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SALT STRESS AFFECTS THE AGRONOMIC TRAITS, PHYTIC ACID, AND AROMA OF RICE (*Oryza sativa* L.) M1 MUTANT LINES

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SUMMARY

Salinity is one of the main abiotic stresses affecting yield stability and rice production. In our previous study, the M1 lines of two rice cultivars, namely, HATRI-192 (G1) and HATRI-62 (G2), were developed by using different concentrations (0.1, 0.5, and 1.0 mM) of methyl salicylate (MeSA). This study aimed to evaluate the effects of different levels of salt stresses on the agronomic traits, phytic acid content, and aroma of M1 mutant lines developed from HATRI-192 and HATRI-62. A total of 250 M1 seeds of both rice cultivars were germinated and screened for salinity at various NaCl levels (6, 8, 12, 15, and 17 dS/m) at the seedling stage and later evaluated for agronomic traits, phytic acid content, and aroma at the reproductive stage. M1G1-12, the M1 line of cultivar HATRI-192 treated with 0.5 mM MeSA, had the highest filled grain per panicle and grain yield per plant and an aromatic score of 1 at 12 dS/m as compared with the control. M1G2-8, the M1 plant of HATRI-62 treated with 0.1 mM MeSA, had the highest plant height (96 cm) and grain yield per plant (35.9 g) at 8 dS/m. The M1 seeds of cultivar HATRI-192 treated with 0.1 mM MeSA and those of cultivar HATRI-62 treated with 0.5 mM MeSA had low phytic acid contents at 15 dS/m. Overall, 6 and 13 M1 lines of cultivars HATRI-192 and HATRI-62, respectively, had aromatic scores of 1 to 2 in terms of phenotypes. Results revealed that M1 lines under salinity conditions had higher values for grain yield and its components, phytic acid content, and aroma than their parental cultivars. These pieces of relevant information can be utilized to develop salt-tolerant rice cultivars with high yielding potential and good quality.

Keywords: Rice mutant lines, methyl salicylate, salt stresses, grain yield, yield-related traits, phytic acid content, aroma

Key findings: Methyl salicylate (MeSA) was successfully used to generate the M1 mutant rice lines of two rice cultivars, i.e., HATRI-192 and HATRI-62, which were evaluated under different salinity stress conditions. Results revealed that the rice

cultivars HATRI-192 and HATRI-62 treated with 0.1 and 0.5 mM MeSA were less affected by salinity and showed adaptability to saline environments as reflected by their traits of filled grains per panicle, reduced percentage of unfilled grains per panicle, grain yield, phytic acid content, and aroma.

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INTRODUCTION

Rice (*Oryza sativa* L.) is the one of essential staple foods in the world. Rice production is limited by many environmental factors, such as heat, drought, cold, salt, and submergence. It is currently considered as one of the most susceptible cereals to salinity, with the majority of cultivated rice cultivars showing an electrical conductivity (EC) threshold of 3 dS/m (Bahmani *et al*., 2015). Therefore, soil with EC > 4 dS/m is considered as saline (Rengasamy, 2006). Specifically, at EC < 3.5 dS/m, losses of 10% occur in yield, and 50% yield losses are observed at EC 6 > dS/m (Zeng *et al*., 2001). Asia produces and consumes approximately 90% of rice in the world (Virmani *et al*., 2008). However, due to current climate change, rising sea levels have increased salinity levels in rice-growing areas in Asian plains, including the Mekong Delta in Vietnam (Dayton, 2014). Despite some successes in increasing the salt tolerance of rice, overall achievements are modest in the face of current saline intrusions. Therefore, the development of new rice cultivars with salt tolerance and increased yield potential under salt stress conditions is urgently needed for sustainable high rice production in the future.

Aromatic rice is a special small group that is highly priced owing to its excellent cooking quality and aroma. Most aromatic rice cultivars have long growth durations and low yields and are susceptible to biotic and abiotic stresses. Many studies reported changes in aroma when rice genotypes are grown in salt-affected areas (Gay *et al*., 2010). Therefore, further extensive research in a salt-stressful environment is required for improving rice yield and aroma to cope with the food demand of the increasing population worldwide.

Breeding programs mainly focus on increasing the productivity of crops. However, improving grain quality traits requires specific emphasis. Phytic acid (myo-inositol 1,2,3,4,5,6-hexakisphosphate-IP 6) content has been ignored during the past few decades. Phytic acid is the main storage compound of phosphorus in crop plants. Typically, in cereal grains, excess phytic acid content can strongly absorb metal ions and reduce the ability of humans and animals to consume minerals, i.e., iron, zinc, calcium, and magnesium (Ha and Xuan, 2018). Phytic acid impairs the absorption of iron, zinc, and calcium in the human body and promotes mineral deficiencies. Phytic acid content in seeds is essentially affected by genetic and environmental factors (Liu, 2005), which include climatic conditions; crop management and fertilizer application practices; and soil features, such as soil physical, chemical, and biological factors (Kaya *et al*., 2009; Brankovic *et al*., 2015; Dhole and Reddy, 2015). Some recent studies have revealed enhancements in the genetic potential of rice genotypes for increased grain yield and reduced phytic acid content (Liu, 2005; Lang *et al*., 2007; Brankovic *et al*., 2015; Dhole and Reddy, 2015; Ha and Xuan, 2018).

Improved rice productivity in salt-affected areas can be used as a basis for anticipating the increasing rice demand in Vietnam. Conventional natural selection methods for salinity tolerance are difficult because of their large associated environmental effects and genetic mechanisms (Gregorio, 1997). The use of mutagenesis to improve abiotic stress tolerance has recently been successfully employed in crops (Gaswanto *et al*., 2016; Kask *et al*., 2016; Yusuff *et al*., 2016; Liu *et al*., 2018).

Methyl salicylate (MeSA) is a volatile plant secondary metabolite that is synthesized from salicylic acid (SA) (Kalaivani *et al*., 2016). MeSA and SA protect plants directly and indirectly and are major compounds of the shikimic acid pathway (Dicke and Hilker, 2003). SA is extensively studied due to its important role as a signaling molecule in the expression of many plant stress resistance mechanisms (Khan *et al*., 2003). SA has been used to induce the expression of alternative oxidase and to increase antioxidant activities in cells (Poór, 2020). When the reactions of proline, antioxidants, heat shock proteins, secondary metabolites, and sugars are increased, SA reduces plant stress (Amal *et al*., 2007). SA confers plant tolerance against stresses, such as salt, heavy metals, drought, cold, heat, and ultraviolet rays (Ahmad *et al*., 2019).

Salinity stress is one of the main stresses that significantly reduce crop yields. Yan *et al*. (2018) suggested that the application of SA (0.5–1.5 mM) decreases salt stress by enhancing the activity of reactive oxygen species, ascorbate peroxidase, and glutathione reductase in *Nitraria tangutorum* at seedling stage. Endogenous signaling molecules, such as SA and MeSA, play a key role in regulating plant responses to environmental stress (Hasanuzzaman *et al*., 2019). Treatment with SA and MeSA is inexpensive, simple, and can be applied to many crops (Asghari and Aghdam, 2010). AtMES7-encoded and AtMES9-encoded proteins display high MeSA esterase activity (Park *et al*., 2009), and past findings have revealed the pathway for SA signaling in crop plants (Loake and Grant, 2007).

Moreover, several studies have concentrated on the relationship between exogenous MeSA doses in stressed plants. The use of MeSA for mutagenesis affects the mechanisms of MeSA conversion into SA such that plants can increase their tolerance to salinity, coldness, and disease resistance (Ding *et al*., 2002). MeSA has been applied in mutation breeding as a chemical mutagen to improve tolerance to salt stress in rice (Ha *et al*., 2020) and heat stress in *Brassica nigra* (Kask *et al*., 2016). Ha *et al*. (2019) utilized MeSA to improve salt tolerance in rice at the germination stage and in mustard at the seedling stage. However, information about the effects of MeSA on rice salt tolerance at the reproductive stage is limited.

Therefore, this study aimed to evaluate rice M1 mutant lines developed from two rice cultivars via MeSA treatment for yield-related traits and grain yield, phytic acid content, and aroma under different salinity levels at the reproductive stage. The identified and selected salt-tolerant rice M1 lines were grown to produce M2 populations and further used as a base genetic material for improving the salt tolerance, yield, and quality traits of rice.

MATERIALS AND METHODS

Plant materials and procedures

Plant material was obtained from the laboratory of Genetics and Plant Breeding, Genomic Research Institute and Seed, Ton Duc Thang University, Vietnam. The first-generation M1 mutant lines were created from two rice cultivars, namely, HATRI-192 and HATRI-62, by using MeSA with various concentrations (0.1, 0.5, and 1.0 mM) (Ha *et al*., 2020). Rice seeds that were not treated with MeSA were considered as control-1; those treated with 0.1 mM MeSA were control-2; those treated with 0.5 mM MeSA were control-3; and those treated with 1 mM MeSA were control-4. Nonsalinity stress was used for experiment. In this study, the seeds of 250 M1 mutant lines of each rice cultivar were germinated and screened for salinity at various NaCl levels (6, 8, 12, and 17 dS/m) at the seedling stage. Each rice seedling was coded as M1G1 and M1G2 to indicate the M1 lines of cultivars HATRI-192 (G1) and HATRI-62 (G2), respectively. Subsequently, these lines were recovered and transferred to normal conditions. During the flowering of the rice lines, salinity treatments were carried out at different concentrations (6, 8, 12, 15, and 17 dS/m) until harvest. M1 lines with salt tolerance were selected from the plant population under different salinity levels at the reproductive stage (Table 1).

Data recorded

Data for plant height, panicle length, tiller per plant, filled grains per panicle, unfilled grains per panicle, 1000-grain weight, and grain yield per plant were recorded. Phytic acid content and aroma analyses were conducted as follows.

Phytic acid content

Phytic acid content was estimated in accordance with Ha and Xuan (2018) as modified from Chen's reagent method (Chen *et al*., 1956). Three seeds from each treatment were dried and ground into a fine powder. Each sample was mixed with 2 mL of HCl 0.4 M and kept overnight at 4 °C. Next, 100 µL of the sample was removed from the test tube and was added to 900 µL of distilled water and 1 mL of Chen's reagent (H_2SO_4:ammonium molybdate: ascorbic acid:H_2O at a ratio of 1:1:1:2). Subsequently, the mixture was mixed in a shaker and incubated for 1.5 h at 37 °C in the dark. Finally, the optical density (OD) of the samples was measured at 820 nm by using a spectrophotometer. A calibration curve for determining phytic acid content was prepared by using inorganic phosphate (KH_2PO_4, 1 mM) with volumes of 50, 100, 150, 200, and 250 µL.

Aroma analysis

The leaves of the M1 lines of both rice cultivars were cut into small pieces (approximately 5 mm) and kept in test tubes. Subsequently, 5 mL of 1.7% KOH solution was added into the test

Table 1. Salt-tolerant rice M1 lines exposed to 6, 12, 15, and 17 dS/m NaCl at the reproductive stage.

Treatments	M1 lines	MeSA doses
Control (HATRI-192)		0-MeSA
		0.1-MeSA
		0.5-MeSA
		1-MeSA
6 dS/m	M1G1-1	0-MeSA
	M1G1-2	0.1-MeSA
	M1G1-3	0.5-MeSA
	M1G1-4	1-MeSA
	M1G1-5	1-MeSA
8 dS/m	M1G1-6	0-MeSA
	M1G1-7	0.1-MeSA
	M1G1-8	0.5-MeSA
	M1G1-9	1-MeSA
12 dS/m	M1G1-10	0-MeSA
	M1G1-11	0.1-MeSA
	M1G1-12	0.5-MeSA
	M1G1-13	1-MeSA
15 dS/m	M1G1-14	0-MeSA
17 dS/m	M1G1-15	0-MeSA
	M1G1-16	0.1-MeSA
	M1G1-17	0.5-MeSA
	M1G1-18	1-MeSA

Treatments	M1 lines	MeSA doses
Control - HATRI-62		0-MeSA
		0.1-MeSA
		0.1-MeSA
		0.5-MeSA
		1-MeSA
6 dS/m	M1G2-1	0-MeSA
	M1G2-2	0.1- MeSA
	M1G2-3	0.5-MeSA
	M1G2-4	0.5-MeSA
	M1G2-5	1-MeSA
	M1G2-6	1-MeSA
8 dS/m	M1G2-7	0-MeSA
	M1G2-8	0.1-MeSA
	M1G2-9	0.5-MeSA
	M1G2-10	1-MeSA
12 dS/m	M1G2-11	0-MeSA
	M1G2-12	0.1-MeSA
	M1G2-13	0.5-MeSA
	M1G2-14	1-MeSA
15 dS/m	M1G2-15	0-MeSA
	M1G2-16	0.1-MeSA
	M1G2-17	0.5-MeSA
	M1G2-18	1-MeSA
17 dS/m	M1G2-19	0.1-MeSA
	M1G2-20	0.5-MeSA
	M1G2-21	1-MeSA

tubes, which were then incubated at 50 °C for 25 min. The solutions were assessed by using a smelling method consisting of three levels: nonaromatic (0), slightly aromatic (1), and very aromatic (2) (Lang, 2002).

Data analysis

Data were displayed as the mean ± standard deviation of at least triplicate measurements. Means were separated by using Tukey's least significant difference (LSD) test at $P \leq 0.05$. Data on phytic acid content were analyzed by using the SPSS 20.0 program (SPSS Inc., Chicago, IL, USA) for testing the significance of NaCl treatments.

RESULTS AND DISCUSSION

Phenotyping evaluation

The purpose of this study was to evaluate the effects of salinity levels on the agronomic traits, phytic acid content, and aroma of the M1 populations of two rice cultivars at the reproductive stage compared with those of control treatments. This study will also support future breeding programs. Therefore, the desirable M1 rice lines were selected and evaluated for yield components, phytic acid content, and aroma at the reproductive stage after exposure to different salinity levels at the seedling stage and compared with control plants.

In this study, significant variations were observed among 22 M1 individuals of HATRI-192 and 26 M1 individuals of HATRI-62, including control lines for yield and yield-related traits (Table 1). Under salinized and nonsalinized conditions, cultivars HATRI-192 and HATRI-62 treated with the MeSA mutagen showed reduced plant height as compared with the controls (Tables 2 and 3). Overall, all the MeSA treatments resulted in reduced plant height under different salinity conditions. A salt tolerance test was applied to screen the rice populations for salt tolerance during the reproductive stage. Our previous study demonstrated that rice treated with MeSA show increased shoot length when exposed to 6 and 8 dS/m NaCl at the seedling stage (Ha *et al*., 2020). However, some other past findings revealed that rice seeds treated with MeSA present increased stem and root length compared with the control (Kalaivani *et al*., 2016). The plant height and root length of crop plants under saline conditions decrease due to salt accumulation in cell walls; this phenomenon limits cell wall growth, springiness, and elongation (Naseer *et al*., 2001; Taghipour and Salehi, 2008; Colla *et al*., 2012) and leads to underdeveloped shoots and roots (Aslam *et al*., 1993).

Past studies demonstrated the effects of MeSA at the germination and early seedling growth stages of rice and concluded that MeSA's positive effects depends on its different doses (Kalaivani *et al*., 2016). Hence, such promising findings for crop plants should be saved for further research in the future. Mutation effects always occur differently and randomly in the genome. Therefore, positive effects should be isolated as much as possible. This will assist the breeders in selecting and isolating the best-performing mutant populations in addition to mutant traits based on a series of crop plants. This study revealed that rice cultivars have salt tolerance, and hence individual seedlings were selected within the M1 populations of both rice cultivars. Salt stress treatments also significantly reduced the number of the branches of both rice cultivars. Under different salinized conditions, the highest number of tillers per plant (13) was exhibited by the HATRI-192 M1 line M1G1-4 treated with 1 mM MeSA at 6 dS/m NaCl (Table 2).

The results are also supported by the past findings of Nicolas *et al*. (1994), Zhao *et al*. (2009), Shahzad *et al*. (2012), and Bagues *et al*. (2018), who observed similar observations in M1 rice populations treated with different salinity levels. Panicle length is an important morphological parameter that contributes to increased yield in crops. Counce *et al*. (2000) reported that panicle length is unaffected by salinity stress at the reproductive stage in all screening steps because the development of the panicle has already been completed. However, in this study, the highest panicle length (27.33 cm) was observed in line M1G1-17 treated with 0.1 mM MeSA at 17 dS/m NaCl (Table 2). This result proved that the panicle length of M1

Table 2. Mean performance of M1 lines developed from rice cultivar HATRI-192 (treated with MeSA) for agronomic traits under salinity stress at the reproductive stage.

Treat ments	M1 lines	MeSA doses	Plant height (cm)	Tillers plant^{-1}	Panicle length (cm)	Filled grains panicle^{-1}	Unfilled grains (%)	1000-grain weight (g)	Grain yield plant^{-1} (g)
Control (HATRI-192)		0-MeSA	97	10	24.67	94	49.28	25.9	24.35
		0.1-MeSA	95	11	25.00	94	17.30	26.1	26.78
		0.5-MeSA	94	7	26.00	90	36.47	25.9	16.32
		1-MeSA	79	12	25.33	84	26.53	23.9	26.11
6 dS/m	M1G1-1	0-MeSA	108	7	26.17	80	38.78	26.9	14.50
	M1G1-2	0.1-MeSA	97	10	26.00	67	48.98	25.9	17.35
	M1G1-3	0.5-MeSA	92	12	25.33	72	31.43	26.9	22.38
	M1G1-4	1-MeSA	89	13	24.67	67	50.25	26.0	22.56
	M1G1-5	1-MeSA	90	5	25.33	29	67.42	23.1	3.76
8 dS/m	M1G1-6	0-MeSA	105	6	24.33	16	81.25	25.2	2.49
	M1G1-7	0.1-MeSA	87	8	25.00	74	58.45	27.1	15.33
	M1G1-8	0.5-MeSA	95	9	26.00	59	37.85	26.1	13.75
	M1G1-9	1-MeSA	78	7	25.67	41	70.29	26.2	7.43
12 dS/m	M1G1-10	0-MeSA	105	8	24.33	91	39.60	24.0	18.86
	M1G1-11	0.1-MeSA	87	7	26.00	93	32.45	27.1	16.86
	M1G1-12	0.5-MeSA	93	11	25.33	96	41.04	25.3	27.35
	M1G1-13	1-MeSA	87	6	25.67	86	33.16	24.8	13.36
15 dS/m	M1G1-14	0-MeSA	103	6	25.33	90	36.62	26.5	13.99
17 dS/m	M1G1-15	0-MeSA	97	6	25.33	91	31.92	26.7	14.14
	M1G1-16	0.5-MeSA	87	8	23.67	64	46.52	27.3	13.26
	M1G1-17	0.1-MeSA	115	9	27.33	57	40.32	26.1	13.29
	M1G1-18	1-MeSA	87	4	25.00	90	36.17	27.3	9.32

rice was positively affected in the saline environment especially at the highest salinity level (17 dS/m).

The results revealed the effect of salt stresses on the yield components of rice M1 mutant populations at the reproductive stage. The trait filled grain per panicle was also affected by salt stress, and this trait could be used to screen M1 populations under different salt stresses. At the reproductive stage and 12 dS/m, the highest filled grain per panicle (96) was recorded for line M1G1-12 treated with 0.5 mM MeSA (Table 2). This result implied that MeSA could play a good role in improving rice cultivars with salinity tolerance at the reproductive stage. At the reproductive stage, M1 lines exhibited varied values of unfilled grains under different salinity stress levels; this trait was found as the best criterion for the evaluation of mutated rice genotypes. The results revealed that at 6 dS/m, the lowest percentage of unfilled grains per panicle (31.43%) was exhibited by line M1G1-3 treated with 0.5 mM MeSA. Overall, at 17 dS/m, the highest grain weight (27.3 g) was achieved by M1G1-16 and M1G1-18 treated with 0.5 and 1.0 mM

Table 3. Mean performance of M1 lines developed from rice cultivar HATRI-62 (treated with MeSA) for agronomic traits under salinity stress at the reproductive stage.

Treat ments	M1 lines	MeSA doses	Plant height (cm)	Tillers plant^{-1}	Panicle length (cm)	Filled grains panicle^{-1}	Unfilled grains (%)	1000-grain weight (g)	Grain yield plant^{-1} (g)
Control (HATRI-62)		0-MeSA	110	10	24.33	77	34.70	24.6	28.51
		0.1-MeSA	84	7	22.00	94	42.98	25.9	30.44
		0.5-MeSA	99	6	24.33	92	44.77	24.4	14.95
		1-MeSA	85	9	24.00	63	72.51	22.8	13.08
6 dS/m	M1G2-1	0-MeSA	87	9	22.33	85	41.69	25.3	29.53
	M1G2-2	0.1- MeSA	85	7	23.83	76	46.40	25.6	16.36
	M1G2-3	0.5-MeSA	95	6	22.00	62	67.79	29.4	24.75
	M1G2-4	0.5-MeSA	89	6	22.17	56	70.16	21.9	3.78
	M1G2-5	1-MeSA	88	8	24.17	39	83.54	22.4	9.82
	M1G2-6	1-MeSA	80	8	21.33	25	82.80	23.6	8.39
8 dS/m	M1G2-7	0-MeSA	87	7	21.83	57	51.61	21.6	16.86
	M1G2-8	0.1-MeSA	85	7	24.17	96	37.70	25.0	35.90
	M1G2-9	0.5-MeSA	88	6	23.17	65	49.78	25.9	26.87
	M1G2-10	1-MeSA	83	5	22.17	34	80.87	21.6	7.19
12 dS/m	M1G2-11	0-MeSA	84	10	24.00	67	46.82	22.4	21.77
	M1G2-12	0.1-MeSA	88	8	25.67	69	43.21	25.3	29.25
	M1G2-13	0.5-MeSA	98	5	18.17	28	62.00	23.5	4.21
	M1G2-14	1-MeSA	83	6	23.83	31	82.30	23.0	2.52
15 dS/m	M1G2-15	0-MeSA	88	7	23.83	43	54.87	24.7	8.46
	M1G2-16	0.1-MeSA	86	10	23.50	57	54.00	25.7	20.6
	M1G2-17	0.5-MeSA	87	9	25.00	31	50.10	23.5	31.23
	M1G2-18	1-MeSA	80	8	20.00	4	96.15	22.5	4.26
17 dS/m	M1G2-19	0.1-MeSA	89	6	25.67	69	61.78	24.9	10.33
	M1G2-20	0.5-MeSA	85	7	21.00	53	53.16	23.8	13.06
	M1G2-21	1-MeSA	87	5	24.67	72	58.70	24.2	10.35

MeSA, respectively. However, at 12 dS/m, the highest seed yield per plant (27.35 g) was attained by line M1G1-12 treated with 0.5 mM MeSA.

The results regarding the effects of salinity stress on M1 lines in cultivar HATRI-62 for grain yield and its component traits at the reproductive stage are presented in Table 3. Two lines, i.e., M1G2-12 and M1G2-19 treated with 0.1 mM MeSA, showed the highest panicle length (25.67 cm) at 12 and 17 dS/m. At the salinity level of 8 dS/m, line M1G2-8 treated with 0.1 mM MeSA showed the highest number of filled grains per panicle (6) and grain yield per plant (35.9 g) with the lowest percentage of unfilled grains (37.7%). Line M1G2-3 treated with 0.5 mM MeSA presented the highest grain weight (29.4 g) at 6 dS/m. However, no M1 line of the rice cultivar HATRI-62 showed a higher number of tillers per plant under salt stress compared with the control. These results further suggested that salinity levels severely affected the tiller development of the M1 lines of both rice cultivars. In this study, the M1 lines were found to be the most sensitive to various salt stresses at the reproductive stage, and this criterion was used to screen mutant populations through comparison with their parental cultivars as the control.

In rice, the seedling, vegetative, and reproductive stages are seriously affected by saline environments, which eventually affect grain yield (Ghosh *et al*., 2016). The flowering and grain-filling stages of rice crops are also more sensitive to salinity stress (Munns and Tester, 2008), resulting in a significant decrease in productivity (Kumar *et al*., 2008). Grain yield is dependent on spikelet fertility and other yield components that are severely affected by salinity; thus, seed setting decreased in the rice panicle (Hasamuzzaman *et al*., 2009). The findings showed that M1 lines provided varied values for yield and yield components under different salinity levels (Tables 2 and 3). Line M1G1-12 of the rice cultivar HATRI-192 treated with 0.5 mM MeSA at 12 dS/m NaCl had the highest filled grains per panicle and grain yield per plant. These findings suggested that mutation induction with MeSA could be used for enhancing salt tolerance in rice at the reproductive stage in future breeding programs. The results also confirmed that some of the selected M1 lines revealed enhanced values for yield components and grain yield compared with their parental cultivars. These selected lines can be utilized for the development of new rice tolerant cultivars with increased yield in future breeding programs.

Evaluation of M1 lines for phytic acid content

Phytic acid is a natural compound that is mainly found in grains, beans, and oilseeds (Garcia-Estepa *et al*., 1999). Zinc and iron deficiencies related to phytic acid content causes micronutrient malnutrition effects in humans (Al-Hasan *et al*., 2016). In the current context of climate change, the majority of breeders only focus on increasing productivity, and very few have focused on grain quality characteristics, especially phytic acid content. Thus, in this study, the main objective was to study the effects of environmental salinity related to phytic acid content and productivity and to select the best M1 rice lines for further breeding. Salinity stress affects rice production and grain quality parameters. Genetic and environmental factors are the major causal agents affecting the phytic acid content of rice seeds (Liu, 2005). They include climate change; crop and fertilizer management practices; and soil features, such as soil physical, chemical, and biological factors (Brankovic *et al*., 2015; Dhole and Reddy, 2015). In rice, phytic acid usually accumulates at all stages of development (Rose *et al*., 2010). Gopal *et al*. (1983) reported that salinity affects the groundnut at the germination stage and decreases phytic acid content. However, information regarding the effects of salinity on phytic acid accumulation in rice grains is limited.

The results revealed that salinity stress not only affected productivity but also created significant variability in the phytic acid content of rice. MeSA treatment resulted in significant variation in the seed phytic acid content of the M1

Table 4. Mean performance of M1 lines developed from rice cultivars HATRI-192 and HATRI-62 (treated with MeSA) for phytic acid content (mg/mL) under salinity stress.

Treatments	M1 lines	MeSA doses	HATRI-192
Control		0-MeSA	0.0145 d
		0.1-MeSA	0.0137 f
		0.5-MeSA	0.0132 h
		1-MeSA	0.0117 k
6 dS/m	M1G1-1	0-MeSA	0.0099 q
	M1G1-2	0.1-MeSA	0.0144 d
	M1G1-3	0.5-MeSA	0.0120 j
	M1G1-4	1-MeSA	0.0102 op
	M1G1-5	1-MeSA	0.0128 i
8 dS/m	M1G1-6	0-MeSA	0.0093 r
	M1G1-7	0.1-MeSA	0.0105 n
	M1G1-8	0.5-MeSA	0.0179 b
	M1G1-9	1-MeSA	0.0102 op
12 dS/m	M1G1-10	0-MeSA	0.0101 p
	M1G1-11	0.1-MeSA	0.0152 c
	M1G1-12	0.5-MeSA	0.0109 m
	M1G1-13	1-MeSA	0.0092 s
15 dS/m	M1G1-14	0-MeSA	0.0187 a
17 dS/m	M1G1-15	0-MeSA	0.0135g
	M1G1-16	0.1-MeSA	0.0139 e
	M1G1-17	0.5-MeSA	0.0103 o
	M1G1-18	1-MeSA	0.0114 l
Means			0.015270513
CV (%)			0.399298865
$LSD_{0.05}$			3.46E-05

Treatments	M1 lines	MeSA doses	HATRI-62
Control		0-MeSA	0.0104 u
		0.1-MeSA	0.0115 t
		0.1-MeSA	0.0173 h
		0.5-MeSA	0.0093 x
		1-MeSA	0.0101v
6 dS/m	M1G2-1	0-MeSA	0.0187 e
	M1G2-2	0.1- MeSA	0.0125 p
	M1G2-3	0.5-MeSA	0.0183 f
	M1G2-4	0.5-MeSA	0.0122 r
	M1G2-5	1-MeSA	0.0134 m
	M1G2-6	1-MeSA	0.0131 n
8 dS/m	M1G2-7	0-MeSA	0.0241c
	M1G2-8	0.1-MeSA	0.0245 b
	M1G2-9	0.5-MeSA	0.0125 p
	M1G2-10	1-MeSA	0.0118 s
12 dS/m	M1G2-11	0-MeSA	0.0194 d
	M1G2-12	0.1-MeSA	0.0123 q
	M1G2-13	0.5-MeSA	0.0250 a
	M1G2-14	1-MeSA	0.0139 l
15 dS/m	M1G2-15	0-MeSA	0.0128 o
	M1G2-16	0.1-MeSA	0.0187e
	M1G2-17	0.5-MeSA	0.0098 w
	M1G2-18	1-MeSA	0.0172 i
17 dS/m	M1G2-19	0.1-MeSA	0.0160 f
	M1G2-20	0.5-MeSA	0.0181 g
	M1G2-21	1-MeSA	0.0142 k
Means			0.01243
CV (%)			0.64936837 1
$LSD_{0.05}$			5.02E-05

Note: Mean values followed by same letters were not significantly ($P < 0.05$) different according to Tukey's test.

lines of rice cultivars HATRI-192 and HATRI-62 under different salt stress treatments (Table 4). The lowest phytic acid content (0.0092 mg/g) was shown by line M1G1-13 of the rice cultivar HATRI-192 treated with 1 mM MeSA at 15 dS/m NaCl, whereas the highest phytic acid content (0.0187 mg/g) was found in line M0G1-14 (not treated with MeSA) at 15 dS/m NaCl (Table 4). In cultivar HATRI-62, the lowest phytic acid content (0.0098 mg/g) was found in line M1G2-17 treated with 0.5 mM MeSA at 15 dS/m NaCl. However, the highest phytic acid content (0.0250 mg/g) was recorded for line M0G2-13 treated with 0.5 MeSA at 12 dS/m NaCl.

Table 5. Aroma scores of M1 lines (developed from rice cultivars HATRI-192 and HATRI-62 and treated with MeSA) under salinity stress.

M1 Lines - HATRI-192		Score
Control	KDML105	2
	0-MeSA	0
M1G1-1	0-MeSA	0
M1G1-2	0.1-MeSA	0
M1G1-3	0.5-MeSA	0
M1G1-4	1-MeSA	1
M1G1-5	1-MeSA	1
M1G1-6	0-MeSA	0
M1G1-7	0.1-MeSA	2
M1G1-8	0.5-MeSA	2
M1G1-9	1-MeSA	1
M1G1-10	0-MeSA	0
M1G1-11	0.1-MeSA	0
M1G1-12	0.5-MeSA	1
M1G1-13	1-MeSA	1
M1G1-14	0-MeSA	0
M1G1-15	0-MeSA	0
M1G1-16	0.1-MeSA	0
M1G1-17	0.5-MeSA	0
M1G1-18	1-MeSA-B	1

M1 Lines - HATRI-62		Score
Control	KDML105	2
	0-MeSA	0
M1G2-1	0-MeSA	1
M1G2-2	0.1- MeSA	0
M1G2-3	0.5-MeSA	2
M1G2-4	0.5-MeSA	2
M1G2-5	1-MeSA	1
M1G2-6	1-MeSA	1
M1G2-7	0-MeSA	0
M1G2-8	0.1-MeSA	0
M1G2-9	0.5-MeSA	2
M1G2-10	1-MeSA	2
M1G2-11	0-MeSA	1
M1G2-12	0.1-MeSA	0
M1G2-13	0.5-MeSA	2
M1G2-14	1-MeSA	1
M1G2-15	0-MeSA	0
M1G2-16	0.1-MeSA	2
M1G2-17	0.5-MeSA	1
M1G2-18	1-MeSA	1
M1G2-19	0.1-MeSA	1
M1G2-20	0.5-MeSA	1
M1G2-21	1-MeSA	1

Phytic acid content and inorganic acid phosphorus usually account for approximately 75% and 5% of the total phosphorus in cereal grains, respectively (Lott *et al*., 1995). Rice grains contain phytic acid and oxalic acid that are antinutritional secondary components. Phytic acid is an important source of inositol and stored phosphorus in plants and plays an important role in the prevention of oxidation. Several recessive genes that can control and reduce phytic acid content have been created through chemical mutations (Lang *et al*., 2007). Past findings revealed that phytic acid content is reduced by treating seeds with MeSA, which can convert phytate into phosphates in rice seeds (Kalaivani *et al*., 2016). This study confirmed that MeSA could play an important role as a mutagen for developing improved rice cultivars for phytic acid content in rice under salt stress conditions.

Evaluation of M1 lines for aroma

In this study, in addition to the effects of salinity on yield and yield components and phytic acid content in M1 lines, aroma characteristics were assessed on the basis of the aromatic phenotype of M1 lines under saline conditions. Aroma is the most important quality property of rice, and fragrant rice cultivars are highly priced. Aroma may largely depend on particular environmental factors, including temperature, soil type, abiotic stress, water, CO_2, light, salinity, and shade (Itani *et al*., 2004, Cha-um *et al*., 2007; Mo *et al*., 2015), or special attention from the market and farmers. MeSA had positive and negative effects on managing the aroma of M1 lines compared with that of the parental cultivars. The cultivar 'Basmati' expresses strong aroma when grown in cold environmental conditions, and the rice cultivar KDML105 shows the best aroma when grown under drought conditions (Yoshihashi *et al*., 2002).

In this study, the aroma traits of 18 and 21 lines of irradiated M1 populations derived from rice cultivars HATRI-192 and HATRI-62, respectively, were assessed (Table 5). In cultivar HATRI-192, eight lines treated with MeSA showed aroma scores of 1 and 2. These lines included two lines (M1G1-7 and M1G1-8) with a score of 2 and six lines with a score of 1. In cultivar HATRI-62, 13 lines treated with MeSA produced aroma, wherein six lines (M1G2-3, M1G2-4, M1G2-9, M1G2-10, M1G2-13, and M1G2-16) had an aroma of score 2, similar to KDML105. This research showed that M1 lines with desirable aroma were obtained and highlighted the fact that the expression of aroma in lines treated with MeSA (i.e., 8 lines in HATRI-192 and 13 lines in HATRI-62) compared with populations that were not treated with MeSA. This result implied that MeSA could play an important role in the development of improved aromatic rice genotypes under salt-stressed conditions. The effects of MeSA on the aroma quality trait of rice have not been studied extensively. These findings may contribute and provide a basis for genetic information for future breeding to improve the aroma characteristics of rice.

CONCLUSION

This investigation demonstrated that the rice cultivars HATRI-192 and HATRI-62 showed improved adaptation to saline environments when treated with 0.1 and 0.5 mM MeSA as reflected by their filled grains per panicle, percentage of unfilled grains per panicle, grain yield, phytic acid content, and aroma. Therefore, the findings might contribute to the evaluation and selection of M1 rice genetic material with improved salt tolerance and grain yield and quality traits in future generations.

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