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# Exogenous application of gibberellic acid participates in up-regulation of lipid biosynthesis under salt stress in rice

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**Abstract** Alterations in membrane lipid composition lead to improved plant salt tolerance. Gibberellic acid ($GA_3$) has also been widely reported to reduce growth inhibition induced by increased salinity. However, little is known about whether exogenous application of $GA_3$ participates in up-regulation of lipid biosynthesis under salt stress. In this study, one of the major lipid biosynthesis genes in rice (*Oryza sativa* L. cv. Nipponbare), monogalactosyldiacylglycerol synthase (*OsMGD*) was found to be significantly up-regulated by $GA_3$ treatment. Lipid analysis showed that after salt disturbance, the concentrations of all the measured lipids, including monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and phospholipid lipids + sulfoquinovosyl diacylglycerol (PL + SQDG) were decreased significantly. However, $GA_3$ treatment prior to salt disturbance caused those lipids to remain at high levels, as well as preserving a high DGDG/MGDG ratio. The desaturation of DGDG (DBI) was also increased in $GA_3$ pre-treatment plants as compared with no $GA_3$ pre-treatment, primarily due to a decrease of 16:0 fatty acids and an increase of 18:3 fatty acids in DGDG. Plants pre-treated with $GA_3$ prior to salt disturbance had higher dry weights than those without pre-treatment. The chlorophyll concentration was also higher in $GA_3$ treated plants than in untreated plants under salt disturbance. Taken together, these results indicate that exogenous application of $GA_3$ participates in up-regulation of chloroplast lipid biosynthesis under salt disturbance in rice.

**Keywords** Chloroplast membrane lipids · Fatty acids · Gibberellic acid · Salt stress · Rice

**Abbreviations**

| | |
|---|---|
| $GA_3$ | Gibberellic acid |
| MGD | Monogalactosyldiacylglycerol synthase |
| MGDG | Monogalactosyldiacylglycerol |
| DGDG | Digalactosyldiacylglycerol |
| PL | Phospholipid lipids |
| SQDG | Sulfoquinovosyl diacylglycerol |
| DBI | Double bond index |

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| | |
|---|---|
| PG | Phosphatidylglycerol |
| ABA | Abscisic acid |
| MeJA | Methyl jasmonate |
| TLC | Thin Layer Chromatography |
| FAME | Fatty acyl methylester |
| FID | Flame ionization detector |
| LHCII | Light-harvesting chlorophyll complex II |
| PSII | Photosystem II |

## 1 Introduction

Plants are sessile organisms that are frequently exposed to numerous unfavorable environmental conditions including salinity. Salt stress adversely limits plant growth and productivity worldwide (Flowers et al. 1977; Allakhverdiev et al. 2000). The primary obstacles of salinity stress are ion imbalance, hyperosmotic stress and oxidative damage (Zhu 2001). These obstacles usually affect basic biosynthetic and metabolic pathways such as photosynthesis, protein synthesis, lipid metabolism and membrane repair (Parida and Das 2004; Ruiz-Lozano et al. 2012).

Membranes are the first barriers for plant defense against various adverse environmental conditions. Lipids are the predominant constituents of all membranous structure in plants. Among those membrane lipids, two galactolipids, monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), are the most abundant photosynthetic membrane lipid building blocks, accounting for 50% and 25% of total chloroplast thylakoid membrane lipids, respectively (Block et al. 1983). Previous studies showed that salt disturbance decreases the proportion of galactolipids in chloroplast membranes (Sui et al. 2010). Moreover, overexpression of a rice MGDG synthase gene (*OsMGD*) led to an increased galactolipid content in leaves, which endowed the plants with enhanced salt tolerance in tobacco (Wang et al. 2014). Besides, upregulation of galactolipid biosynthesis genes are also involved in plant drought response in maize. Chen et al. (2018) showed that the expression of *ZmMGD1*, *ZmMGD2* and *ZmMGD3* genes were significantly up-regulated by drought, and as a result, the concentration of total lipid was well maintained in drought tolerant cultivar.

Another variable that affects membrane fluidity and flexibility is the level of unsaturation of the fatty acid components of lipids. Salt stress increases the degree of unsaturation of membrane lipid fatty acids, remarkably protecting the photosynthetic machinery from damage (Allakhverdiev et al. 2001). Consistent with those observations is the finding of Sun et al. (2010), who reported that an increase in unsaturation of fatty acids in phosphatidylglycerol (PG) in the chloroplast membrane enhances salt tolerance in tomato.

Phytohormones are reported to have crucial roles in plant stress responses and alleviation (Shaterian et al. 2005). The abscisic acid (ABA) has been reported to play a mediator in plant responses to various stresses such as salt stress (Keskin et al. 2010). Jasmonic acids (JA) are important cellular regulators. It has been shown that treatment of JA could alleviate salt stress in rice and barley (Kang et al. 2005; Walia et al. 2007). Gibberellic acid (GA) generally participates in plant growth and development. Increased GA biosynthesis and signaling improves growth of plants escaping from shading or submergence stresses (Colebrook et al. 2014). Exogenous application of GA had a benefit role in moderating adverse effects of salt stress and enhanced plant growth and yield (Afzal et al. 2005; Egamberdieva 2009). It has been reported that exogenous application of $GA_3$ was involved in enhancing tomato plants to adapt to a saline environment (Maggio et al. 2010). In rice, many studies have shown that $GA_3$ can mitigate the toxic effects of NaCl on plant growth (Acharya 1983; Prakash and Prathapasenan 1990; Wen et al. 2010). Application of $GA_3$ also alleviates the salt-induced reduction of chlorophyll content (Ashraf et al. 2000), enabling the maintenance of photosynthetic capacities under saline conditions. It is known that lipids are the matrix of chloroplast photosynthetic membranes, and plants easily alter their chloroplast membrane lipid compositions in response to various abiotic conditions (Erdei et al. 1980; Stevanovic et al. 1992; Wang and Lin 2006; Sui et al. 2010). However, little is known about the relationship between $GA_3$ application and the regulation of chloroplast membrane lipid content and composition, especially under salt disturbance.

As one of the key enzymes in the biosynthesis of chloroplast membrane lipids, MGDG synthase (*MGD*) participates in photosynthetic membrane formation (Nakamura et al. 2010; Jarvis et al. 2000). Losing *MGD* function in plants cause disruption of photosynthetic membranes, and impairment of photosynthetic capability (Kobayashi et al. 2007). Moellering and Benning (2011) reported that the *MGD* plays a

crucial role under phosphorous deficiency. Besides, as mentioned previously, overexpression of *OsMGD* enhances salt tolerance in tobacco (Wang et al. 2014). Interestingly, it was shown that the expression of *OsMGD* (AB112060, partial sequence of Os02g0802700 gene) was up-regulated by both $GA_3$ application and salt treatment in rice (Qi et al. 2004). However, whether exogenous application of $GA_3$ was involved in alleviating salt stress through affecting chloroplast lipid contents were still unknown. In this study, we investigated the expression of all three *OsMGD* genes, and found that one of the *OsMGD* gene expression was up-regulated by $GA_3$ pre-treatment. Thus, we hypothesize that exogenous application of $GA_3$ may participate in up-regulation of chloroplast membrane lipid biosynthesis under salt treatment. To test our hypothesis, we investigated plant biomass accumulation, chlorophyll contents, chloroplast membrane lipids concentration, and fatty acids composition of rice after $GA_3$ pre-treatment and subsequent exposure to salt disturbance.

## 2 Material and methods

### 2.1 CDS sequence alignment

Three *MGD* isoenzymes have been isolated from *Arabidopsis*, including *AtMGD*1 (At4g31780), *AtMGD*2 (At5g20410), and *AtMGD*3 (At2g11810), respectively (Awai et al. 2001). Using sequences from the *Arabidopsis* genes to search the NCBI database, three japonica rice (*Oryza sativa* L. cv. Nipponbare) MGDG synthase (*OsMGD*) genes were identified. Three genes are referred to as "probable *MGD*" genes, they are *Os02g0802700* (probable *MGD3*), *Os08g0299400* (probable *MGD2*), and *Os09g0423600* (probable *MGD1*) (https://www.ncbi.nlm.nih.gov/). In this study, they are abbreviated as *OsMGD02 g*, *OsMGD08 g*, and *OsMGD09 g*, respectively.

### 2.2 Plant material and treatments

Hydroponic culture was used to investigate the effects of salt treatment and plant hormones on the expression of *MGD* genes in rice. Rice seeds (*Oryza sativa* L. cv. Nipponbare) were sterilized with 2.5% (w/v) NaClO for 25 min, and germinated using vermiculite as substrate in a growth chamber at 25 °C. Five days after germination, seedlings were transferred to half strength Hoagland solution (Yoshida et al. 1976) in a plastic container (40 × 28 × 14 cm) with 5 L of culture solution for 10 days. All experiments were carried out in a controlled growth chamber under the following growth conditions: photosynthetically active radiation of 600 μmol $m^{-2}s^{-1}$, with day/night temperature of 25 °C/20 °C, and 14 h/10 h of light/-dark photoperiod. The culture solution was changed every 3 days and aerated continuously. Ten days after hydroponic culture, uniform seedlings were selected and transplanted into plastic containers with different plant hormones (10 μM $GA_3$, 100 μM abscisic acid (ABA) and 10 μM methyl jasmonate (MeJA) and salt (100 mM NaCl) added, respectively. After 24 h pre-treatments, leaves were collected and frozen in liquid nitrogen.

### 2.3 Expression analysis of *OsMGD* genes

Total RNA was extracted from 100 mg of frozen leaves using RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and then treated with Recombinant DNase I (RNase-free; Takara Bio Shiga, Japan) to remove the remaining genomic DNA. Reverse transcription (1 μg RNA) was carried out using the iScriptTM cDNA Synthesis Kit (BioRad Hercules, CA, USA) according to the manufacturer's instructions. The quantitative RT-PCR was carried out according to the method of Liu et al. (2014), with 2 μL of cDNA. The genes and the sequences of the specific primers are shown in Table 1. *Ubiquitin* was used as the internal reference. Each pair of primers has been confirmed to be specific to the target gene through DNA sequence comparisons and PCR (Fig. 1a). The expression levels of target genes were calculated using the $2^{-\triangle\triangle t}$ method. The relative expression level of each gene was calculated based on the expression level of *OsUbq* constitutive control. Each treatment contained three replications and each replication included three repeats.

### 2.4 $GA_3$ treatment prior to exposure to saline conditions

Based on previous results, seedlings were treated with and without 10 μM $GA_3$ for 24 h in Hoagland solution. Leaves were sampled from one set of

**Table 1** Primers used for the analysis of the *MGD* expression by real-time quantitative PCR

| Gene | Product size (bp) | Direction | Primer sequence (5′–3′) |
|---|---|---|---|
| *OsMGD02g* | 84 | Forward | GGAAGTTGGCAATGTCCCTTAT |
| | | Reverse | GCAACAAGTTTAGCAGTTTCCC |
| *OsMGD08g* | 145 | Forward | GTCATCTGTGGCAGGAACCA |
| | | Reverse | GTACCAGGACCAGCCTTTGT |
| *OsMGD09g* | 117 | Forward | GGAAGCTGGCAATGTTCCAT |
| | | Reverse | TTGAGCTCGTCTGACCTAGG |
| *OsUbq* | 179 | Forward | CACCCTGGCTGACTACAACA |
| | | Reverse | TTCTTCTTGCGGCAGTTGAC |

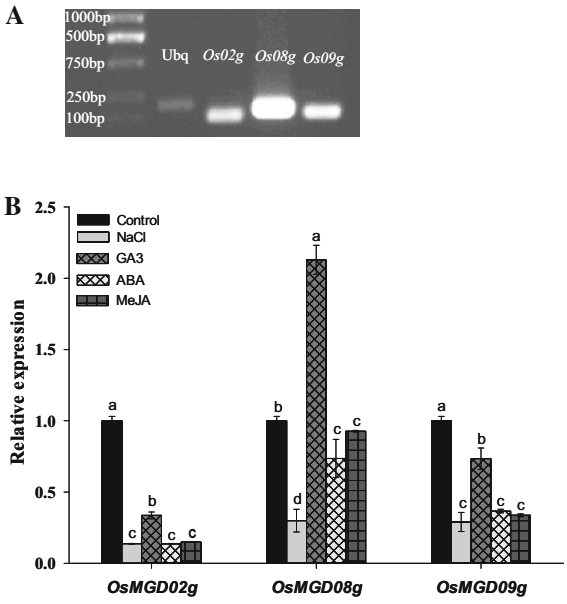


**Fig. 1** Confirmation of primer efficiency by genome PCR (**a**), Effect of NaCl (100 mM), $GA_3$ (10 μM), ABA(100 μM), and MeJA (10 μM) treatment on the expression level of *OsMGD* genes in rice (**b**). Values are presented as the mean ± SE (n = 3). Different letters within the same group indicate statistically significant differences at $p < 0.05$

seedlings; other seedlings were transplanted into Hoagland solution with 100 mM NaCl added. After 72 h of salt treatment, the seedlings were sampled, and the following parameters were measured. Each treatment included four replicates, with each replicate from different individual plant.

## 2.5 Total dry weight

To obtain dry weight of individual plants, the whole plant was washed with distilled water and then dried at 80 °C until constant weight.

## 2.6 Chlorophyll content

The fully expanded upper fresh leaves ( ~ 0.2 g) were used for measuring the chlorophyll concentration. The leaves were cut into small pieces and extracted with 80% acetone on a shaker at room temperature until the leaves were completely bleached. After centrifugation at 5000 g for 5 min, the supernatant was gathered for absorbance measurement at 663 nm and 645 nm by spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan). The chlorophyll *a* and chlorophyll *b* content were calculated according the equation described by Lichtenthaler (1987), and total chlorophyll content was then calculated.

## 2.7 Lipid analysis

Lipids were extracted according to the method described by Wewer et al. (2013) and Zhang et al. (2016). Lipids were first separated by thin layer chromatography (TLC) on silica gel plates (G60; Merck, Germany), then, methylated with HCl in methanol and transferred into fatty acyl methylester (FAME), and the result of the FAMEs were quantified by gas chromatography (GC-2010; Shimadzu, Japan) with flame ionization detector (FID). The lipids were quantified based on the internal standards of pentadecanoic acid (15:0). The fatty acid double bond index (DBI) was calculated by the equation described by Rawyler et al. (1999): DBI = (0 × mol%16:0 + 1× mol %16:1 + 2×mol %16:2 + 3×mol %16:3 + 0× mol %18:0 + 1×mol %18:1 + 2×mol %18:2 + 3× mol %18:3)/100.

### 2.8 Data analysis

Differences between the means were analyzed by analysis of variance (ANOVA) using the least significant difference (LSD) test at $p < 0.05$. All experiments were repeated at least three times. All plots were created using Sigmaplot12.5 and different letters in the same group indicate statistically significant difference at $p < 0.05$.

## 3 Results

### 3.1 Expression of *OsMGD* genes under NaCl, $GA_3$, ABA and MeJA treatment

The expression patterns of *OsMGD02 g*, *OsMGD08 g* and *OsMGD09 g* genes were analyzed by qRT-PCR after 24 h of NaCl, $GA_3$, ABA and MeJA treatments. The expression levels of all three *OsMGD* genes were higher in $GA_3$ treated plants, as compared to plants from the treatments of ABA and MeJA (Fig. 1b). Therefore, $GA_3$ was selected and used for further experiments. Moreover, the expression of *OsMGD* genes were decreased after 24 h of salt treatment, which was different with the short-term (1 h) salt treatment (Supplementary material; Fig. 1). This may be due to a possible stress caused by the long-term salt treatment.

### 3.2 Effects of salt stress and $GA_3$ pre-treatment on plant growth

Salt treatment is known to inhibit plant growth (Munns and Tester 2008). Our results confirmed those findings as shoot and root dry weights of rice seedlings were decreased after salt stress (Fig. 2). However, previous $GA_3$ application significantly alleviated the growth inhibition induced by salt. In the absence of salt, $GA_3$ application had no effect on rice seedling growth. Specifically, the biomass of $GA_3$ pre-treated rice was 24% higher than that of plants without $GA_3$ application under salt conditions. The concentration of chlorophyll *a* and *b* increased by $GA_3$ application alone (Fig. 3a), and $GA_3$-treatment prior to salt conditions significantly increased the concentrations of chlorophyll *a* and *b* and total chlorophyll by 29.3%, 25.2% and 25.2%, respectively ($p < 0.05$) as compared with plants not pre-exposed to $GA_3$ (Fig. 3b).

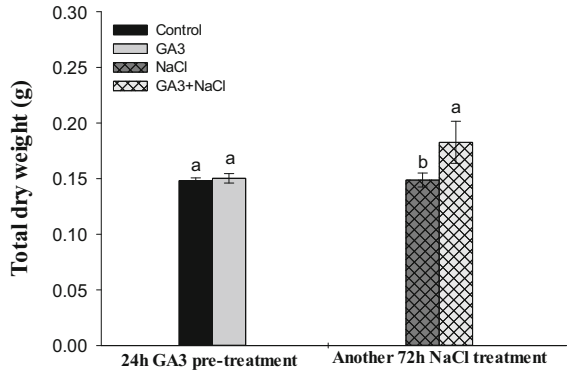


**Fig. 2** Effect of 24 h $GA_3$ pretreatment (before salt treatment) and NaCl treatment (exposed to NaCl solution for another 72 h, in the treatment of salt stress) on the total dry weight of rice plants. Values are presented as the mean ± SE (n = 3). Different letters within the same group indicate statistically significant differences at $p < 0.05$

Therefore, $GA_3$ application ameliorated the salt-induced reduction of the chlorophyll concentration.

### 3.3 Effect of salt and $GA_3$ pre-treatment on lipid content

The membrane lipids, MGDG, DGDG, and PL + SQDG concentrations were analyzed to investigate the regulation of lipid biosynthesis by $GA_3$ pre-treatment both before and after salt disturbance. In the absence of salt, there was no difference in the lipid concentration of PL + SQDG between with and without $GA_3$ treatment, except for the increase of MGDG and DGDG concentrations (Fig. 4a). Under salt conditions, the lipid concentrations relative to biomass were all decreased. However, pre-application of $GA_3$ prevented the decrease in lipid concentration under salt treatment. In $GA_3$-treated plants, the concentration of MGDG was 15.7% ($p < 0.05$) higher than that of plants without $GA_3$ (Fig. 4b). $GA_3$ application also maintained the higher concentrations of DGDG and PL + SQDG, as compared with no $GA_3$ pre-treatment under salt conditions. As a result, $GA_3$ treatment had no effect on the DGDG/MGDG ratio compared with the control conditions before salt treatment (Fig. 5). However, exogenous pre-application of $GA_3$ followed by exposure to NaCl significantly increased the ratio of DGDG/MGDG (Fig. 5). The fatty acid double bond index (DBI) did not change under the simple salt treatment and $GA_3$ treatment, but the DBI of

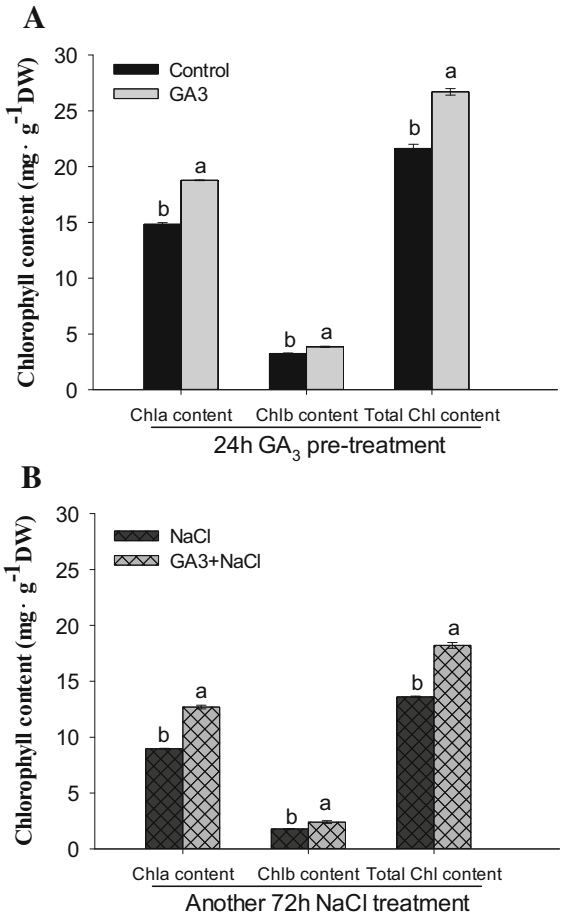


**Fig. 3** Effect of 24 h $GA_3$ pre-treatment (**a**) (before salt treatment) and NaCl treatment (**b**) (exposed to NaCl solution for another 72 h, in the treatment of salt stress) on chlorophyll concentration of rice plants. Values are presented as the mean ± SE (n = 3). Different letters within the same group indicate statistically significant differences at $p < 0.05$

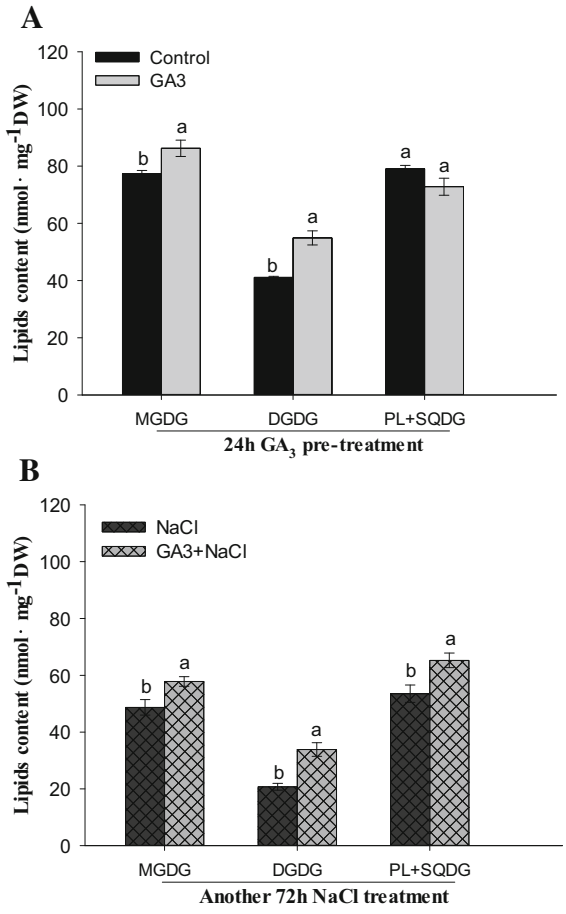


**Fig. 4** Effect of 24 h $GA_3$ pre-treatment (**a**) (before salt treatment) and NaCl treatment (**b**) (exposed to NaCl solution for another 72 h, in the treatment of salt) on galactolipid and phospholipid composition of rice leaves. Values are presented as the mean ± SE (n = 3). Different letters within the same group indicate statistically significant differences at $p < 0.05$

DGDG increased significantly when plants were treated with $GA_3$ prior to salt conditions (Fig. 6).

### 3.4 Effect of $GA_3$ pre-treatment and salt on the chloroplast fatty acid composition

In this study, eight fatty acids from MGDG, DGDG, PL + SQDG [16:0, 16:1, 16:2, 16:3, 18:0, 18:1, 18:2, 18:3(carbon number: double bond number)] were detected in rice (Fig. 7). $GA_3$ pre-treatment followed by salt treatment resulted in a decrease in the 16:0 content and an increase in 18:2 content of MGDG. Regarding DGDG, the contents of both 16:0 and 18:0 decreased significantly with the $GA_3$ pre-treatment. Specifically, the contents of 16:0 and 18:0 were 38.6% and 27.9% ($p < 0.05$) lower than in plants without $GA_3$ pre-treatment under salt conditions, respectively. The decrease in the fully saturated fatty acids was compensated by a significant increase in the unsaturated fatty acid (18:3) in DGDG. As for PL + SQDG, $GA_3$ application did not affect the content of these fatty acids under salt conditions.

## 4 Discussion

Salt treatment severely inhibits plant growth. In the present study, compared with no $GA_3$ pre-treatment

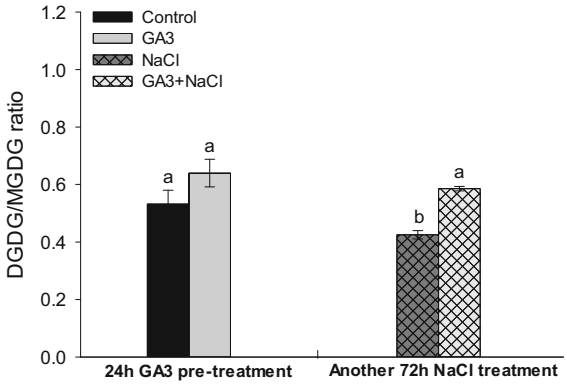


**Fig. 5** Effect of 24 h $GA_3$ pre-treatment (before salt treatment) and NaCl treatment (exposed to NaCl solution for another 72 h, in the treatment of salt) on the ratio of DGDG to MGDG in rice leaves. Values are presented as the mean ± SE (n = 3). Different letters within the same group indicate statistically significant differences at $p < 0.05$

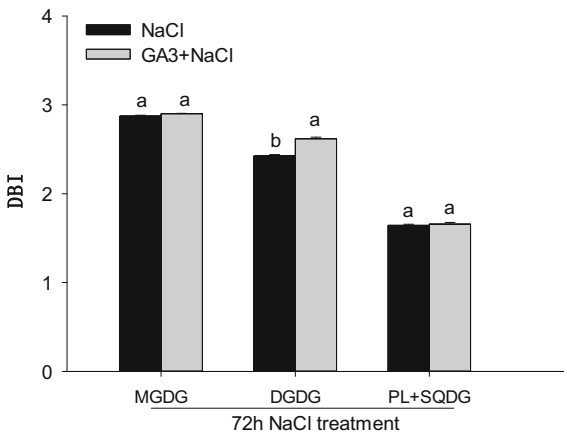


**Fig. 6** DBI of galactolipids and phospholipids in rice leaves after salt treatment at 72 h. Values are presented as the mean ± SE (n = 3). Different letters within the same group indicate statistically significant differences at $p < 0.05$

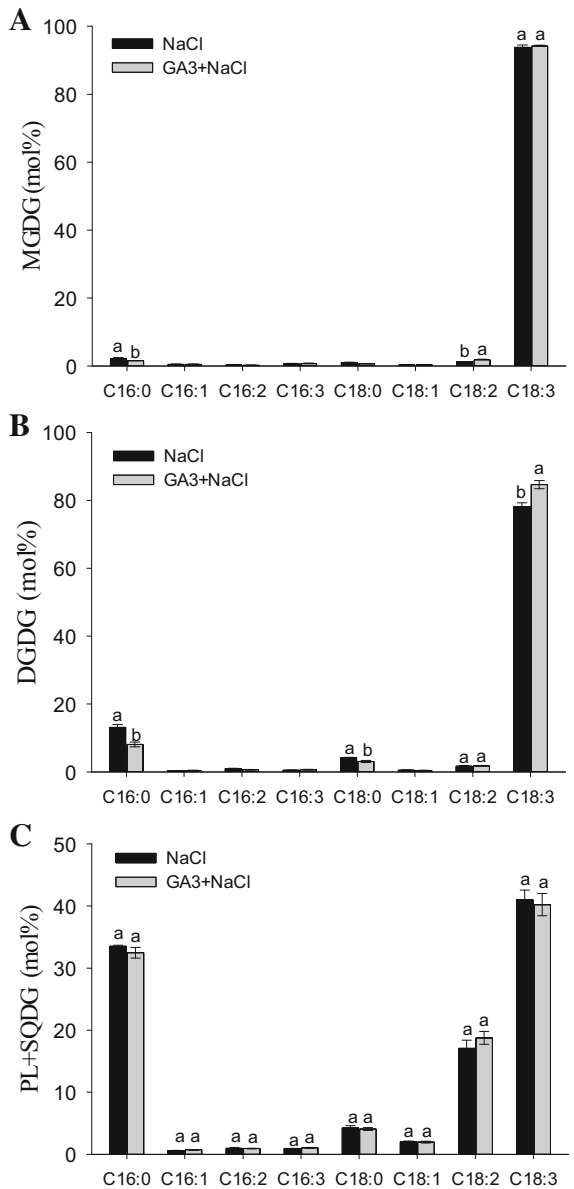


**Fig. 7** Effect of salt stress and $GA_3$ pre-treatment on the free fatty acid composition (mol%) in the lipids of rice leaves. Values are presented as the mean ± SE (n = 3). Different letters within the same group indicate statistically significant differences at $p < 0.05$

under salt condition, $GA_3$ pre-treatment was involved in enhancement of several plant growth parameters under the same condition. The total dry weight was significantly higher in plants that were pre-treated with $GA_3$ than that without $GA_3$ pre-treatment under salt conditions (Fig. 2), indicating that $GA_3$ may participate in maintaining the plant growth under salt conditions. In wheat, it was also shown that $GA_3$ treatment significantly improves plant height and shoot dry weight under saline conditions (Iqbal and Ashraf 2013). Meanwhile, the chlorophyll concentration of seedlings was higher in $GA_3$ pre-treated plants than that without $GA_3$ pre-treatment after 72 h of NaCl treatment (Fig. 3b). In addition, our results showed that $GA_3$ pre-treatment alone also enhanced chlorophyll concentrations (Fig. 3a). Similar results have been reported that the effects of $GA_3$ on chlorophyll concentration were similar both with and without salt treatment in other crops, including rice

and wheat (Prakash and Prathapasenan 1990; Ashraf et al. 2000). Since a reduction in chlorophyll concentration reduces photosynthetic capacity (Hasegawa et al. 2000), $GA_3$ pre-treated plants should have improved vigor when exposed to salt.

Previous studies have indicated that chloroplasts from salt-treated wild-type tobacco are fairly disorganized, with large membrane-free areas (Wang et al. 2014). Those changes are consistent with alterations in lipid content and composition occurring as a consequence of the excessive salt in the metabolism (Wu et al. 1998; Andersson et al. 2003; Gigon et al. 2004). The lipid structure of the chloroplast membrane is important for the structure and function of Chl-protein complexes in thylakoids (Yamamoto et al. 2014). As the major polar lipid constituents of chloroplast membrane, MGDG is synthesized by the UDP-galactose:1,2-diacylglycerol 3-β-galactosyltransferase (MGDG synthase) (Marechal et al. 1994), and is required for energy coupling between light-harvesting chlorophyll complex II (LHCII) and photosystem II (PSII). MGDG is a non-bilayer galactolipid that is vital for maintaining the stability of the chloroplast membrane. Our results showed that after 72 h of salt treatment, the concentration of MGDG (48.71 nmol $mg^{-1}$ DW) was lower than that before salt treatment (77.39 nmol $mg^{-1}$ DW). Previous reports indicate that MGDG concentrations in mesophyll cell chloroplasts are notably lower under salt disturbance, compared with no salt conditions (Omoto et al. 2016). It was proposed that low concentration of MGDG in bundle sheath cell chloroplasts and the relative insensitivity of MGDG concentration to salt stress causes them to be more tolerant to salinity than are mesophyll cell chloroplasts. Huynh et al. (2012) reported that MGDG is one of the lipids most affected when rice seedlings are exposed to aluminum. Again, in this case, an abiotic disturbance results in a disruption of chloroplast lipid biosynthesis and an alteration in the normal lipid composition of chloroplasts.

The other major chloroplast galactolipid DGDG also has an indispensable function in chloroplast membrane structure. DGDG is a bilayer-forming lipid, which is crucial for the stability of the lamellar structure of the chloroplast membrane. It has been reported that a decreased level of DGDG results in the degradation of PS I complexes and shortening of chlorophyll fluorescence half-life, which reduces the stability of the thylakoid membrane (Krumova et al. 2010). In the present study, the concentration of DGDG and PL + SQDG were increased by 38.8% and 36.8%, respectively, when plants were treated with $GA_3$ prior to the NaCl treatment, compared with no $GA_3$ pre-treatment under the same condition (Fig. 4b). It has been reported that when plants are exposed to salt conditions, the proportions of MGDG and DGDG are decreased by 9.4% and 5.6%, respectively, and the concentration of unsaturated fatty acids in membrane lipids increase (Sui et al. 2010). In another investigation, MGDG concentration was significantly decreased by 15% in mesophyll cells under salt conditions compared with plants not experiencing salt effects (Omoto et al. 2016). Thus, even slight changes in membrane lipids could affect plant vigor and/or enhance their tolerance to the disturbance. The decreased concentrations of DGDG and PL + SQDG may be critical to a disordered chloroplast membrane under NaCl treatment. Therefore, these results indicate that application of $GA_3$ prior to NaCl treatment could elevate the concentrations of DGDG and PL + SQDG, which play a crucial role in enhancing salt tolerance under saline conditions.

Regulating the ratio of DGDG to MGDG in chloroplast lipid membrane is a strategy for maintaining the structure of the membrane and the accumulation of phospholipids. A relatively high content of bilayer-forming lipids could improve lamellar membrane stability. Thus, a higher DGDG/MGDG ratio might protect the photosynthetic apparatus and stabilize photosynthetic process, such as ATP synthesis and light-harvesting complex (Sui and Han 2014). In the present study, before the salt treatment, only $GA_3$ application did not cause significant increase in DGDG/MGDG ratio, however, after 72 h of NaCl treatment, this ratio was higher in $GA_3$ pre-treated plants than that without $GA_3$ pre-treatment (Fig. 5). In *Sulla carnosa*, the chloroplast structure was severely damaged by NaCl stress, and the DGDG/MGDG ratio also decreased (Bejaoui et al. 2016). Overexpressing the *OsMGD* gene of tobacco maintains a higher ratio of DGDG to MGDG than the wild-type under salt stress (Wang et al. 2014). An increased ratio of DGDG to MGDG also enhances the stability of the thylakoid membrane at elevated temperatures (Chen et al. 2006). In addition to the changes in DGDG and MGDG contents, when plants were pre-treated with $GA_3$ prior to salt treatment, the DBI of DGDG also

increased (Fig. 6). Therefore, we suggest that the increased concentrations of DGDG and its increased composition of unsaturated fatty acids may be an adaptive strategy for plants to overcome adverse environmental disturbances.

Fatty acid composition and the degree of unsaturation influence chloroplast membrane stability, fluidity and permeability, and both factors are vital for maintaining homeostasis of membranes (Spychalla and Desborough 1990). Increased concentrations of unsaturated fatty acids in chloroplast membrane lipids improves the tolerance of photosystem II under salt stress (Sui and Han 2014). It has been reported that decreased unsaturated lipids may result in damage to thylakoid membranes, and decrease the stability of proteins in membranes (Thomas et al. 1986). Increased content of unsaturated fatty acids may enhance plant tolerance to various stresses (Dakhma et al. 1995; Matos et al. 2002; Sui et al. 2007; Popov et al. 2012). In this study, we found that $GA_3$ pre-treatment decreased the concentrations of 16:0 and increased the concentration of 18:2 fatty acid in MGDG. However, the DBI in MGDG was not affected by $GA_3$ pre-treatment. For DGDG, both 16:0 and 18:0 fatty acid concentrations were reduced by $GA_3$ in the presence of salt, and the 18:3 fatty acid was increased. As a result, the DBI in DGDG increased significantly as a consequence of $GA_3$ pre-treatment in the presence of salt (Fig. 7). It has been found that in the halophyte Thellungiella, DBI is notably increased under NaCl treatment (Sui and Han 2014). Furthermore, the unsaturated fatty acids were significantly increased by $GA_3$-pretratment, which could result in an increase in the chloroplast lipid membrane fluidity and thus possibly affect chloroplast membrane permeability and stability. In turn, the improved fluidity of chloroplast membrane may improve the activity of $H^+$-ATPase(s) (Allakhverdiev et al. 2001). Thus, we believe that fatty acid composition is regulated when $GA_3$ pre-treatment is followed by salt treatment, consequently protecting the structure and function of chloroplasts.

Taken together, we found that pretreatment with $GA_3$ prior to salt conditions did affect the content of lipids and the unsaturation of fatty acids in chloroplast membranes. Combining the present finding with those of previous studies, we propose that (1) $GA_3$ activates the expression of MGDG synthase, which further participates in the catalysis and synthesis of MGDG, thus affecting chloroplast lipid composition; (2) the alterations of lipid composition contributes to a transition of membrane structure, helping to maintain the stability of the photosynthetic systems, and that (3) the higher stability of chloroplast membranes results in an increase in chlorophyll abundance, and both will counteract the growth inhibition caused by salt. Therefore, here we evidenced that exogenous application of $GA_3$ before salt treatment could affect the chloroplast membrane lipids biosynthesis, and that it could maintain plant growth and consequently facilitate plants adaptation to a saline environment. To understand the molecular mechanism in the regulation of lipids by exogenous application of $GA_3$ under salt treatment, the detailed signal pathways that are involved still require further studies.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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