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[1]Department of Biology, College of Science, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia
[2]Basic and Applied Scientific Research Center, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

Up-modulation of membrane lipid composition and functionality by seed priming under salinity in the Hasawi rice variety

Nabil Ben Youssef[1,2,*], Reem Mohammed Shaher Al-Moaikal[1,2], Ghadah Hamad Saleh Al-Hawas[1,2], Farah Mustafa Alrammah[1,2]

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Summary

In this study, we investigated the role of seed priming in mitigating depressive effects of high salinity on membrane lipid composition and membrane functionality in seedlings of Hasawi rice cultivar (*Oryza sativa* L). Results indicated that seed pre-treatment with hydropriming (HP) and priming with 50 mM NaCl solution enhanced germination performance and early seedling growth under salinity condition. Priming treatments were found effective in maintaining membrane stability and integrity by increasing total membrane lipid content and reducing membrane damage. Concentrations of monogalactosyldiacylglycerol, digalactosyldiacylglycerol and phosphatidylglycerol were greatly increased when seeds were primed. Priming also increased phosphatidylcholine PC (lipid forming lamellar structure) and maintained phosphatidylethanolamine PE (non-bilayer-forming lipid) levels resulting in enhanced PC/PE ratio under salinity condition. Membrane unsaturation level was also increased, suggesting an improvement in membrane fluidity under salinity conditions. HP and NaCl-priming with 50 mM also induced increased contents of total phenolics, total soluble sugar and proline as compared to unprimed seedlings subjected to salinity. It is concluded that HP and priming with 50 mM NaCl solution can offer perspectives to improve germination and early growth of Hasawi rice under high salinity. This could be achieved through down-regulation of oxidative stress, accumulation of osmoprotectant compounds and improving cell membrane fluidity and integrity.

Keywords: Phosphatidylcholine, Linolenic acid, Peroxidation, Glycolipids, Osmoprtectants.

Introduction

Salinity is a common abiotic stress that affects agricultural production and quality. It has been estimated that one-fifth of total cultivated areas and one-third of irrigated agricultural lands are already salt-affected causing huge economic losses. Rice (*Oryza sativa* L.), belonging to the Poaceae family, is the most important cereal food crops in the world. Abiotic stresses alone are responsible for 50% of the total yield loss in rice crops and salinity and drought are the main hindrances to global rice production (Ghosh et al., 2016).

Plants can suffer direct negative impacts from salinity through osmotic effects and direct ion toxicity. Excessive salinity increases accumulation of reactive oxygen species (ROS), inflicting injury to biomolecules such as lipids, proteins and DNA which in turn result in negative effects on metabolism and cellular structures (Ibrahim, 2016). Biological membranes are generally regarded as one of the main sites of salt injury. Lipids as main components of cell membranes play a crucial role in controlling membrane integrity, fluidity and permeability. Salt-induced changes in the lipid composition of cell membrane were suggested to contribute to salinity adaptation (Guo et al., 2019). Physicochemical properties of membranes are strongly influenced by the fatty acid composition and a high level of membrane lipid unsaturation is assumed to maintain the membrane fluidity and functionality. Some changes in the fatty acid composition under stress conditions modulate the physiological properties of membranes to better deal with the environmental constraint (Guo et al., 2019).

The need to develop more salt-tolerant crops has been amplified intensely in recent decades due to increased salinity problems around the world. Many approaches have been applied to improve plant salt tolerance; some are labor and time-consuming (e.g., traditional breeding) and others are cost intensive as well as currently intolerable in many countries of the world (e.g genetically modify crops). As alternative, seed priming, which is easily applied and low-cost, is thought to be an effective technique to enhance germination performance under both optimal and adverse environments. It consists of a controlled seed hydration in a specific environment accomplished by the subsequent drying so that the germination processes begins, but radicle emergence is prevented. Several priming methods are currently used including hydro-priming, osmo-priming, chemical-priming, hormonal-priming, UV radiation priming, biological-priming and solid matrix priming (Thomas and Puthur, 2017; Masondo et al., 2018; Lingyun et al., 2019; Wang et al., 2019). However, these techniques have different properties, effectiveness, and required optimization for each crop species (Horii et al., 2007; Savvides et al., 2016).

Evaluating the effectiveness of priming on biological membrane stability and functionality under high salinity, a common condition in the Middle East, may represent an approach to deal with the environmental constraint and the problem of limiting production. Such studies were not done before with rice in the Saudi agricultural backgrounds conditions. Therefore, the current study aimed to examine the effects of hydropriming and NaCl-priming on the stability of membrane structure and function during early seedling development in Hasawi rice.

Materials and methods

Plant material and priming

'Hasawi' rice variety, used as seed material in our study, is a landrace adapted to the climate of eastern Saudi Arabia. Experiments were carried out in the Basic and Applied Scientific Research Center of Dammam (26°24'23.8" N, 50°05'04.2" E, 10 m). Seeds with initial moisture content of about 10% (dry weight basis) were surface sterilized with 0.5% sodium hypochlorite solution for 15 min and washed several times with distilled water to remove the traces of the disinfectant. Hydropriming (HP) using double distilled water and NaCl-priming with NaCl solution (P 50: 50 mM, P 100: 100 mM, P 150: 150 mM) were performed at 20 °C in darkness for 24 h. The seed to solution ratio was 1:4 (w/v). After priming, seeds were washed with distilled water for 2 min, surface-dried using blotting

* Corresponding author

paper and then dried back to their initial moisture contents at room temperature. An unprimed dry seeds were maintained as the control treatment for a comparison.

Seed germination

Rice primed (HP, P 50, P100 and P 150) and unprimed (UP) seeds were incubated in germination chamber set at 25 °C with a 14 h light/10 h dark photoperiod for 7 days in sterile plastic Petri dish (90 × 15 mm) containing two sterilized layers of filter paper moistened with distilled water and high salt solution (200 mM NaCl). Five replicates of 20 seeds per treatment were executed. Seeds were considered germinated when 2 mm length radicle protruded through the seed coat. Seven days after sowing, length and weight of seedlings (roots and shoots) were measured and seedlings were washed in distilled water and used for biochemical analysis. Germinated seeds were recorded daily for 7 days and at the end of experiment, the final germination percentage was calculated. Germination index (GI) was calculated according to the method established by the Association of Official Seed Analysis (1990). Mean germination time (MGT), and seedling vigour were estimated as described by NOORHOSSEINI et al. (2018).

Lipid and fatty acid analysis

Shoot and root samples were fixed in boiling water for 5 min to stop lipolytic activities. Total lipids were extracted in a chloroform/methanol/water (1/1/1, v/v/v) mixture (BLIGH and DYER, 1959). Lipid classes were separated by thin layer chromatography using the solvent system as described by LEPAGE (1967). After identification by comparison with standards, individual lipids were scraped and then methylated. Methyl esters of fatty acids were separated and quantified by gas chromatography using a Hewlett–Packard chromatograph (4890D) equipped with capillary column (Supelcowax-10: 30 m × 0.53 mm × 0.25 lm) and coupled to a flame ionization detector (FID) and injector. Fatty acid identification was carried out by comparison of their retention times with those of known standards. To quantify fatty acids, heptadecanoic acid (C17:0) was added as internal standard before methylation.

Lipid peroxidation

Lipid peroxidation was evaluated in germinated rice seedlings by malondialdehyde (MDA) quantification using the thiobarbituric acid (TBA) method. Samples were ground in a mortar with 5mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged for 5 min at 10 000 g. 250 µl supernatant was mixed with 1 ml of 0.5% TBA prepared in TCA solution (20%). The mixture was incubated at 95 °C for 30 min and quickly cooled in an ice bath. After centrifugation at 10,000 × g for 10 min, the absorbance of the supernatant was measured at 532 and 600 nm and the value for unspecific turbidity at 600 nm was subtracted. The MDA concentration was calculated using an extinction coefficient of 155 $mM^{-1}cm^{-1}$.

Electrolyte leakage

The Relative membrane leakage was estimated as electrolyte leakage and determined as described by GHOULAM et al. (2002). Fresh rice seedlings were excised and immersed in double distilled water at 25 °C for 24 h. Following incubation, initial electrical conductivity of solution (EC1) was measured. Then, samples were autoclaved at 120 °C for 20 min to release all electrolytes. After cooling, the final electrical conductivity (EC2) was measured and the electrolyte leakage (EL) was calculated using the formula:

EL = (EC1/EC2) × 100.

Proline content

Free proline content was estimated according to the method described by BATES et al. (1973). Samples of rice seedlings were homogenized in 3% aqueous sulfosalicylic acid and centrifuged at 6,000 rpm for 10 min. Equal volume of 2.5% ninhydrin solution and glacial acetic acid were added to the supernatant and incubated in water bath at 100 °C for 1 h. After cooling, toluene was added to the reaction mixture and vortexed. Absorbance of chromophore containing toluene was measured at 520 nm. Proline content was estimated by referring to a standard curve of L-proline.

Total phenolic content (TPC)

Total phenolic content (TPC) in rice seedling was estimated according to the Folin-Ciocalteu method. Phenolic compounds were isolated by methalonic extraction (80%). The Folin-Ciocalteau reagent was added to a suitable aliquot of the extracts, and the absorption of the mixture at 650 nm was measured. Total phenolic contents in samples were calculated from the calibration plot and expressed as mg galic acid equivalents per gram of sample.

Total soluble sugar contents

Soluble sugar contents were determined according to the anthrone colorimetric method. Samples of rice seedling were extracted in ethanol 80%. 3% Anthron reagent (Sigma Aldrich) was added to a suitable aliquot of the extracts. After incubation and the absorption of the mixture in boiling water bath for 1 min, the absorbance was measured at 630 nm. The content of soluble sugar was determined using glucose as standard curve.

Statistics

The experimental design was two factors factorial arranged in a completely randomized design. The first factor was seed priming (UP, HP, P 50, P 100 and P 150). Salinity was the second factor with 0 and 200 mM NaCl. Experiments on seed germination consisted of five replications of 20 seeds. Seven days after sowing, fresh material from each Petri dish were pooled to get a mean value and used as a replication for biochemical studies. Lipid peroxidation, electrolyte leakage, proline, total soluble sugar and total phenol were carried out in four replicates. Lipid and fatty acid analysis were conducted with three replicates. A two-way analysis of variance (ANOVA) was implemented using IBM SPSS Statistics (Version: 20). Data were presented as mean ± standard deviation (SD) and the significance of differences was evaluated by Duncan's multiple range test at $p = 0.05$.

Results and Discussion

Priming improves seed germination and early seedling growth under high salinity

Analysis of variance showed high significant effect of salinity on all studied germination parameters ($p < 0.001$) (Tab. 1). Severe salinity caused an approximately 42 and 71% reduction in FGP and GI, respectively and delayed germination as indicated by a 17.5% increase in MGT (Tab. 2). These results are in agreement with the findings on wheat (BAJWA et al., 2018). SHEREEN et al. (2011) reported that rice lines germinated after a delay of 3 days at higher salinity levels. Recently, ROY et al. (2019) revealed decreased germination of two high yielding rice varieties under salinity condition. In germination, imbibition is essential for the restoration of enzymatic activity and both salt induced-poor hydration and ion toxicity delay the imbibition and other physiological processes entail less or delayed germination. Further, statistical analysis points out towards the significant effects of priming and its interaction with salinity

Tab. 1: Two-way ANOVA analysis of priming, salinity, and their interactions for seed germination (FGP: final germination percentage, MGT: mean germination time, GI: germination index) and morphological parameters of rice seedlings (Shoot length, Root length, biomass and vigour index)

Parameter	Source of variance	d.f.	Mean square	F	Sig.
FGP	Salinity (S)	1	8450.000	348.454	0.000***
	Priming (P)	4	98.250	4.052	0.008**
	(S) × (P)	4	121.250	5.000	0.002**
	Error	40	24.250		
MGT	Salinity (S)	1	3.923	612.767	0.000***
	Priming (P)	4	0.412	64.321	0.000***
	(S) × (P)	4	0.100	15.545	0.000***
	Error	40	0.006		
GI	Salinity (S)	1	1576.278	3060.262	0.000***
	Priming (P)	4	52.099	101.148	0.000***
	(S) × (P)	4	4.210	8.173	0.000***
	Error	40	0.515		
Shoot length	Salinity (S)	1	198.403	5376.780	0.000***
	Priming (P)	4	0.552	14.951	0.000***
	(S) × (P)	4	0.663	17.959	0.000***
	Error	40	0.037		
Root length	Salinity (S)	1	265.882	1664.883	0.000***
	Priming (P)	4	0.340	2.128	0.095ns
	(S) × (P)	4	0.256	1.602	0.193ns
	Error	40	0.160		
Biomass	Salinity (S)	1	31100.180	3344.105	0.000***
	Priming (P)	4	44.680	4.804	0.003**
	(S) × (P)	4	195.380	21.009	0.000***
	Error	40	9.300		
SeVI	Salinity (S)	1	9938665.280	3232.360	0.000***
	Priming (P)	4	12485.350	4.061	0.007**
	(S) × (P)	4	13729.430	4.465	0.004**
	Error	40	3074.740		

*, **, *** and ns denote significance at $P < 0.05, 0.01, 0.001$ probability level and no significance, respectively

Tab. 2: Effect of priming treatments on the final germination percentage (FGP), mean germination time (MGT), germination index (GI), Shoot and root length,, biomass and seedling vigour index of rice seeds germinated under normal and high salinity condition.

Priming treatment	Salinity NaCl mM	FGP (%)	MGT (days)	GI	Shoot lenght (cm)	Root lenght (cm)	Biomass (mg)	SeVI
UP	0	99 a	5.17 a	18.98 a	5.12 a	5.3 a	96.6 a	1031.0 a
	200	63 b	6.07 b	5.77 b	0.78 b	0.48 b	32.2 b	79.4 b
HP	0	98 a	4.86 c	23.84 c	5.16 a	5.5 a	90.4 c	1045.6 a
	200	80 c	5.4 d	12.46 d	1.84 c	1.14 c	46.4 d	283.3 c
P 50	0	98 a	4.87 c	23.47 c	5.18 a	5.22 a	88.0 c	1019.9 a
	200	77 cd	5.42 d	12.04 de	1.64 c	1.02 bc	45.6 d	205.5 c
P 100	0	98 a	4.99 e	21.16 f	5.24 a	5.6 a	86.6 c	1062.8 a
	200	71 de	5.39 d	11.17 eg	0.96 b	0.64 bc	39.6 e	114.0 b
P 150	0	98 a	5.01 e	20.97 f	5.3 a	5.24 a	90.4 c	1033.4 a
	200	70 e	5.43 d	10.84 g	0.86 b	0.52 b	38.8 e	97.0 b

UP: unprimed, Hp: hydroprimed, OsP 50: osmoprimed with 50 mM NaCl, OsP 100: osmoprimed with 100 mM NaCl and OsP 150: osmoprimed with 150 mM NaCl. Means in a column with the same letter are not significantly different at $p < 0.05$ level according to Duncan's Multiple Range Test.

(priming × salinity) on FGT, MGT and GI (Tab. 1). Seed priming was found to be efficient in alleviating the salt-induced adversities on rice germination. Maximum effect was observed with hydropriming (HP) and NaCl-priming with 50 mM for which FGP are respectively 80 and 77% as compared to the umprimed seeds (63%) under salinity stress. Likewise, HP and P 50 induced more uniform and faster germination as indicated by increased GI and decreased MGT (Tab. 2). Results also showed that priming promoted rapid seedling emergence both under non-salt and salt solutions (Fig. 1). Primed seeds exhibited, under non-stress condition, higher cumulative germination percentage from the second to the third day of germination, but this difference was equalized to the late days of germination in comparison with controls. However, priming significantly accelerated seed germination under severe salinity condition since the number of days to first germination decreased and the germination percentage increased compared to unprimed seeds (Fig. 1). Better germination

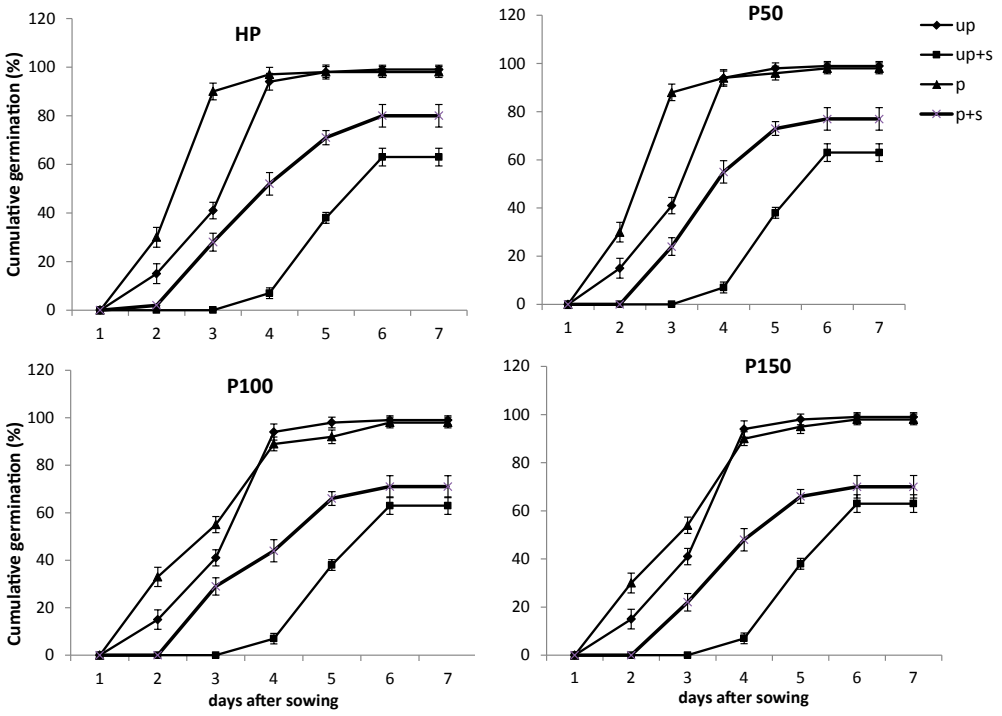

Fig. 1: Cumulative seed germination of unprimed and primed rice seeds germinated under high salinity. UP: unprimed seeds germinated under no salinity, UP+S: unprimed seeds germinated under salinity, P: primed seeds germinated under no salinity, P+S: primed seeds germinated under salinity. HP: hydropriming, P50: NaCl-priming with 50 mM NaCl, P100: NaCl-priming with 100 mM NaCl. P150: NaCl-priming with 150 mM NaCl.

performance of primed seeds might be due to priming stimulation of the pre-germination metabolic processes that makes seeds ready for radicle protrusion, repairs membranes and leaches emergence inhibitors (Balestrazzi et al., 2011). Moreover, a moderate abiotic stress generated by priming treatments during soaking step could be allowing the seeds to cope with environmental stresses during germination (Ibrahim, 2016).

Early seedling growth is a critical stage in the crop growing season and investigation in this phase is still the interest of several researches. Our results showed that salinity had highly significant effect ($p < 0.001$) on shoot length, root length, seedling biomass and seedling vigour index (Tab. 1). Priming significantly affected shoot length ($P < 0.001$), seedling biomass ($p < 0.05$) and seedling vigour index ($p < 0.05$). A significant two-way interaction (Priming and salinity) was found for shoot length ($p < 0.001$), seedling biomass ($p < 0.05$) and seedling vigour index ($p < 0.05$) (Tab. 1). In salinity condition, priming significantly reduced the salt negative impact. For shoot length, maximum effects were observed with HP and and P 50 for which reduction rate were about 64 and 68%, respectively compared to stressed control (84%). The same tendency was found in seedling biomass were salt-induced reduction was lower (about 48% for both HP and P 50) than that of stressed control (67%). Cell division and cell elongation require turgor pressure that could be reduced by salinity. This will, therefore, decrease the length of the shoots and roots, thus leading to stunted growth. As observed in germination parameters, priming treatments were effective in alleviating the negative impact of salinity on early seedling growth. HP and P 50 appear even more effective than P 100 and P 150 since they have led to significant enhancement in seedling growth as compared to stressed control (Tab. 2). These results are supported by Ahmadvand et al. (2012) who found that hydropriming and osmopriming with potassium nitrate improved early seedling growth of cotton under saline conditions. Also, similar findings were reported by Keshavarza and Moghadamb (2017) in *Phaseolus*. Subramanyam et al. (2019) stated that seed priming with sodium selenate improved growth in rice seedling under high salinity. Promoted early seedling growth by HP and P 50 under saline condition might be related to earlier emergence stimulated seedling and the enhancement of cell division and cell enlargement. Induction of antioxidative defense system and synthesis of osmoprotectants could also contribute to enhance germination performance and early seedling growth (Paul et al, 2017; Savvides et al, 2016).

Effect of priming on membrane lipids

Membrane lipids are dynamic compounds responsible for the major biological properties of cell membranes which are often considered as the first target of salt injury. Variations in membrane lipids are thought to be involved in the salinity adaptation process and might contribute to plant resistance. The present results indicate that total membrane lipids in roots and shoots were significantly affected by salinity ($P < 0.001$) and priming ($p < 0.01$) whereas the interaction between these two factors was only significant for lipid content in shoot ($p < 0.05$) (Tab. 3). The high salinity significantly decreased total membrane lipid content both in shoots and roots by 49 and 53% as compared to the respective controls (Fig. 2a, b). Several investigations have shown the depressive effect of salinity on membranes lipids (Chalbi et al., 2013; Mansour, 2013). The observed reduction of membrane lipids could result from salt-induced enhancement of lipolytic and peroxidative activities as well as inhibition lipid biosynthesis pathways (Guo et al., 2019). Our findings confirm these observations and showed, under high salinity condition, enhanced membrane damage. The extent of membrane injury could be assessed by an indirect measurement of electrolyte leakage and lipid peroxidation evaluated by the MDA accumulation. These two parameters were significantly affected by salinity and priming and were significantly increased in salt-stressed seedlings as compared with controls (Tab. 3; Fig. 3a, b). This may reflect the high level of membrane

Tab. 3: Two-way ANOVA analysis of priming, salinity, and their interactions for the biochemical attributes (electrical conductivity, lipid peroxidation, proline content, total phenol, soluble sugar and lipids) of rice seedlings under salinity.

Parameter	Source of variance	d.f.	Mean square	F	Sig.
MDA	Salinity (S)	1	2155.616	709.707	0.000***
	Priming (P)	4	87.544	28.823	0.000***
	(S) × (P)	4	75.086	24.721	0.000***
	Error	20	3.037		
Electrolyte leakage	Salinity (S)	1	9249.036	199.571	0.000***
	Priming (P)	4	186.301	4.020	0.015*
	(S) × (P)	4	187.081	4.037	0.015*
	Error	20	46.345		
Proline	Salinity (S)	1	73408.533	981.398	0.000***
	Priming (P)	4	1411.533	18.871	0.000***
	(S) × (P)	4	536.533	7.173	0.001**
	Error	20	74.800		
Total phenol	Salinity (S)	1	0.727	21.731	0.000***
	Priming (P)	4	0.192	5,739	0.003**
	(S) × (P)	4	0.053	1.575	0.219ns
	Error	20	0.033		
soluble sugar	Salinity (S)	1	789.507	310.666	0.000***
	Priming (P)	4	28.473	11.204	0.000***
	(S) × (P)	4	17.582	6.918	0.001**
	Error	20	2.541		
Root lipids	Salinity (S)	1	117.019	199.713	0.000***
	Priming (P)	4	1.938	3.308	0.031*
	(S) × (P)	4	0.689	1.177	0.351ns
	Error	20	0.586		
Shoot lipids	Salinity (S)	1	282.072	316.147	0.000***
	Priming (P)	4	5.481	6.143	0.002**
	(S) × (P)	4	2.678	3.001	0.043*
	Error	20	0.892		

*, **, *** and ns denote significance at $P < 0.05, 0.01, 0.001$ probability level and no significance, respectively

injury as a result of oxidative damage. Additionally, the observed increased lipid peroxidation could result from the salt-induced overproduction of ROS causing peroxidation of unsaturated fatty acids in the biological membranes. This in turn leads to disruption of the physicochemical membrane properties and consequently declined seedling growth (Ibrahim, 2016). Similar results were found by Chalbi et al. (2013) who showed that electrolyte leakage and MDA accumulation were negatively correlated with membrane integrity under salinity conditions in *Hordeum vulgare*.

Our study showed that priming treatments were effective in decreasing salt induced membrane damage, since total lipid contents were significantly increased in both seedling shoots and roots raised from primed seeds as compared to stressed control. Hence, 40% and 23% increase in lipid content were observed in shoot seedlings from rice seeds treated with HP and P 50 respectively, as compared to stressed control (Fig. 2a). Similarly, about 40% increase was seen in root seedlings raised from rice seeds treated with HP as compared to stressed control (Fig. 2b). Such variations could be expected to promote maintenance of membrane integrity and cellular homeostasis in salt conditions and hence postulating that lipids might be involved in the protection against salinity. This finding was supported by the significant priming-induced decrease in electrolyte leakage and lipid peroxidation observed in stressed seedlings compared to the stressed controls (Fig. 3a, b). Results showed again that HP and P 50 were more effective than P 100 and P 150 in terms of reducing membrane damage. Hence, increases in electrolyte leakage are about 98 and 82% in seedlings from primed seeds with H_2O and 50 mM NaCl respectively while in stressed control the increase was more marked (175%). Furthermore, as much as 139% and 88% lesser MDA accumulation can be observed in seedlings from rice seeds treated with HP and P 50 respectively, as compared to the stressed control. These improvements may be related to the induction of antioxidant responses which provide protection against oxidative damage (Paul et al., 2017). Reorganization of cellular membrane and maintenance of membrane integrity during priming would be responsible for the observed reduction in electrolyte leakage. Our observations are well supported by several other reports where hydropriming and osmopriming significantly reduced membrane damage and the MDA concentration in salt-stressed *Cucumis melo* and *Glycine max* (Farhoudi et al., 2011; Yan Dai et al., 2017).

Shoot membrane lipids are mainly composed of monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), and phosphatidylcholine (PC), followed by phosphatidylethanolamine (PE), and phosphatidylglycerol (PG) and a small amount of phosphatidylinositol (PI) and Sulfoquinovosyldiacylglycerol (SQDG) (Tab. 4). On exposure to high salinity, all lipid classes showed a significant decrease which was dramatic in chloroplast lipids (MGDG, DGDG and PG) as compared to major phospholipids (PC and PE). These changes may negatively affect photosystem stability and activity leading to photosynthesis inhibition (Liu et al., 2018). Our findings are in line with those obtained by Liu et al. (2018) and Bejaoui et al. (2016) who revealed a sharped decrease in glycolipids under salinity conditions. Salt treatment decreased lipids forming lamellar structure (PC) against a stability of non-bilayer-forming lipids (PE) leading to the reduction of the PC to PE ratio. Such modifications impair the cell membrane proprieties and activities (Salama and

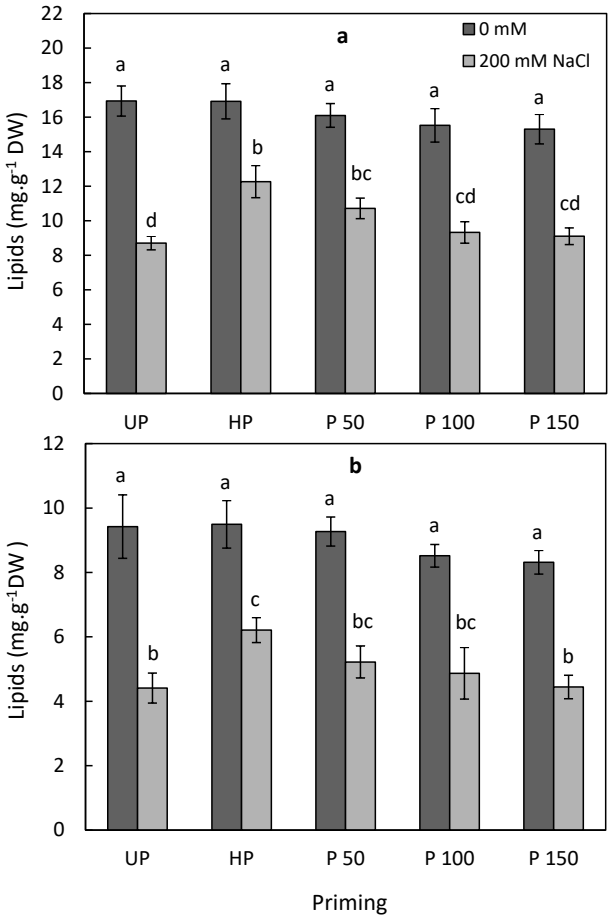

Fig. 2: Membrane lipid content in shoots (a) and roots (b) of early rice seedlings from unprimed and primed seeds grown under normal and high salinity. UP: unpriming, Hp: hydropriming, P 50: priming with 50 mM NaCl, P 100: priming with 100 mM NaCl and P 150: priming with 150 mM NaCl. Bars with different letters are significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

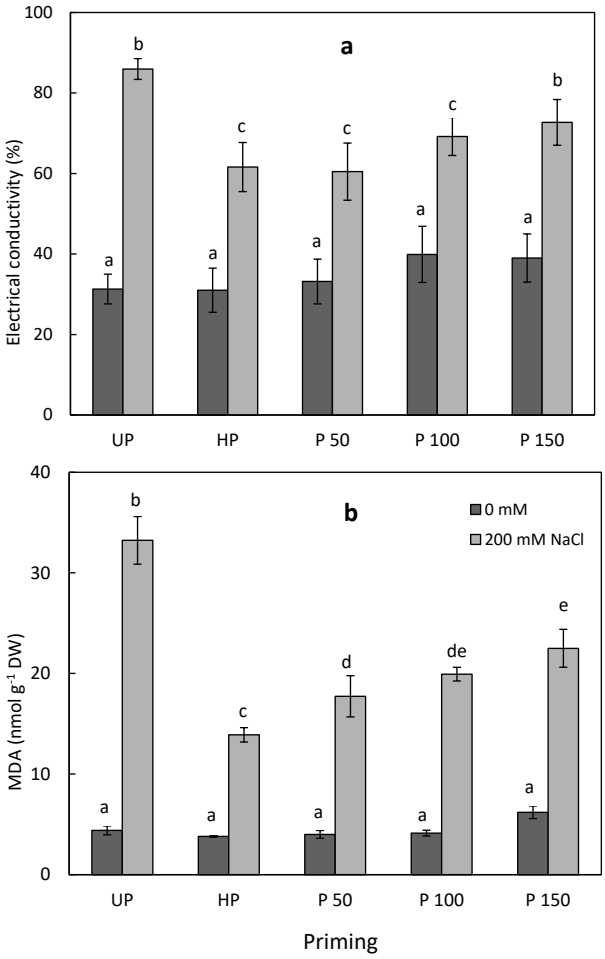

Fig. 3: Electrical leakage (a) and MDA content (b) of early rice seedlings from unprimed and primed seeds grown under normal and high salinity. UP: unpriming, Hp: hydropriming, P 50: priming with 50 mM NaCl, P 100: priming with 100 mM NaCl and P 150: priming with 150 mM NaCl. Bars with different letters are significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

Mansour, 2015). Under salinity stress, concentrations of MGDG, DGDG and PG were greatly increased in seedling shoot raised from primed seeds (especially HP and P 50), as compared with stressed controls (Tab. 4). Such changes could contribute to improving the chloroplast membrane stability and functionality under saline conditions (Liu et al., 2018). However, MGDG to DGDG ratio remained unchanged suggesting the upkeep of a relative functionality, even with a reduced membrane structure under salinity (Tab. 4). Priming also increased bilayer-forming lipids (PC) and maintained PE level resulting in enhanced PC/PE ratio under salinity condition (Tab. 4). These results argue for improved membrane stability and functionality required in salt acclimation (Salama and Mansour, 2015).

Physicochemical properties of cell membranes are also determined by the fatty acyl composition of membrane lipids. Modifications in fatty acid composition are believed to affect membrane fluidity, permeability, and cellular metabolic functions and therefore might be strongly considered in the stress resistance process. Our results showed that membrane lipids in untreated shoots had a high level of unsaturated fatty acids (UFAs) with 75% of total fatty acids while saturated fatty acids (SFAs) accounted only for about 25% (Tab. 5). Linolenic acid, (C18:3) was the most abundant fatty acid followed by linoleic (C18:2) and stearic (C16:0) acids which have relatively equal proportions. Hexadecenoic (C16:1), stearic (C18:0) and oleic (C18:1) acids are faintly represented. The high salinity induced a significant decrease in membrane unsaturation level. This was indicated by the reduced double-bond index and unsaturated-to-saturated fatty acid ratio due to salt-induced decrease in polyunsaturated fatty acid C18:3 in favour of C18:1 and C16:0 (Tab. 5). Saturated lipids generate liquid-order phases while unsaturated lipids lead to liquid-disordered phases, thereby the presence of fatty acid residues and their state of saturation can directly affect membrane fluidity (Guo et al., 2019). Hence, decreasing membrane fatty acid unsaturation and unsaturated-to-saturated fatty acid ratio could induce a phase separation in the cell membrane thereby affecting its fluidity and permeability (Mansour, 2013; Guo et al., 2019). Results revealed that priming treatments increased membrane unsaturation level of stressed seedling shoots as indicated by the enhancement of the double-bond index and unsaturated-to-saturated fatty acid ratio in comparison with the stressed controls (Tab. 5). Such findings suggest a possible priming-induced stimulation of desaturase activity leading accordingly to an improving in membrane fluidity under saline conditions.

Priming elicits accumulation of proline, soluble sugar and total phenol

Proline accumulation is recognized as a common metabolic reaction of plants exposed to abiotic stress (Ibrahim, 2016). Our results supported this observation since it was significantly affected by salin-

Tab. 4: Lipid classes in shoot seedlings from unprimed and primed rice seeds grown under normal and high salinity.

Priming treatment	Salinity NaCl mM	MGDG	DGDG	PC	PE	PG	PI	SQDG	PC/PE	DGDG/ MGDG
UP	0	6.04 a	2.88 a	3.41 a	1.86 ab	1.49 a	0.87 a	0.37 a	1.83 a	2.1 ab
	200	2.4 g	1.19 e	2.35 e	1.87 ab	0.44 f	0.32 e	0.14 e	1.26 b	2.02 ab
HP	0	6.01 a	2.85 a	3.45 a	1.86 ab	1.5 a	0.84 a	0.39 a	1.85 a	2.11 ab
	200	3.85 d	2.04 c	2.9 c	1.77 b	0.97 d	0.49 cd	0.26 b	1.61 ab	1.89 a
P 50	0	5.73 b	2.73 a	3.19 b	1.85 ab	1.42 ab	0.8 a	0.37a	1.72 a	2.1 ab
	200	3.40 e	1.66 d	2.58 d	1.55 c	0.86 d	0.45 de	0.25 bc	1.66 ab	2.05 ab
P 100	0	5.56 bc	2.52 b	3.15 b	1.9 ab	1.35 bc	0.69 b	0.36 a	1.65 ab	2.21 ab
	200	2.79 f	1.25 e	2.53 de	1.58 c	0.58 e	0.38 de	0.20 cd	1.60 ab	2.34 b
P 150	0	5.43 c	2.47 b	3.18 b	1.97 a	1.28 c	0.61 bc	0.37 a	1.61 ab	2.2 ab
	200	2.54 fg	1.56 e	2.54 de	1.55 c	0.6 e	0.36 e	0.36 de	1.63 ab	2.05 ab

UP: unpriming, Hp: hydropriming, P 50: priming with 50 mM NaCl, P 100: priming with 100 mM NaCl and P 150: priming with 150 mM NaCl. MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SQDG, Sulfoquinovosyldiacylglycerol; PC/PE, ratio of PC to PE; DGDG/MGDG, ratio DGDG to MGDG. Means in a column with the same letter are not significantly different at p<0.05 level according to Duncan's Multiple Range Test.

Tab. 5: Fatty acid composition of total membrane lipids in shoot seedlings from unprimed and primed rice seeds grown under normal and high salinity.

Fatty acids									
Priming	Salinity NaCl mM	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	DBI $	UFAs : SFAs*
UP	0	21.53 ab	1.12 ab	1.77 a	1.89 a	21.52 a	52.17 a	202.6 a	5.33 a
	200	25.06 c	1.44 ab	1.60 ab	4.23 b	22.91 a	44.73 b	185.7 b	4.53 b
HP	0	21.32 ab	1.22 ab	2.05 abc	2.29 ac	21.93 a	51.20 ac	200.9 a	5.64 ac
	200	23.05 abc	0.95 a	2.15 bcd	3 abc	22.06 a	48.8 abc	194.5 ab	5.39 a
P 50	0	20.82 a	1.61 b	1.85 ab	2.02 ac	22.28 a	51.43 ac	202.5 a	5.56 ac
	200	23.15 abc	1.44 ab	2.02 abc	3.48 abc	22.88 a	46.89 bc	191.4 ab	5.24 a
P 100	0	20.88 ab	1.02 ab	2.48 d	2.25 abc	23.28 a	50.1 abc	200.1 a	6.15 c
	200	23.03 abc	1.18 ab	2.45 cd	3.41 bc	23.02 a	46.9 bc	191.2 ab	5.68 ac
P 150	0	21.75 ab	1.04 a	2.07 bc	2.12 ac	22.85 a	50.17 ac	199.4 ab	5.57 ac
	200	23.55 bc	1.35 ab	2.16 bcd	3.38 bc	22.85 a	46.7 bc	190.6 ab	5.31 a

UP: unpriming, Hp: hydropriming, P 50: priming with 50 mM NaCl, P 100: priming with 100 mM NaCl and P 150: priming with 150 mM NaCl.
$DBI, double-bond index = ∑(unsaturated fatty acid × number of double bonds), * UFAs : SFAs, unsaturated–to–saturated fatty acid ratio.
Means in a column with the same letter are not significantly different at p<0.05 level according to Duncan's Multiple Range Test.

ity ($P < 0.001$), priming ($p < 0.001$) and their interaction ($p < 0.05$) (Tab. 3). Salinity stress significantly increased proline concentrations in unprimed seedlings when compared to controls (Fig. 4a). Such findings were corroborated with previous studies on different species (Farhoudi et al., 2011; Espanany et al., 2016). Interestingly, priming induced further accumulation of proline in early seedlings exposed to high salinity. HP and P 50 appeared once again as the most effective priming treatments as they led to the largest increases under high salinity (Fig. 4a). These results are in agreement with those of Farhoudi et al. (2011) who showed that seed-NaCl priming enhanced proline accumulation in muskmelon seedling under salinity. Kubala et al. (2015) reported that the improved germination performance of osmoprimed rape seeds was accompanied by a significant increase in proline content under salinity stress. In addition to its contribution to osmotic adjustment, proline acts as an antioxidant and a stabilizer of subcellular structures and macromolecules under stress condition (Hayat et al., 2012; Kubala et al., 2015). Therefore, the observed increase in proline content in early rice seedlings may provide protection and stability to macromolecules and may reflect the improvement salinity resistance.

Total phenol content was significantly affected by salinity ($P < 0.001$) and priming ($p < 0.05$) whereas the interaction between these two factors was insignificant (Tab. 3). Our results revealed enhanced accumulation of total phenols in rice seedlings under high salinity condition. However, as observed in proline content, seed pre-treatment by hydropriming induced further accumulation in total phenolics (Fig. 4b). Several studies have showed increased accumulation in phenolics under various biotic and abiotic stresses (Moulick et al., 2016; Farooq et al., 2017). Du et al. (2019) reported that rice seed priming with sodium selenate induced higher phenol content in seedlings. The protective role of phenolics in plants is renowned by their aromatic structure which stabilizes biomembranes during stress and scavenges ROS in cells (Taiz et al., 2015). Thus, our results suggest that seed pre-treatment, particularly hydropriming, could contribute to membrane stability and antioxidative protection in early seedling development through stimulation of phenol synthesis.

Sugar are crucial for germination since it is involved in regulating metabolic activities as well as providing carbon source for young seedling growth and contributing to the osmoregulation required for the growth during germination under salinity. As indicated in Tab. 3, total soluble sugar was significantly affected by salinity ($P < 0.001$), priming ($p < 0.001$) and their interaction ($p < 0.05$). Sever salinity considerably reduced soluble sugar content as compared to control (Fig. 4c). Previous studies revealed both increase and decrease in sugar content depending on genotypes and stress intensity (Paul et al., 2017; Bajwa et al., 2018). Our data also indicated that all

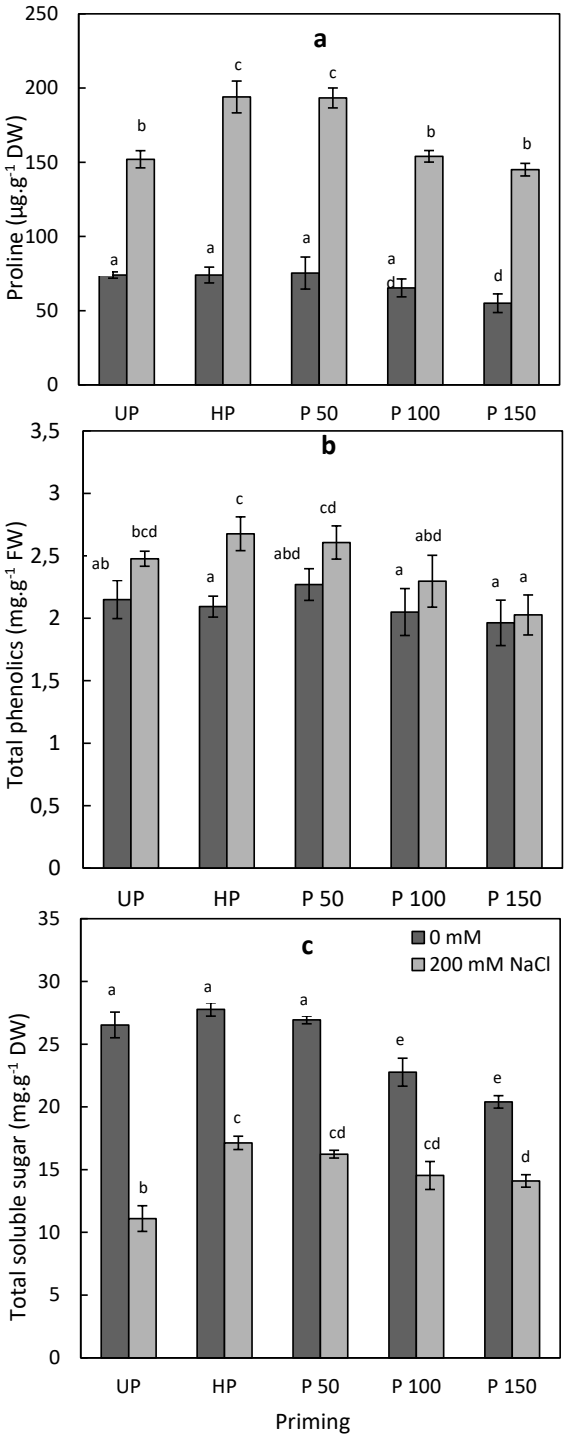

Fig. 4: Proline (a), total phenolic (b) and soluble sugar contents (c) of rice seedlings from unprimed and primed seeds grown under normal and high salinity. UP: unpriming, Hp: hydropriming, P 50: priming with 50 mM NaCl, P 100: priming with 100 mM NaCl and P 150: priming with 150 mM NaCl. Bars with different letters are significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

priming treatments increased total soluble sugars as compared to stressed control and HP and P 50 were obviously the most effective treatments. The results are in line with the findings of Bajwa et al. (2018) in wheat and Wang et al. (2019) in cotton. The priming-induced increase in soluble sugars probably reduced the salt-caused damages and enhanced the resistance of rice to salinity.

Conclusion

The present work showed that high salinity causes severe damage during the germination phase and early seedling growth of Hasawi rice. Seed pre-treatment with hydropriming and NaCl-priming with 50 mM has been revealed to significantly alleviate the harmful effect of salinity. Thus, primed seeds became could manage salinity with a better ability to maintain cell membrane integrity and fluidity. In addition, accumulation of phenols and osmoprotectants may reflect a more activated antioxidant system, thus reducing the oxidative stress generated by salinity. These resulted in lower levels of MDA and preserve the integrity and stability of biological membranes. On the basis of our experimental data, seed pre-treatment with HP and P 50 reduced the lethal effect of NaCl leading to the improvement of germination performance and early seedling growth of Hasawi rice. This could be promising for the exploitation of marginalized lands affected by salt.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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ORCID
Nabil Ben Youssef https://orcid.org/0000-0003-2597-8067

Address of the corresponding author:
Nabil Ben Youssef, Imam Abdulrahman Bin Faisal University, P.O Box 1982, 31441, Dammam, Saudi Arabia
E-mail: nabilbtn@gmail.com