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ORIGINAL ARTICLE

Choline priming-induced plasma membrane lipid alterations contributed to improved wheat salt tolerance

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Abstract Salt stress is a major environmental threat influencing crop growth and yield. The plasma membrane (PM) is believed to be one facet of the cellular mechanisms of salt adaptation. Choline priming has been reported to enhance salt tolerance of the sensitive wheat cultivar used in this work. The study was, therefore, undertaken to examine whether changes in the PM lipids will participate in choline-improved salt tolerance. The caryopses were primed in choline chloride (0, 5 and 10 mM) for 24 h. They were then germinated in sand for 10 days, watered with 1/4-strength modified Hoagland solution (MHS). The seedlings were grown in the sand, watered with MHS containing 150 mM NaCl for 3 weeks. Root PM was isolated by two-phase partitioning method and its lipid classes were determined. Choline maintained the PM total lipids, sterol and phospholipids, which were altered by NaCl. The PM sterols/phospholipids ratio was decreased by NaCl, whereas choline retained this ratio. Salt stress reduced the PM unsaturated fatty acids while increased its saturated fatty acids. Choline alleviated PM unsaturated/saturated ratio reduction. NaCl declined the PM phosphatidylcholine (PC), phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) whereas increased phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylethanolamine (PE). Choline decreased PS and PE, while increased PC level. The PM cholesterol, campesterol and β-sitosterol were increased while stigmasterol was declined under NaCl. Choline increased stigmasterol whereas decreased cholesterol and campesterol. The alterations in the PM lipids were discussed in relation to choline-enhanced salt tolerance.

Keywords Choline · Lipids · Plasma membrane · Priming · Salt stress

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Introduction

Salinity is a major abiotic stress affecting plant growth and productivity. Salt stress has an injurious effect on plants through osmotic and ionic stresses, nutrient imbalance as well as oxidative stress (Munns and Tester 2008). Unfortunately, crop plants are grouped as relatively salt sensitive and their ability to tolerate low level of salinity is minimal. Evidence indicates that tolerance to salt stress in glycophytes (the majority of crop plants is glycophytes) and halophytes is working at the cellular level (Hasegawa et al. 2000; Mansour 2014). One facet of the cellular level mechanism of salt acclimation is the plasma membrane (PM), which is believed to be a primary site of salt injury (Cramer et al. 1985; Lauchli 1990; Mansour and Salama 2004; Flowers and Flowers 2005; Mansour 2013, 2014). Understanding the mechanism of salt tolerance can, therefore, be achieved through studying salt responses at the cellular level of plants differing in their sensitivity to salinity (Hasegawa et al. 2000; Mansour and Salama 2004; Mansour et al. 2005). Because of its critical roles in adaptation of plants to saline conditions, the PM components are believed to have or undergo certain compositional and structural alterations to maintain the PM stability and thus withstand high salt (Hasegawa et al. 2000; Mansour 2014). This contention is supported by the fact that changes in the composition/structure of the PM have been

found in saline environment which were proposed to contribute to salinity adaptation (Kuiper 1984; Mansour et al. 1994, 2002; Kerkeb et al. 2001; Wu et al. 2005; Salama et al. 2007; Zamani et al. 2010).

The involvement of choline in stress tolerance was observed under salt stress (Mansour et al. 1993; Salama et al. 2011), chilling stress (Sheng et al. 2006), heat and radiation stress (Kreslavski et al. 2001). The studies demonstrate that choline alters membrane lipid composition, and also protects membranes against lipid peroxidation. Choline priming has been reported to enhance salt tolerance of a sensitive wheat cultivar (Salama et al. 2011) that is used in the present investigation. Choline is an important cell metabolite that can be synthesized through two pathways into an osmoprotectant (glycine betaine) and a membrane phospholipid constituent (i.e., phosphatidylcholine, PC) (Su et al. 2006; Salama et al. 2011). Glycine betaine has been shown to play a prime role in salt tolerance (Mansour 2000; Munns and Tester 2008), and PC is an important membrane component that may have significance in regulating ion absorption under salt conditions (Mansour 2013; Mansour et al. 2015).

Presowing seed treatment using different agents (i.e., seed priming) has been demonstrated to be a useful approach to enhance salt tolerance in different crop plants under salinity (Ashraf and Foolad 2005). Based on the above evidence, choline priming of wheat caryopsis was adopted in the current work to study its influence in the PM lipid alterations and whether these alterations will participate to improved salt tolerance reported by Salama et al. (2011). The data obtained in this investigation are clearly supporting the contention that the PM components are crucial feature in salt adaptation mechanism. That is, favorable alterations in the PM lipids maintained its integrity and thus ion homeostasis (Salama et al. 2011) under saline conditions, which is a fundamental determinant in salt tolerance mechanism (Mansour 2014; Mansour et al. 2015).

Materials and methods

Growth conditions

Salt-sensitive cultivar of *Triticum sativum* L. (Gomeza 7) was used in this investigation. Caryopses were surface sterilized by 0.1 % $HgCl_2$ for 5 min and then rinsed with tap water several times. Caryopses were then divided into three groups. Group I was presoaked in distilled water for 24 h. Groups II and III were presoaked in 5 and 10 mM choline chloride for 24 h, respectively. Choline chloride and distilled water were renewed every 4 h. Caryopses were next cultivated in sand which previously wet with 1/4-strength modified Hoagland solution (MHS) (Epstein 1972). Each pot contained 12 plants. All plants were irrigated with MHS and maintained under natural environmental conditions (13-h light period, 46 % relative humidity, 335 μmol m^{-2} s^{-1} irradiation (400–700 nm), day/night temperatures of 36/20 ± 3 °C) for 10 days. After 10 days, group I was further divided into two groups: one group was grown only in MHS for 21 days (non-treated plants, NT) whereas the other group was grown in MHS containing 150 mM NaCl for 21 days (salt control, SC). Groups II and III (presoaked in choline) were also treated with 150 mM NaCl added to MHS for 21 days. Each treatment was replicated three times and each replicate consists of five pots. The plants were irrigated every 3 days, and the sand was washed with tap water every 7 days to prevent salt accumulation during the 21-day treatment period.

The PM isolation

Two-phase partitioning method was used to isolate the root PM as described by Mansour et al. (1994, 2002). In short, the PM was prepared by partitioning of microsomal suspension in 27 g aqueous polymer two-phase system containing 6.5 % dextran T-500 (Pharmacia), 6.5 % polyethylene glycol 3350 (Sigma). After several steps of centrifugation, the microsomal pellet was subjected to three successive phase partitioning steps. The upper phase, containing the PM fraction, centrifuged again at 50,000*g* for 1 h, and the pellet resuspended in a buffer (pH 7.5). All steps of the PM isolation were carried out at 0–4 °C. The purity of our PM preparation was based on Mansour et al. (1994, 2002).

The PM lipid extraction and separation

Boiled isopropanol was immediately added to the PM suspension to inhibit the activity of lipase (Kates 1972). Lipids were then extracted with 3.75 mM chloroform:isopropanol (2:1, v/v) and 2.25 mL of 0.1 M KCl was added to enhance the chloroform phase separation. The mixture was then centrifuged in cold room at 1000*g* for 5 min. The upper water phase was re-extracted with 2 mL chloroform. The first and second chloroform phases (containing lipids) were collected and dried under CO_2 stream. The dried lipids were dissolved in 2.5 mL chloroform and stored at −80 °C until analysis.

Determination of the PM total lipid, total sterols and total phospholipids

The protocol of March and Weinstein (1966) was adopted to determine the total lipids, using olive oil as standard. Total sterols were determined according to Zlatkis and Zak (1969), with cholesterol as standard. Total phospholipids were determined according to Ames (1966) using phosphate as the standard.

Determination of the PM phospholipid classes

Phospholipids were assayed according to Deinstrop and Weinheim (2000). Briefly, the lipid extract was spotted along a glass thin-layer chromatography plate (TLC, Merk, Germany), and phospholipid classes were separated by two-dimensional TLC. Individual phospholipids were identified by co-chromatography with authentic standards. The area on TLC corresponding to each individual phospholipid was marked, scarped and assayed according to Ames (1966).

Determination of the PM fatty acids and sterols

The method of Mansour et al. (2002) was used for analysis of the PM fatty acids and sterols. One mL of lipid extract, 6 mL of benzene and 1.5 mL of 10 % alcoholic KOH were mixed together. The tubes were refluxed for 4 h in a boiling water bath and then the mixture was evaporated. Excess of diethylether was added and shaken well. The organic phase (upper phase) was pipetted and the aqueous phase (lower phase) was further washed three times with diethylether. The organic phase was used to determine the different classes of sterols and aqueous phase was acidified to determine the different fatty acids. The aqueous phase containing fatty acids was saponified and fatty acids were methylated with 1 N H_2SO_4 and methanol according to Mansour et al. (2002). Fatty acid methyl esters were quantified by gas chromatography (HP-5890, Hewlett Packard, Little Falls, DE).

Lipid samples from the organic phase were loaded on silica gel plates and the plates developed in hexane:diethylether (50:50, v/v). Sterols were identified by co-chromatography with authentic standards and their identity was confirmed by spraying the plates with a solution containing 2,7-dichlorofluorescein in ethanol (0.2 %). Spots were marked, scraped, eluted into chloroform and centrifuged. The free sterol components were identified by gas–liquid chromatography (GLC, Vista 6000, Palo Alto, USA) as described by Mansour et al. (2002).

Statistical analysis

The data were statistically tested by analysis of variance (ANOVA). To compare the means of non-treated and treated samples, the software Excel was used.

Results

Salt stress induced a significant decrease in the total lipids and total sterols of the SC root PM comparing with NT (Table 1). Priming of caryopses with 5 mM choline alleviated salt-induced reduction, whereas 10 mM choline increased the reduction in both parameters. After salt imposition (SC plants), the root PM total phospholipids was increased (Table 1). Choline priming (in particular 5 mM) significantly returned the level of the PM total phospholipids near to that of NT plants. Choline at 10 mM had no significant effect on the PM total phospholipids (Table 1). The PM sterols/phospholipids ratio was decreased in SC plants in response to 150 mM NaCl treatment (Table 1). This ratio was maintained near to that of NT plants after 5 mM choline priming. Pretreatment caryopses with 10 mM choline had no effect on this ratio (Table 1).

Salt stress altered the mol percentage of the root PM fatty acids: increased saturated fatty acids (except 17:0) while reduced unsaturated fatty acids, resulting in reduced unsaturated/saturated ratio (Table 2). Priming of caryopses in choline significantly increased unsaturated fatty acids (in particular 16:1 and 18:1) and decreased saturated fatty acids, leading to elevated unsaturated/saturated ratio comparing with SC plants (Table 2).

Phosphatidylglycerol (PG), diphosphatidylglycerol (DPG) and PC were the most abundant phospholipid classes in the PM of NT plants (Table 3). After salt treatment (SC plants), the PM PC, PG and DGP were decreased whereas phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylethanolamine (PE) were increased. This resulted in a significant reduction of PC/PE ratio of the PM of SC plants.

Table 1 The root PM total lipids (μmol g^{-1}), total sterols (nmol g^{-1}) and total phospholipids (nmol g^{-1}) of wheat primed with 5 or 10 mM choline chloride (CC) and then exposed to 150 mM NaCl for 21 days

Treatment	Total lipids (μmol g^{-1})	Total sterols (nmol g^{-1})	Total phospholipids (nmol g^{-1})	Sterols/phospholipids ratio
0 mM CC → 0 mM NaCl (NT)	29.07 ± 1.71	10.58 ± 0.47	36.35 ± 0.65	0.29 ± 0.01
0 mM CC → 150 mM NaCl (SC)	14.57 ± 1.99[a]	8.33 ± 0.42[a]	60.3 ± 1.05[a]	0.14 ± 0.02[a]
5 mM CC → 150 mM NaCl	25.97 ± 1.60[b]	10.40 ± 0.32[b]	34.94 ± 0.92[b]	0.29 ± 0.02[b]
10 mM CC → 150 mM NaCl	8.47 ± 0.46[b]	6.48 ± 0.46[b]	62.07 ± 3.60	0.11 ± 0.02

Each value is the mean ± SD of three replicates

[a] Significantly different from NT plants at least at $P = 0.05$

[b] Significantly different from SC plants at least at $P = 0.05$

Table 2 Fatty acids composition (mol%) of the root PM of wheat primed with 5 or 10 mM choline chloride (CC) for 24 h and then exposed to 150 mM NaCl for 21 days

Fatty acid	0 mM CC → 0 mM NaCl (NT)	0 mM CC → 150 mM NaCl (SC)	5 mM CC → 150 mM NaCl	10 mM CC → 150 mM NaCl
C 16:0	17.37 ± 0.10	33.04 ± 0.61[a]	17.70 ± 0.56[b]	14.83 ± 0.31[b]
C 16:1	9.56 ± 0.30	3.46 ± 0.21[a]	13.40 ± 0.35[b]	12.52 ± 0.36[b]
C 17:0	13.22 ± 0.80	6.68 ± 0.74[a]	26.95 ± 1.10[b]	17.76 ± 0.70[b]
C 18:0	13.60 ± 0.76	39.66 ± 1.03[a]	11.43 ± 0.35[b]	30.10 ± 1.42
C 18:1	12.33 ± 0.60	5.23 ± 0.45[a]	14.11 ± 0.79[b]	6.88 ± 0.38[b]
C 18:2	14.56 ± 0.40	7.18 ± 0.44[a]	1.80 ± 0.20[b]	4.74 ± 0.56
C 20:0	19.34 ± 0.35	4.76 ± 0.53[a]	14.60 ± 0.92[b]	13.24 ± 0.95[b]
Unsaturated/saturated	0.57 ± 0.02	0.19 ± 0.03[a]	0.42 ± 0.04[b]	0.32 ± 0.01[b]

Each value is the mean ± SD of three replicates

[a] Significantly different from NT plants at least at $P = 0.05$

[b] Significantly different from SC plants at least at $P = 0.05$

Table 3 Phospholipid composition (mol%) of the root PM of wheat caryopses primed with 5 or 10 mM choline chloride (CC) for 24 h and then exposed to 150 mM NaCl for 21 days

Phospholipid class	0 mM CC → 0 mM NaCl (NT)	0 mM CC → 150 mM NaCl (SC)	5 mM CC → 150 mM NaCl	10 mM CC → 150 mM NaCl
PA	13.08 ± 1.01	9.72 ± 1.10	12.04 ± 0.83	12.87 ± 0.7[b]
PI	5.88 ± 1.67	8.81 ± 1.16[a]	10.10 ± 0.61	10.55 ± 0.99
PS	9.17 ± 0.85	19.88 ± 1.46[a]	12.30 ± 0.71[b]	14.23 ± 1.19[b]
PC	18.03 ± 1.11	12.02 ± 0.46[a]	17.91 ± 1.10[b]	13.10 ± 1.23
PE	9.97 ± 0.46	17.61 ± 0.65[a]	8.35 ± 1.04[b]	13.65 ± 1.75[b]
PG	28.26 ± 2.05	21.62 ± 1.06	25.26 ± 1.19	23.53 ± 1.00
DPG	15.60 ± 1.21	10.35 ± 0.96[a]	14.05 ± 0.51	12.08 ± 0.15
PC/PE	1.81 ± 0.21	0.68 ± 0.03[a]	2.15 ± 0.31[b]	0.96 ± 0.11[b]

Each value is the mean ± SD of three replicates

PA phosphatidic acid, *PI* phosphatidylinositol, *PS* phosphatidylserine, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *DPG* diphosphatidylglycerol

[a] Significantly different from NT plants at least at $P = 0.05$

[b] Significantly different from SC plants at least at $P = 0.05$

Choline priming significantly decreased PS and PE, and increased PC level of the root PM leading to an increase in PC/PE ratio, more so with 5 mM choline (Table 3). The mol percentage of PG was maintained by choline priming.

Salt stress increased the relative proportion of the PM cholesterol, campesterol and β-sitosterol, and reduced that of stigmasterol (Table 4). Presoaking of wheat caryopses in choline decreased the level of cholesterol, campesterol and increased that of stigmasterol (Table 4).

Discussion

Choline priming (in particular 5 mM) retained the PM total lipids, total phospholipids and total sterol under saline stress may have an adaptive significance since preservation of the PM integrity under salinity has been reported to result from maintained or increased lipid level of the PM (López-Perez et al. 2009; Zamani et al. 2010; Lu et al. 2012; Mansour 2013; Mansour et al. 2015). Alteration in the PM lipids observed here in response to choline priming may suggest a stimulation of membrane biosynthesis to accommodate the PM stability under NaCl stress. Salt-tolerant species/genotypes showed increased (Huang 2006; Liang et al. 2006; Kumari et al. 2013; Mansour et al. 2015) or absence of variation (Huang 2006; Kumari et al. 2013; Mansour et al. 2015) in total free sterols and phospholipid content of the PM under salinity, suggesting that maintenance of the PM sterol and phospholipids is essential for its function under salinity. It is, therefore, proposed that impaired PM lipid contents under NaCl stress might relate to salt sensitivity of the studied salt-sensitive wheat

Table 4 Sterols composition (mol%) of the root PM of wheat caryopses primed with 5 or 10 mM choline chloride (CC) for 24 h and then exposed to 150 mM NaCl for 21 days

Sterol class	0 mM CC → 0 mM NaCl (NT)	0 mM CC → 150 mM NaCl (SC)	5 mM CC → 150 mM NaCl	10 mM CC → 150 mM NaCl
Cholesterol	4.21 ± 0.75	10.83 ± 0.99[a]	4.24 ± 0.31[b]	7.32 ± 1.21[b]
Stigmasterol	48.45 ± 1.46	23.71 ± 1.90[a]	43.13 ± 1.99[b]	38.83 ± 1.11[b]
Campesterol	30.81 ± 1.92	44.74 ± 1.40[a]	31.17 ± 1.45[b]	35.05 ± 1.83[b]
β-Sitosterol	16.53 ± 0.38	20.72 ± 1.76[a]	21.46 ± 1.74	18.80 ± 1.82

Each value is the mean ± SD of three replicates

[a] Significantly different from NT plants at least at $P = 0.05$

[b] Significantly different from SC plants at least at $P = 0.05$

cultivar. On the other hand, maintenance or increased PM total lipids, sterols and phospholipids under salinity may correlate with salt adaptation. Elevated total PM phospholipids observed here in response to salt stress were similarly reported in salt-sensitive dwarf cashew root PM under high salinity (Alvarez-Pizarro et al. 2009). Higher PM-free sterols/phospholipids ratio reported in several salt-tolerant species/cultivars (Mansour et al. 1994, 2002, 2015; Kerkeb et al. 2001; Salama et al. 2007; Zamani et al. 2010, Mansour 2013) also has been found in this study, which probably contributed to improved salt tolerance demonstrated by Salama et al. (2011) in response to choline priming. Different response obtained with the different concentrations of CC regarding the PM lipid changes is unclear and warrants further investigations. Possibly, CC influence may involve complex cross-talk between, e.g., adjustment of metabolism and gene expression for enhanced physiological adaptation, and high concentration of CC may have unfavorable impact on that. This is not established in plants because of the limited data. However, high choline intake has been shown to have adverse effect in human (The National Academies 1998). In the same trend, proline pretreatment with low concentration (1 mM) was found to be effective and stimulated cellular activities, whereas high concentration (10 mM) was ineffective in improving plant growth under high level of NaCl (Hasanuzzaman et al. 2013).

One crucial impact of the fatty acids on membrane is their degree of unsaturation/saturation (Mansour et al. 2015). The ability of cells to alter the degree of unsaturation/saturation in their membranes is anticipated to be an important factor in cellular acclimatization to environmental conditions (Mansour 2013). NaCl induced reduction in the PM unsaturated fatty acids and increased saturated fatty acids has been previously reported in different plant species in saline conditions (Wu et al. 2005; Salama et al. 2007; Zamani et al. 2010; Mansour 2013; Mansour et al. 2015). An increase in fatty acid saturation may induce formation of a gel phase and phase separation in the PM (Senaratna et al. 1984), which impairs the PM's proper functioning and properties and hence may participate in salt sensitivity of the wheat cultivar. Choline priming, however, increased the PM unsaturation/saturation ratio which most probably plays important role in induced salt adaptation by choline. Our conclusion is supported by the fact that many reports show correlation between increased PM fatty acid unsaturation and acclimation to high salinity (Upchurch 2008; Hajlaoui et al. 2009; López-Perez et al. 2009; Lu et al. 2012; Kumari et al. 2013; Mansour 2013). Increased PM unsaturation may affect salt tolerance through retaining proper fluidity, which is an essential determinant for the transport system activities required for ion homeostasis in saline environment (Mansour 2014; Mansour et al. 2015; Morales-Cedillo et al. 2015).

Salt treatment increased non-bilayer-forming lipids (e.g., PE) and decreased those forming lamellar structure (e.g., PC, PG) resulting in reduced PC/PE ratio, which also impairs the PM functions and properties (Russell 1989; Lu et al. 2012; Mansour 2013; Mansour et al. 2015). Non-lamellar domain in the PM causes interruption of the bilayer structure and hence renders high permeability (Russell 1989), which may increase toxic ion absorption under salinity as indicated in the study of Salama et al. (2011). In support to that, high level of PE was related to chloride accumulation in grape root (Kuiper 1984). PI and PS increased by NaCl treatment are in agreement with previous reports illustrating possible relationship between these phospholipid classes and salt sensitivity in various crop plants (Mansour et al. 1994; Racagni et al. 2003; Salama et al. 2007; Zamani et al. 2010; Bybordi 2011). It is interesting to mention that choline presoaking of caryopses declined the abundance of PI and PS in the PM under salinity. Furthermore, choline priming also decreased PE, while increased PC and maintained PG level of the PM, leading to increased PC/PE ratio. Increased bilayer-forming lipids (PC) and PC/PE ratio has been suggested to play a role in salt acclimation (Kuiper 1984; Racagni et al.

2003; Mansour 2013; Mansour et al. 2015). In addition, alteration in specific PM phospholipid classes under salt stress was comparable with increased ion contents in wheat and maize under salt stress (Mansour et al. 2002; Salama et al. 2007). This holds true in this study and that of Salama et al. (2011): increased non-lamellar structure forming lipids (this study) was associated with increased Na^+ and Cl^- (Salama et al. 2011) under NaCl stress. Further supporting for the crucial role of the PM lipid classes in salinity adaptation comes from the finding that increased PM PC and decreased PE and PI as a result of choline priming (this study) were related to declined Na^+ and Cl^- and increased K^+ in presence of NaCl (Salama et al. 2011). Maintaining (5 mM CC) or increasing (10 mM CC) PA level of the PM may be interpreted by the fact that PA is a biologically active lipid molecule playing a role as key signaling molecule in response to salinity (Munnik and Testerink 2009; Mansour et al. 2015). Taken together, choline-induced alterations in the PM phospholipid species observed here seems to be in a favorable direction to sustain ion homeostasis and hence improved adaptation to salinity.

As for membrane sterols, some sterol classes are planar (e.g., stigmasterol, campesterol, cholesterol) while others are less planar (e.g., β-sitosterol) (Mansour et al. 1994, 2002). Less planar sterols disrupt membrane packing resulting in increased permeability. Priming caryopsis with choline (5 mM) increased planar sterol (stigmasterol), and 10 mM choline increased, in addition to stigmasterol, both campesterol and a minor planar sterol, cholesterol. This change resulted in an increase in the ratio of more planar/less planar sterol species (β-sitosterol). A shift to more planar sterols might be advantageous in ion exclusion as demonstrated by Douglas and Walker (1983), and Mansour et al. (2002). Furthermore, planar sterols integrate more readily into the liquid lipid phase of the membranes than less planar sterols, and the latter thus allow higher Cl^- permeability (Douglas 1985; Douglas and Sykes 1985). In citrus, free sterols have been shown to regulate the degree of Cl^- exclusion, which essentially depend upon whether they are planar or less planar sterols (Douglas 1985). Similarly, NaCl increased Cl^- and Na^+ in the same sensitive wheat cultivar (Salama et al. 2011), which was consistently associated with increasing the relative distribution of less planar sterol species in the PM (this study). On the other hand, choline priming declined the toxic ion Na^+ and Cl^- (Salama et al. 2011), which is most likely attributable to increased more planar sterol classes (this study). It is important to note that free sterols are of great importance during salt stress because they can also regulate membrane enzyme activities (Ros et al. 1990; Grandmougin-Ferjani et al. 1997; Morales-Cedillo et al. 2015), membrane fluidity (Kerkeb et al. 2001; Morales-Cedillo et al. 2015), and thus greatly affecting membrane ion absorption and homeostasis.

In summary, choline priming-enhanced salt tolerance (Salama et al. 2011) of the same salt-sensitive wheat cultivar and under the same growth conditions as those adopted in the current work can be interpreted by the choline-induced alterations in the PM lipids. These alterations in the PM lipids in presence of NaCl stress obviously were in a favorable direction to maintain the PM stability, fluidity and hence ion homeostasis, which is of special importance in the adaptation to salinity. Although more cultivars should be studied in the future research for a general and final conclusion to be drawn, the study confirms and supports the crucial role of the PM in salt tolerance.

Author contribution statement KHAS and MMFM contributed equally to this work.

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