et al., 1998). In previous research, MDA content was lower while more efficient radical scavenging enzymes were observed in plants. MDA is one of the main products of membrane lipid peroxidation, which was usually as an indicator of the degree of plant oxidative stress and the structural integrity of the membranes of plants subjected to low temperatures (Posmyk et al., 2005). Meanwhile, plants contain various enzymatic antioxidants, such as SOD, APX, and CAT, and non-enzymatic antioxidants, such as ascorbate, glutathione, flavonoids and carotenoids (Song et al., 2005). The activity of SOD was the key enzymes to scavenge $O_2^{·-}$ in leaves, and the APX activity in leaves was mainly involved in the removal of H_2O_2 in leaves (Asada, 1992; Guo et al., 2006; Scandalios, 1993; Xu et al., 2010). Chilling resistant varieties could maintain higher SOD and APX activity than the varieties with weak chilling resistance. In this study, under chilling treatment, the content of MDA in leaves of M-81E was lower than Roma (Figure 2), which showed that the damage to Roma plants was serious. This phenomenon is related to the protective enzyme system in plants was involved in the process of scavenging active oxygen. The SOD and APX activity of the two sweet sorghum inbred lines increased under chilling stress. But the SOD and APX of M-81E were higher than that of Roma (Figure 3). Therefore, M-81E had a higher antioxidative enzymes activity than Roma under chilling stress.

Hydrophobic membrane lipids are internally used as barriers through many ions and macromolecules (Upchurch, 2008). In addition, membrane integrity and functional integrity of membrane proteins are maintained by membrane structure and fluidity. Zhang et al. (2005) reported that modulated fatty acid desaturation via over expression of two distinct ω-3 desaturases differentially alters tolerance to various abiotic stresses in transgenic tobacco cells and whole plants. The different substrate selectivity of GPAT enzyme can also affect the cold resistance of plants (Sui et al., 2007). Introduction of the GPAT gene into rice increased the unsaturated fatty acid levels and led to cold resistance (Ariizumi et al., 2002). In the present study, after treatment at 10 °C for 24 h, the

unsaturated fatty acid content and the DBI of the major membrane lipid of MGDG, DGDG, SQDG, PC, PE and PG (Table 1) were significantly increased in M-81E. Previous studies have indicated that in higher plants, PG is a major factor determining the temperature of phase transition of membrane lipids and it contributes to the development of chloroplasts (Hagio et al., 2002). The contents of PG in cucumber decrease when plants are exposed to chilling stress (Lei et al., 2010).The content of saturated fatty acids within PG was closely related to the sensitivity of plants to chilling stress, which was further correlated with membrane fluidity. Increasing cis-unsaturated fatty acid contents of PG can increase plant tolerance to cold stress (Nishida & Murata, 1996). SQDG is also important for the structure and function of thylakoid membrane (Yu & Benning, 2003). The primary role of SQDG is to replace the PG so as to ensure that the balance of negative lipids being short of the phosphorus (Boudière et al., 2014). In this study, we have found that the levels of 18:2 of SQDG and PG increased in M-81E (Table 1), which might stabilize the photosynthetic processes and improve the light-harvesting complex II and the normal functioning of ATP synthesis (Minoda et al., 2003). After treatment at 10 ^oC for 24 h, the levels of 18:3 of M-81E and Roma increased by 20.8 and 5.2%, respectively (Table 2). The level of PG in leaves of Roma decreased 49.4%, while it increased 65.6% in leaves of M-81E (Table 3). Previous studies have found that by increasing of unsaturated fatty acids in membrane lipids can alleviate photoinhibition of PSII in plants (Sui & Han, 2014; Sun et al., 2011). These results suggesting that increasing unsaturated fatty acids in membrane lipids could protect the photosynthetic apparatus, and stabilizes the photosynthetic processes.

5. Conclusion

In conclusion, after a chilling treatment (10 ^oC) for 24 h, the M-81E antioxidant systems were enhanced compared with Roma, and the activity of antioxidant enzymes was increased, which can effectively remove reactive oxygen species and alleviated Photoinhibition. The unsaturated fatty acid content and the DBI of the major membrane lipid of MGDG, DGDG, SQDG, PC, PE and PG (Table 1) were significantly increased in M-81E, while the DBI of MGDG, DGDG, SQDG, PC and PG of Roma significantly decreased. Consequently, M-81E might have a higher degree of fatty acid desaturation which protects the photosynthetic system. In our future studies, the mechanism of membrane lipid and unsaturated fatty acid content in alleviating chilling stress would be elucidated in sweet sorghum.

References

Agarie, S., Hanaoka, N., Ueno, O., Miyazaki, A., Kubota, F., Agata, W., & Kaufman, P. B. (1998). Effects of silicon on tolerance to water deficit and heat stress in rice plants (*Oryza sativa* L.), monitored by electrolyte leakage. *Plant Production Science, 1*, 96-103. http://dx.doi.org/10.1626/pps.1.96

Ariizumi, T., Kishitani, S., Inatsugi, R., Nishida, I., Murata, N., & Toriyama, K. (2002). An increase in unsaturation of fatty acids in phosphatidylglycerol from leaves improves the rates of photosynthesis and growth at low temperatures in transgenic rice seedlings. *Plant and Cell Physiology, 43*, 751-758. http://dx.doi.org/10.1093/pcp/pcf087

Aro, E. M., Virgin, I., & Andersson, B. (1993). Photoinhibition of photosystem II. Inactivation, protein damage and turnover. *Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1143*, 113-134. http://dx.doi.org/10.1016/0005-2728(93)90134-2

Asada, K. (1992). Ascorbate peroxidase–a hydrogen peroxide-scavenging enzyme in plants. *Physiologia Plantarum, 85*, 235-241. http://dx.doi.org/10.1111/j.1399-3054.1992.tb04728.x

Baker, N. R. (1991). A possible role for photosystem II in environmental perturbations of photosynthesis. *Physiologia Plantarum, 81*, 563-570. http://dx.doi.org/10.1111/j.1399-3054.1991.tb05101.x

Berberich, T., Harada, M., Sugawara, K., Kodama, H., Iba, K., & Kusano, T. (1998). Two maize genes encoding ω-3 fatty acid desaturase and their differential expression to temperature. *Plant Molecular Biology, 36*, 297-306. http://dx.doi.org/10.1023/A:1005993408270

Boudière, L., Michaud, M., Petroutsos, D., Rébeillé, F., Falconet, D., Bastien, O., ... Jouhet, J. (2014). Glycerolipids in photosynthesis: Composition, synthesis and trafficking. *Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1837*, 470-480. http://dx.doi.org/10.1016/j.bbabio.2013.09.007

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248-254. http://dx.doi.org/10.1016/0003-2697(76)90527-3

Chen, Z., Xu, C., Chen, M., Xu, L., Wang, K., Lin, S., & Kuang, T. (1993). Effect of cold-hardening on thylakoid membrane lipids and proteins of spring wheat and winter wheat. *Acta Botanica Sinica, 36*, 423-429. Retrieved from http://europepmc.org/abstract/cba/264779

Cheng, S., Yang, Z., Wang, M., Song, J., Sui, N., & Fan, H. (2014). Salinity improves chilling resistance in *Suaeda salsa*. *Acta Physiologiae Plantarum, 36*, 1823-1830. http://dx.doi.org/10.1007/s11738-014-1555-3

Cushman, J. C., & Bohnert, H. J. (2000). Genomic approaches to plant stress tolerance. *Current Opinion in Plant Biology, 3*, 117-124. http://dx.doi.org/10.1016/S1369-5266(99)00052-7

Dakhma, W. S., Zarrouk, M., & Cherif, A. (1995). Effects of drought-stress on lipids in rape leaves. *Phytochemistry, 40*, 1383-1386. http://dx.doi.org/10.1016/0031-9422(95)00459-K

Domonkos, I., Malec, P., Sallai, A., Kovács, L., Itoh, K., Shen, G., … Kis, M. (2004). Phosphatidylglycerol is essential for oligomerization of photosystem I reaction center. *Plant Physiology, 134*, 1471-1478. http://dx.doi.org/10.1104/pp.103.037754

Duan, M., Feng, H. L., Wang, L. Y., Li, D., & Meng, Q. W. (2012). Overexpression of thylakoidal ascorbate peroxidase shows enhanced resistance to chilling stress in tomato. *Journal of Plant Physiology, 169*, 867-877. http://dx.doi.org/10.1016/j.jplph.2012.02.012

Ercoli, L., Mariotti, M., Masoni, A., & Arduini, I. (2004). Growth responses of sorghum plants to chilling temperature and duration of exposure. *European Journal of Agronomy, 21*, 93-103. http://dx.doi.org/10.1016/S1161-0301(03)00093-5

Graham, D., & Patterson, B. D. (1982). Responses of plants to low, nonfreezing temperatures: Proteins, metabolism, and acclimation. *Annual Review of Plant Physiology, 33*, 347-372. http://dx.doi.org/10.1146/annurev.pp.33.060182.002023

Guo, Z., Ou, W., Lu, S., & Zhong, Q. (2006). Differential responses of antioxidative system to chilling and drought in four rice cultivars differing in sensitivity. *Plant Physiology and Biochemistry, 44*, 828-836. http://dx.doi.org/10.1016/j.plaphy.2006.10.024

Hagio, M., Sakurai, I., Sato, S., Kato, T., Tabata, S., & Wada, H. (2002). Phosphatidylglycerol is essential for the development of thylakoid membranes in *Arabidopsis thaliana. Plant and Cell Physiology, 43*, 1456-1464. http://dx.doi.org/10.1093/pcp/pcf185

Havaux, M., & Kloppstech, K. (2001). The protective functions of carotenoid and flavonoid pigments against excess visible radiation at chilling temperature investigated in Arabidopsis npq and tt mutants. *Planta, 213*, 953-966. http://dx.doi.org/10.1007/s004250100572

Hetherington, S. E., He, J., & Smillie, R. M. (1989). Photoinhibition at low temperature in chilling-sensitive and-resistant plants. *Plant Physiology, 90*, 1609-1615. http://dx.doi.org/10.1104/pp.90.4.1609

Hu, W., Wu, Y., Zeng, J., He, L., & Zeng, Q. (2010). Chill-induced inhibition of photosynthesis was alleviated by 24-epibrassinolide pretreatment in cucumber during chilling and subsequent recovery. *Photosynthetica, 48*, 537-544. http://dx.doi.org/10.1007/s11099-010-0071-y

Ivanov, A. G., Hurry, V., Sane, P. V., Öquist, G., & Huner, N. P. (2008). Reaction centre quenching of excess light energy and photoprotection of photosystem II. *Journal of Plant Biology, 51*, 85-96. http://dx.doi.org/10.1007/BF03030716

Jimenez, A., Hernandez, J. A., del Río, L. A., & Sevilla, F. (1997). Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. *Plant Physiology, 114*, 275-284. Retrieved from http://www.plantphysiol.org/content/114/1/275.short

Jordan, W., Dugas, W., & Shouse, P. (1983). Strategies for crop improvement for drought-prone regions. *Agricultural Water Management, 7*, 281-299. http://dx.doi.org/10.1016/0378-3774(83)90090-2

Kong, F., Deng, Y., Zhou, B., Wang, G., Wang, Y., & Meng, Q. (2013). A chloroplast-targeted DnaJ protein contributes to maintenance of photosystem II under chilling stress. *Journal of Experimental Botany, 65*, 143-158. http://dx.doi.org/10.1093/jxb/ert357

Kooten, O., & Snel, J. F. (1990). The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Research, 25*, 147-150. http://dx.doi.org/10.1007/BF00033156

Kratsch, H., & Wise, R. (2000). The ultrastructure of chilling stress. *Plant, Cell & Environment, 23*, 337-350. http://dx.doi.org/10.1046/j.1365-3040.2000.00560.x

Lei, T., Feng, H., Sun, X., Dai, Q. L., Zhang, F., Liang, H. G., & Lin, H. H. (2010). The alternative pathway in cucumber seedlings under low temperature stress was enhanced by salicylic acid. *Plant Growth Regulation, 60*, 35-42. http://dx.doi.org/10.1007/s10725-009-9416-6

Lin, Z. F., Li, S. S., Lin, G. Z., Sun, G. C., & Guo, J. Y. (1984). Superoxide dismutase activity and lipid peroxidation in relation to senescence of rice leaves. *Acta Botanica Sinica, 26*, 605-615. Retrieved from http://agris.fao.org/agris-search/search.do?recordID=US201302015428

Liu, X. Y., Li, B., Yang, J. H., Sui, N., Yang, X. M., & Meng, Q. W. (2008). Overexpression of tomato chloroplast omega-3 fatty acid desaturase gene alleviates the photoinhibition of photosystems 2 and 1 under chilling stress. *Photosynthetica, 46*, 185-192. http://dx.doi.org/10.1007/s11099-008-0030-z

Matos, M., Campos, P., Ramalho, J., Medeira, M., Maia, M., Semedo, J., … Matos, A. (2002). Photosynthetic activity and cellular integrity of the Andean legume Pachyrhizus ahipa (Wedd.) Parodi under heat and water stress. *Photosynthetica, 40*, 493-501. http://dx.doi.org/10.1023/A:1024331414564

Mikami, K., & Murata, N. (2003). Membrane fluidity and the perception of environmental signals in cyanobacteria and plants. *Progress in Lipid Research, 42*, 527-543. http://dx.doi.org/10.1016/S0163-7827(03)00036-5

Minoda, A., Sonoike, K., Okada, K., Sato, N., & Tsuzuki, M. (2003). Decrease in the efficiency of the electron donation to tyrosine Z of photosystem II in an SQDG-deficient mutant of Chlamydomonas. *FEBS Letters, 553*, 109-112. http://dx.doi.org/10.1016/S0014-5793(03)00981-5

Murata, N. (1983). Molecular species composition of phosphatidylglycerols from chilling-sensitive and chilling-resistant plants. *Plant and Cell Physiology, 24*, 81-86. Retrieved from http://pcp.oxfordjournals.org/content/24/1/81.short

Namdjoyan, S., Khavari-Nejad, R., Bernard, F., Nejadsattari, T., & Shaker, H. (2011). Antioxidant defense mechanisms in response to cadmium treatments in two safflower cultivars. *Russian Journal of Plant Physiology, 58*, 467-477. http://dx.doi.org/10.1134/S1021443711030149

Nishida, I., & Murata, N. (1996). Chilling sensitivity in plants and cyanobacteria: The crucial contribution of membrane lipids. *Annual Review of Plant Biology, 47*, 541-568. http://dx.doi.org/10.1146/annurev.arplant.47.1.541

Noctor, G., & Foyer, C. H. (1998). Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Biology, 49*, 249-279. http://dx.doi.org/10.1146/annurev.arplant.49.1.249

Ogweno, J. O., Song, X. S., Shi, K., Hu, W. H., Mao, W. H., Zhou, Y. H., ... Nogués, S. (2008). Brassinosteroids alleviate heat-induced inhibition of photosynthesis by increasing carboxylation efficiency and enhancing antioxidant systems in *Lycopersicon esculentum*. *Journal of Plant Growth Regulation, 27*, 49-57. http://dx.doi.org/10.1007/s00344-007-9030-7

O'Kane, D., Gill, V., Boyd, P., & Burdon, R. (1996). Chilling, oxidative stress and antioxidant responses in *Arabidopsis thaliana* callus. *Planta, 198*, 371-377. http://dx.doi.org/10.1007/BF00620053

Paterson, A. H., Bowers, J. E., Bruggmann, R., Dubchak, I., Grimwood, J., Gundlach, H., … Poliakov, A. (2009). The Sorghum bicolor genome and the diversification of grasses. *Nature, 457*, 551-556. http://dx.doi.org/10.1038/nature07723

Pinheiro, C., & Chaves, M. (2011). Photosynthesis and drought: can we make metabolic connections from available data? *Journal of Experimental Botany, 62*, 869-882. http://dx.doi.org/10.1093/jxb/erq340

Posmyk, M. M., Bailly, C., Szafrańska, K., Janas, K. M., & Corbineau, F. (2005). Antioxidant enzymes and isoflavonoids in chilled soybean (*Glycine max* (L.) Merr.) seedlings. *Journal of Plant Physiology, 162*, 403-412. http://dx.doi.org/10.1016/j.jplph.2004.08.004

Quinn, P. J. (1988). Effects of temperature on cell membranes. *Symp. Soc. Exp. Biol., 42*, 237-258. Retrieved from https://www.researchgate.net/publication/20078144_Effects_of_temperature_on_cell_membranes

Sala, J. M., & Lafuente, M. A. T. (2004). Antioxidant enzymes activities and rindstaining in '*Navelina*' *oranges* as affected by storage relative humidity and ethylene conditioning. *Postharvest Biology and Technology, 31*, 277-285. http://dx.doi.org/10.1016/j.postharvbio.2003.10.002

Scandalios, J. G. (1993). Oxygen stress and superoxide dismutases. *Plant Physiology, 101*, 7. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC158641/pdf/1010007.pdf

Shigeoka, S., Ishikawa, T., Tamoi, M., Miyagawa, Y., Takeda, T., Yabuta, Y., & Yoshimura, K. (2002). Regulation and function of ascorbate peroxidase isoenzymes. *Journal of Experimental Botany, 53*, 1305-1319. http://dx.doi.org/10.1093/jexbot/53.372.1305

Siegenthaler, P. A., & Eichenberger, W. (1984). *Structure, function, and metabolism of plant lipids*. International Symposium on the Structure, Function, and Metabolism of Plant Lipids 1984: Neuchâtel, Switzerland. Elsevier Science Publishers; Sole distributors for the USA and Canada, Elsevier Science Pub. Co. Retrieved from http://agris.fao.org/agris-search/search.do?recordID=US201300374202

Song, X. S., Hu, W. H., Mao, W. H., Ogweno, J. O., Zhou, Y. H., & Yu, J. Q. (2005). Response of ascorbate peroxidase isoenzymes and ascorbate regeneration system to abiotic stresses in *Cucumis sativus L*. *Plant Physiology and Biochemistry, 43*, 1082-1088. http://dx.doi.org/10.1016/j.plaphy.2005.11.003

Sonoike, K. (1999). The different roles of chilling temperatures in the photoinhibition of photosystem I and photosystem II. *Journal of Photochemistry and Photobiology B: Biology, 48*, 136-141. http://dx.doi.org/10.1016/S1011-1344(99)00030-5

Sui, N. (2015). Photoinhibition of *Suaeda salsa* to chilling stress is related to energy dissipation and water-water cycle. *Photosynthetica, 53*, 207-212. http://dx.doi.org/10.1007/s11099-015-0080-y

Sui, N., & Han, G. (2014). Salt-induced photoinhibition of PSII is alleviated in halophyte *Thellungiella halophila* by increases of unsaturated fatty acids in membrane lipids. *Acta Physiologiae Plantarum, 36*, 983-992. http://dx.doi.org/10.1007/s11738-013-1477-5

Sui, N., Li, M., Zhao, S. J., Li, F., Liang, H., & Meng, Q. W. (2007). Overexpression of glycerol-3-phosphate acyltransferase gene improves chilling tolerance in tomato. *Planta, 226*, 1097-1108. http://dx.doi.org/10.1007/s00425-007-0554-7

Sun, X. L., Yang, S., Wang, L. Y., Zhang, Q. Y., Zhao, S. J., & Meng, Q. W. (2011). The unsaturation of phosphatidylglycerol in thylakoid membrane alleviates PSII photoinhibition under chilling stress. *Plant Cell Reports, 30*, 1939-1947. http://dx.doi.org/10.1007/s00299-011-1102-2

Szalontai, B., Kóta, Z., Nonaka, H., & Murata, N. (2003). Structural consequences of genetically engineered saturation of the fatty acids of phosphatidylglycerol in tobacco thylakoid membranes. An FTIR study. *Biochemistry, 42*, 4292-4299. http://dx.doi.org/10.1021/bi026894c

Upchurch, R. G. (2008). Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. *Biotechnology Letters, 30*, 967-977. http://dx.doi.org/10.1007/s10529-008-9639-z

Vij, S., & Tyagi, A. K. (2007). Emerging trends in the functional genomics of the abiotic stress response in crop plants. *Plant Biotechnology Journal, 5*, 361-380. http://dx.doi.org/10.1111/j.1467-7652.2007.00239.x

Wada, H., & Murata, N. (1998). Membrane lipids in cyanobacteria. *Lipids in photosynthesis: Structure, function and genetics* (pp. 65-81). Springer. Retrieved from http://link.springer.com/chapter/10.1007/0-306-48087-5_4

Wada, H., Gombos, Z., & Murata, N. (1990). Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation. *Nature, 347*, 200-203. http://dx.doi.org/10.1038/347200a0

Wang, G. J., Miao, W., Wang, J. Y., Ma, D. R., Li, J. Q., & Chen, W. F. (2013). Effects of exogenous abscisic acid on antioxidant system in weedy and cultivated rice with different chilling sensitivity under chilling stress. *Journal of Agronomy and Crop Science, 199*, 200-208. http://dx.doi.org/10.1111/jac.12004

Xi, Z., Wang, Z., Fang, Y., Hu, Z., Hu, Y., Deng, M., & Zhang, Z. (2013). Effects of 24-epibrassinolide on antioxidation defense and osmoregulation systems of young grapevines (*V. vinifera* L.) under chilling stress. *Plant Growth Regulation, 71*, 57-65. http://dx.doi.org/10.1007/s10725-013-9809-4

Xu, C. C., Jeon, Y. A., & Lee, C. H. (1999). Relative contributions of photochemical and non-photochemical routes to excitation energy dissipation in rice and barley illuminated at a chilling temperature. *Physiologia Plantarum, 107*, 447-453. http://dx.doi.org/10.1034/j.1399-3054.1999.100411.x

Xu, S. C., Li, Y. P., Jin, H., Guan, Y. J., Zheng, Y. Y., & Zhu, S. J. (2010). Responses of antioxidant enzymes to chilling stress in tobacco seedlings. *Agricultural Sciences in China, 9*, 1594-1601. http://dx.doi.org/10.1016/S1671-2927(09)60256-X

Xu, Y., & Siegenthaler, P. A. (1997). Low temperature treatments induce an increase in the relative content of both linolenic and λ3-hexadecenoic acids in thylakoid membrane phosphatidylglycerol of squash cotyledons. *Plant and Cell Physiology, 38*, 611-618. http://dx.doi.org/10.1093/oxfordjournals.pcp.a029211

Yamamoto, Y., Kobayashi, Y., & Matsumoto, H. (2001). Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots. *Plant Physiology, 125*, 199-208. http://dx.doi.org/10.1104/pp.125.1.199

Yu, B., & Benning, C. (2003). Anionic lipids are required for chloroplast structure and function in *Arabidopsis*. *The Plant Journal, 36*, 762-770. http://dx.doi.org/10.1046/j.1365-313X.2003.01918.x

Zhang, L., Paakkarinen, V., van Wijk, K. J., & Aro, E. M. (2000). Biogenesis of the chloroplast-encoded D1 protein: regulation of translation elongation, insertion, and assembly into photosystem II. *The Plant Cell, 12*, 1769-1781. http://dx.doi.org/10.1105/tpc.12.9.1769

Zhang, M., Barg, R., Yin, M., Gueta-Dahan, Y., Leikin-Frenkel, A., Salts, Y., … Ben-Hayyim, G. (2005). Modulated fatty acid desaturation via overexpression of two distinct ω-3 desaturases differentially alters tolerance to various abiotic stresses in transgenic tobacco cells and plants. *The Plant Journal, 44*, 361-371. http://dx.doi.org/10.1111/j.1365-313X.2005.02536.x

Zhang, Z., Jia, Y., Gao, H., Zhang, L., Li, H., & Meng, Q. (2011). Characterization of PSI recovery after chilling-induced photoinhibition in cucumber (*Cucumis sativus* L.) leaves. *Planta, 234*, 883-889. http://dx.doi.org/10.1007/s00425-011-1447-3

Abbreviations

Fv/Fm: Maximal photochemical efficiency of PSII; MDA: Malondialdehyde; SOD: Superoxide dismutase; APX: Ascorbate peroxidase; 16:0: Palmitic acid; 16:1(3t): ∆3-*trans*-Hexadecenoic; 18:0: stearic acid; 18:1: oleic acid; 18:2: linoleic acid; 18:3: linolenic acid; DBI: double bond index; DGDG: digalactosyldiacylglycerols; MGDG: monogalactosyldiacylglycerols; PG: phosphatidylglycerols; SQDG: sulphoquinovosyldiacylglycerols; Fo: initial fluorescence of the dark adapted state.

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/4.0/).