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Effects of Methyl Salicylate (MeSA) on the Physiology and Biochemical Characteristics of Rice Under Salinity Stress at Seedling Stage

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Salt stress negatively affects crop survival, growth, development, and yield. Methyl salicylate (MeSA) is synthesized from salicylic acid (SA) which is a volatile organic compound that is responsible for inducing defense mechanisms in plants and also has a protective role in stress sensitivity. The objective of this study was to examine the effect of different concentrations of methyl salicylate (MeSA) on the physiological and biochemical characteristics of two rice varieties GRIS4 (G1) and GRIS5 (G2) under salt stress at the seedling stage. Five hundred seeds of each variety were treated with different doses of MeSA (0, 0.1, 0.5, and 1.0 mM) and screening various salinity levels (0, 6, 8, 12, 15, and 17 dS m^{-1}) at the seedling stage (21 days old). The different traits such as survival rates, morphological characteristics including plant height and root and length, the biomass of fresh and dry weights of the shoots and roots, and biochemical parameters (chlorophyll a and b, proline, and phenolic and flavonoid content) were measured. Treatment 0.1 mM MeSA had significantly higher root length and dry weight under 8 dS m^{-1}, 6 dS m^{-1}, higher chlorophyll and phenolic content under 17 dS m^{-1} for G1, whereas the 0.1 mM MeSA-treated plants had significantly higher root length under 15 dS m^{-1} and root fresh weight under 12 dS m^{-1} for G2 compared to control plants. The 0.5 mM MeSA-treated plants had significantly higher seedling length, survival rate, root fresh weight, and shoot fresh weight under 8 dS m^{-1}, 6 dS m^{-1} then also had higher proline and flavonoid content under 17 dS m^{-1} for G1, while G2 had higher shoot dry weight under 17 dS m^{-1}, higher root fresh weight under 8 dS m^{-1}, higher proline content under15 dS m^{-1}, and higher phenolic and flavonoid contents under 12 dS m^{-1} compared to control plants. Treatment 1.0 mM MeSA had higher survival rate under 6 dS m^{-1}, seedling length under 15 dS m^{-1}, shoot fresh weight under 6 dS m^{-1}, root dry weight under 8 dS m^{-1}, chlorophyll a under 15 dS m^{-1}, and chlorophyll b under 8 dS m^{-1} compared to control plants. These results showed that positive effects induced by MeSA increased some physiological and biochemical traits of rice under salt stress at the seedling stage.

Keywords: salt, rice, MeSA, chlorophyll, proline, phenolic, and flavonoid

Abbreviations: G1—GRIS4, G2—GRIS5, MeSA—Methyl salicylate, ROS—reactive oxygen species

INTRODUCTION

Rice is one of the most important cereal crops, providing food for half of the world's population. Therefore, improved rice production is important for food security and economic development. However, climate change and saline intrusions are major threats to rice-growing countries. Salinity is one of the most severe abiotic stresses on rice production in many rice-producing areas due to salty conditions. It can also negatively affect the morphological, physiological, and biochemical attributes of plants (Hasanuzzaman et al. 2017). About one-third of the world's crop area is affected by salinity stress caused by seawater intrusion or saline groundwater when rice paddies are in drought (Gale 2002; Mike 2003; Singh 2018). Salt causes oxidative stress due to excessive

production of reactive oxygen species (ROS) that activate lipid peroxidation, cause damage to photosynthetic pigment, and mineral nutrient status disruption (Turan and Tripathy 2013). High amounts of salt inhibit water absorption, change the effects of stomatal opening and closing, and alter the source of CO_2 absorption into plant leaf cells, causing damage to plant tissues (Lien 2010; Haworth et al. 2016). This affects the growth, survival, development, and productivity of crops. Salinity can affect the different growth and developmental stages of rice. However, the most sensitive to salinity are the seedling and growth stages, which directly affect rice yield (Zeng et al. 2001; Hassan et al. 2012, Khatun et al. 2013). The most effective means of dealing with soil salinity is to establish a cultivable and high-yielding plant variety that can survive under such conditions (Luan et al. 2007; Machado et al. 2017).

Methyl salicylate (MeSA) is a volatile organic compound synthesized from salicylic acid (SA) and is a hormone that can reduce the harmful effects of abiotic stresses such as high salinity (Tari et al. 2002). Salicylic acid (SA) is a natural phenolic compound that plays a role in regulating the physiological and biochemical processes of plants (Kandaswamy et al. 2016). It also has been reported to be accountable for the regulation of certain processes in plants related to abiotic stresses such as those induced by salt, heat, and heavy metals (Kang et al. 2014; Li et al. 2019). In addition, the major characteristics of salicylic acid include the increasing rate of germination, shoots and root length, fresh and dry weight of both shoots and roots of plants, and various antioxidant enzyme activities (Arfan et al. 2007; Hasanuzzaman et al. 2017). Previous studies have reported that SA reduces salt stress by improving photosynthetic properties and increasing salinity tolerance by inducing antioxidant metabolism (Nazar et al. 2015). It acts as signal molecules in the induction of defense mechanisms that contribute to the regulation of many physiological processes in plants such as the germination rate, cell growth, air opening, photosynthesis, and ion absorption (Raskin 1992; Dempsey et al. 2011; Yusuf et al. 2013; Vazirimehr et al. 2014; Wiesel et al. 2015; Kandaswamy et al. 2016). Many previous studies have reported the effect of salicylic acid on the protection of plants under saline conditions. The improvement of crop production by managing abiotic stress is one of the main goals of rice breeding. Previous studies have been undertaken to produce MeSA-induced rice mutant populations that are resistant to abiotic stress (Kasket et al. 2016; Li et al. 2017; Ha et al. 2019; Ha et al. 2020). While our research previously evaluated the role of MeSA in the germination of rice under varying salt stress conditions (Ha et al. 2020), there are still limited studies examining how seed treated with MeSA affects salt tolerance and changes the morphological and biochemical characteristics of rice at the seedling stage. Therefore, the objective of the present study was to examine the positive and negative effects of different concentrations of MeSA (0, 0.1, 0.5, and 1.0 mM) application by seed treatment under the various levels of salinity concentration (0, 6, 8, 12, 15, and 17 dS m^{-1}) through physiological and biochemical characteristics of two rice varieties at the seedling growth stage. The presented data in this research will help to develop a new method for improving the salinity tolerance of rice in a future breeding program.

MATERIALS AND METHODS

Plant Materials

The rice varieties GRIS4 (G1) and GRIS5 (G2) were used for this study. These varieties were selected by our previous study (Ha et al. 2016; Ha et al. 2017) from two lines (45 and 54) of BC_2F_5 were divided from OMCS2000/ IR75499-73-1-B with drought tolerance, resistance to both BPH and blast disease. These lines were continued to be improved in the salinity tolerance for climate change adaptation in Vietnam.

Seed Treatments and Experimental Design

The seeds of the two cultivars (5040 seeds/variety) were treated with the MeSA as described previously by Ha et al. (2020). The seeds were soaked in warm water (approximately 65–70°C) for 24 h at room temperature (about 28–30°C) and were then soaked in 200 mL of MeSA solution consisting of increasing levels of 0, 0.1, 0.5, and 1 mM of MeSA (Table 1), then agitated for 4 h under gentle stirring. The MeSA solution was removed, and the seeds were thoroughly washed under running tap water for 15 min to reduce any residual effect of mutagens sticking to the seed coat. Germinated rice seeds (70 seeds x 4 treatments of MeSA x 3 trays x 6 levels of NaCl) were plated into the floating styrofoam which drilled the holes with diameter 1 cm, length 16-hole, and width 22-hole and put into the spongy tray plastic tray (diameter of 40 x 60 x 10 cm) under sterilized water for 7 days (Fig. 1). The screening seedling stage was cultured hydroponically for 7 days in Yoshida solution (12 mL/L) (Yoshida 1981). On the 14^{th} day after sowing, NaCl solution at various salinity levels (0, 6, 8, 12, 15, and 17 dS m^{-1}) was added to the solution, with the *pH*-controlled at 5.0 -5.5 (Yoshida 1981). The controls without indued MeSA and salinity conditions from the two varieties were also used as a check for comparison. The seedlings were assessed based on different traits after 21 days,

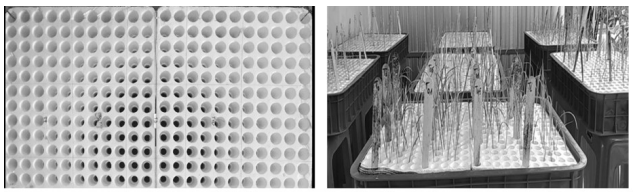


Fig. 1. Experiment design for screening salinity stress of rice seeds treated with concentrations 0, 0.1, 05, and 1.0 mM of MeSA grew under different salt conditions (0, 6, 8, 12, 15, and 17 after seedling 14 days old.

including survival rates, morphological characteristics (plant height, root length, the biomass of fresh, dry weights of the shoots, roots, and biochemical parameters [chlorophyll a and b, proline, and phenolic and flavonoid contents]).

Growth Characteristics

The rice plants were collected after 21 days of sowing. The rice roots were washed under running water to remove any dirt. A ruler was used to measure the length of the shoots and roots. The shoot length (cm) was measured from the root joint to the upper tip of the leaf. The root length (cm) was measured from the original shoot joint to the end of the root tip (Syed et al. 2015). The fresh and dry weights of the shoots and roots were taken first, followed by oven drying at 55°C for 24 h, and lastly, dry weight measurement.

Table 1. Treatments of rice seeds induced with MeSA under salt stress conditions at the seedling stage.

No.	Treatments	No.	Treatments
T0	0 MeSA control under non-salt condition	T12	0.1 mM MeSA under 8 dS m^{-1}
T1	0 MeSA control under 6 dS/m^{-1}	T13	0.5 mM MeSA under 8 dS m^{-1}
T2	0 MeSA control under 8 dS m^{-1}	T14	1.0 mM MeSA under 8 dS m^{-1}
T3	0 MeSA control under 12 dS m^{-1}	T15	0.1 mM MeSA under 12 dS m^{-1}
T4	0 MeSA control under 15 dS m^{-1}	T16	0.5 mM MeSA under 12 dS m^{-1}
T5	0 MeSA control under 17 dS m^{-1}	T17	1.0 mM MeSA under 12 dS m^{-1}
T6	0.1 mM MeSA control under non-salt condition	T18	0.1 mM MeSA under 15 dS m^{-1}
T7	0.5 mM MeSA control under non-salt condition	T19	0.5 mM MeSA under 15 dS m^{-1}
T8	1.0 mM MeSA control under non-salt condition	T20	1.0 mM MeSA under 15 dS m^{-1}
T9	0.1 mM MeSA under 6 dS m^{-1}	T21	0.1 mM MeSA under 17 dS m^{-1}
T10	0.5 mM MeSA under 6 dS m^{-1}	T22	0.5 mM MeSA under 17 dS m^{-1}
T11	1.0 mM MeSA under 6 dS m^{-1}	T23	1.0 mM MeSA under 17 dS m^{-1}

Determination of Chlorophyll Content

Chlorophyll a (Chl a) and b (Chl b) contents were estimated following the method described by Arnon (1949). Fresh leaf samples of rice (1 g) were crushed using mortar and pestle with 20 mL of 80% acetone and 0.5 mg of $MgCO_3$. Then, the homogeneous mixture was placed at 4°C for 4 h. Next, the sample was centrifuged at 500 rpm for 5 min. The supernatant was collected into a volumetric flask and made up to 100 mL with 80% acetone. The absorbance of the sample was read by a spectrophotometer at wavelengths of 645 nm and 663 nm. A blank sample containing only 80% acetone was also used.

Chl a and b (μg/mL) contents were determined by the following formulas:

$$\text{Chl a} = 11.75 \times A_{662.6} - 2.35 \times A_{645.6}$$

$$\text{Chl b} = 18.61 \times A_{645.6} - 3.96 \times A_{662.6}$$

Determination of Proline Content

Proline content was determined following the method described by Chinard (1952). Proline was extracted from 100 g of fresh leaves by homogenization with 3% sulfosalicylic acid (5 μL/mg samples) and centrifuged at 6000 rpm for 5 min. An assay mixture containing 100 μL of 3% sulfosalicylic acid, 200 μL of glacial acetic acid, 200 μL of acidic ninhydrin, and a 100 μL aliquot of the supernatant was heated to 96°C for 1 h and then rapidly cooled in ice to end the reaction. After cooling, 1 mL of toluene was added and vortexed for 20 s. The mixture was allowed to stand for 5 min and the absorbance was measured at 520 nm wavelength with a UV-Vis spectrophotometer. The proline content in the sample was expressed as μg/g or μM/g of fresh weight. L-Proline was used with concentrations of 2, 4, 6, 8, and 10 mg/mL for the calibration curve.

Determination of Phenolic Content

Approximately 3 g of dried leaves were ground with 100 mL of ethanol solution (99.5%) until a homogeneous solution was obtained and then shaken for 16 h at room temperature and centrifuged at 500 rpm for 10 min. The residue was re-extracted under equivalent conditions. The two supernatants were combined and evaporated at 30°C in a rotary evaporator. The extract was dried and then dissolved with methanol and stored at 4°C under dark conditions until subsequent analysis.

The total phenolic content in the extract was determined by the Folin Ciocalteu reagent method described by Dewanto et al. (2002). A 0.125 mL extract

was added to a tube containing 0.5 mL of distilled water and 0.125 ml of the Folin Ciocalteu reagent. The mixture in the tube was allowed to stand for 6 min. Then, the mixture was made up to 2 mL with a 7% sodium carbonate solution (Na_2CO_3) and the absorbance was measured at 760 nm wavelength. The total phenolic concentration was calculated from the calibration curve where gallic acid was used as the standard with different concentrations (0, 0.05, 0.1, 0.15, and 0.2 mg/mL). The results were shown in mg gallic acid equivalent per 100 g of the dry weight of the sample.

Determination of Flavonoid Content

The flavonoid accumulation was determined according to the method described by Dewanto et al. (2002). The total flavonoid content in the extract was determined as follows: 0.5 mL of extract (a similar extraction procedure was done as in phenolic content) was added to a tube containing 0.5 mL of $AlCl_3$ (2% methanol) solution. The mixture could stand for 15 min at room temperature and then the absorbance was read at 430 nm wavelength. The total flavonoid content was calculated from the calibration curve. The result was expressed as mg rutin equivalent per 1 g dry weight of the sample.

Statistical Analysis

Statistical analyses were presented using the SAS software program (version 9.1, SAS Institute). Mean values and standard deviation (SD) were obtained from three replicates analyzed using Duncan's multiple range tests. P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Effects of MeSA Application on the Survival Rate of Rice Seedling Under Saline Stress

Seedling survival is also one of the important pointers of the salt tolerance of a cultivar (Garg et al. 1996; Uçarlı 2020). SA has been revealed to affect salt tolerance in various plant species (Farhangi-Abriz and Ghassemi-Golezani 2017; Methenni et al. 2018). MeSA is produced by salicylic acid carboxyl methyltransferase (Jayakannan et al. 2015) and is also involved in regulating the crosstalk between SA and jasmonic acid (JA) defense pathways (Robert-Seilaniantz et al. 2011). It is a volatile organic compound synthesized from SA, a hormone capable of reducing the harmful effects of abiotic stress such as high salinity (Tari et al. 2002). In this study, the investigation on the effect of MeSA for the salt tolerance seedling stage (Fig. 3) showed that MeSA treatment did not increase the survival rate in both varieties under salt stress conditions (P < 0.05) when compared to normal control. However, the 0.5 MeSA treatment significantly (P < 0.05) increased the survival rate under 12 dS m^{-1} salinity, as 56.67% in G1 seedling and 45.83% in G2 seedling as compared to non-MeSA and other treatments at the same salt conditions. Also, the application of MeSA improved the survival rate of G1 seedlings as it was increased to a highly significant level (P < 0.05) as 52.50 % (1 mM +15 m^{-1}), 40% (0.5 mM+17 m^{-1}) and 37.5% (1 mM+ 17 m^{-1}) when compared to 43.33% (non-MeSA +15 m^{-1}) and 26.67% (non -MeSA +17 m^{-1}), respectively (Fig. 2). It indicated that MeSA application on rice plants exhibited an increase in various salt tolerance dependent on the different concentrations and varieties.

Effects of MeSA on Seedling Height and Root Length of Rice Under Saline Stress

Plant height and root length were generally reduced with salinity stress in rice especially in salt-sensitive varieties (Syed et al. 2015). Results showed that the highest value (16.19 cm) was observed in G1 seedlings treated with 0.5 mM MeSA + 0 dS m^{-1} salinity (Fig. 3). For seedling height of G1 plants treated with 0.5 mM MeSA as T10 (14.17 cm) T19 (13 cm) and T22 (14.08 cm) were significantly and positively affected by NaCl (6, 15 and 17 dS m^{-1}) compared with control (non-MeSA) (P < 0.05). In this way, the seeds treated with 1 mM MeSA of T17 had higher seedling height (13.58 cm) than other treatments and T3 (non-MeSA, 12.58 cm) under 12 dS m^{-1}. However,

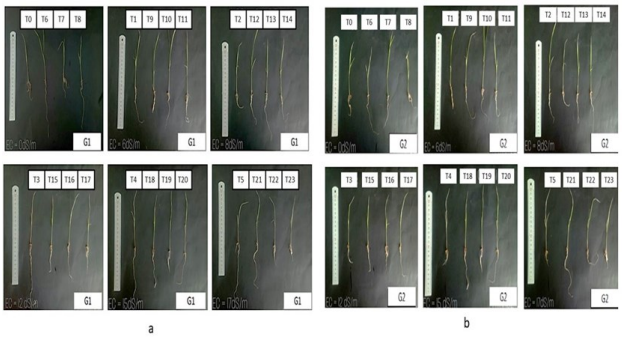


Fig. 2. The phenotype of variety G1 (a) and variety G1 (b) seedlings grew under different salt conditions. treated with concentrations 0, 0.1, 05, and 1.0 mM of MeSA grew under different salt conditions (0, 6, 8, 12, 15, and 17 after seedling 14 days old.

Note: T0 (0 MeSA control under non-salt condition), T1 (0 MeSA control under 6 dS m^{-1}), T2 (0 MeSA control under 8 dS m^{-1}), T3 (0 MeSA control under 12 dS m^{-1}), T4 (0 MeSA control under 15 dS m^{-1}), T5 (0 MeSA control under 17 dS m^{-1}), T6 (0.1 mM MeSA control under non-salt), T7 (0.5 mM MeSA control under non-salt), T8 (1.0 mM MeSA control under non-salt), T9 (0.1 mM MeSA under 6 dS m^{-1}), T10 (0.5 mM MeSA under 6 dS m^{-1}), T11 (1.0 mM MeSA under 6 dS m^{-1}), T12 (0.1 mM MeSA under 8 dS m^{-1}), T13 (0.5 mM MeSA under 8 dS m^{-1}), T14 (1.0 mM MeSA under 8 dS m^{-1}), T15 (0.1 mM MeSA under 12 dS m^{-1}), T16 (0.5 mM MeSA under 12 dS m^{-1}), T17 (1.0 mM MeSA under 12 dS m^{-1}), T18 (0.1 mM MeSA under 15 dS m^{-1}), T19 (0.5 mM MeSA under 15 dS m^{-1}), T20 (1.0 mM MeSA under 15 dS m^{-1}), T21 (0.1 mM MeSA under 17 dS m^{-1}), T22 (0.5 mM MeSA under 17 dS m^{-1}), T23 (1.0 mM MeSA under 17dS m^{-1}).

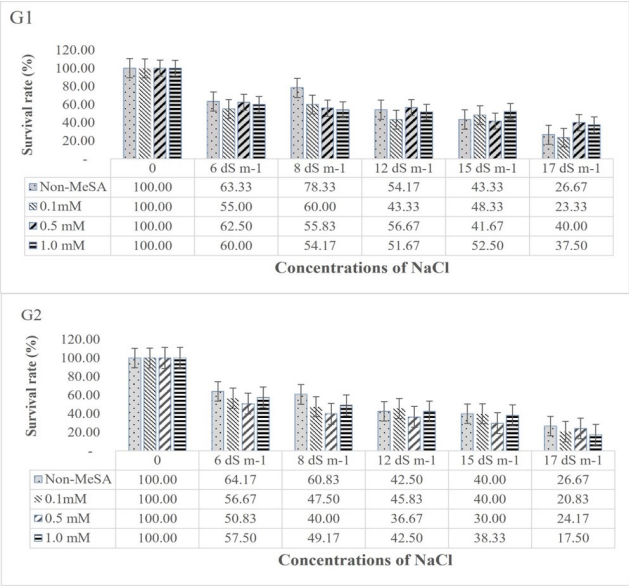


Fig. 3. Effect of MeSA on the survival rate of rice in different salt conditions.

it was observed that the seeds treated with MeSA had negatively affected seedling height as compared to control plants (no-MeSA) at all at 8 dS/m. In the case of G2 seedling, the maximum value of seedlings height was observed in treatment treated with 0.1 mM MeSA under 15 dS m^{-1} (14.19 cm) ($P < 0.05$) when compared with treatments under salt stress (12.61 to 13.86 cm) (Fig. 3). The seed treated with 0.1 meSA (T12: 13.33 cm and T16: 13.33 cm) and 0.5 mM MeSA (T15: 14.19 cm) significantly increased the height of the seedling as compared to treatments non-MeSA (T2: 13.06 cm, T3: 13.33 cm, and T4: 13. 44 cm) under 8 and 15 dS m^{-1}. However, the effect of MeSA on the seedling height of G2 was not significant when compared with that of non-MeSA under 6 and dS m^{-1}. MeSA treatments produced measurable effects on the plant growth of rice varieties. The result of this study demonstrated that increasing doses from 0.5 and 1 mM MeSA affected seeding height under normal conditions (non-salt) (Fig. 3 and Fig. 4). The same effect was observed in the rice varieties IAC165 and Huajingxian (Bi et al. 2007). Furthermore, Martin-Mex and Larqué-Saavedra (2001) reported similar findings with the application of SA for increased shoot growth in *Clitoria sp.* In this study, there was also a slight increase in seedling height by different concentrations of MeSA and rice varieties under various salinity conditions.

The highest root length was observed in the G1 seedling treated with 0.1 mM MeSA (T12) under 8 dS m^{-1} (13.50 cm) as compared to the control treatments ($P < 0.05$) (Fig. 4). For G1 seedling, the root length showed a positive effect on treatments treated with 0.1 (10.28 cm) and 0.5 mM MeSA (12. 44 cm) as compared with control non-MeSA under 6 and 12 (10.08 cm and 10.50 cm), respectively ($P < 0.05$). However, the data obtained revealed that the root length was negatively affected by different concentrations of MeSA as compared to control no-MeSA under 15 and 17 dS m^{-1}. Figures 2 and 5 showed that the effect of various concentrations of MeSA on root length of G2 seedling under salinity stress. G2 seedlings were highest value for the mean of root length (13.42 cm) under 0.1 mM MeSA + 15 dS m^{-1} salinity, while treatments treated with 0.1 mM MeSA were also higher value for the mean of root length (T9: 11.44 cm and T12: 10.58 cm) when compared to control non-MeSA under 6, and 8 dS m^{-1} (9.58 and 10.11 cm), respectively. However, the mean value of the plant height of treatments treated with 0.1, 0.5, and 1 mM MeSA was decreased (6.50,7.47, and 9.19 cm) at 17 dS m^{-1} as compared to control (10.42 cm) (Fig. 2 and Fig. 5). Studies have shown the ability of MeSA to have a positive or negative effect on plant species depending on the MeSA concentration. Results from the study of Muhammad et al. (2018) showed that wheat treated with 0.25 mM of SA overcame the adverse effects of salinity and promoted root length under a saline environment (150 mM NaCl). A similar observation was

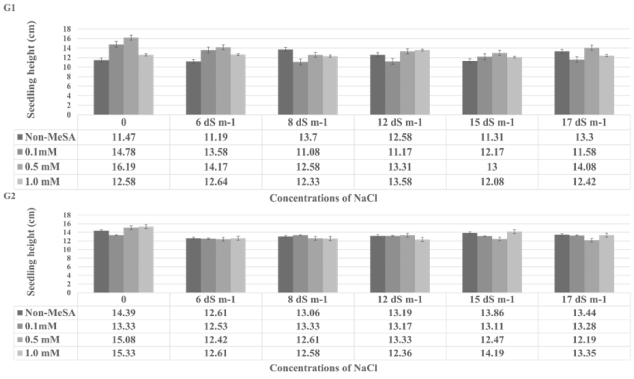


Fig. 4. Effect of MeSA on the seedling height of rice in different salt conditions.

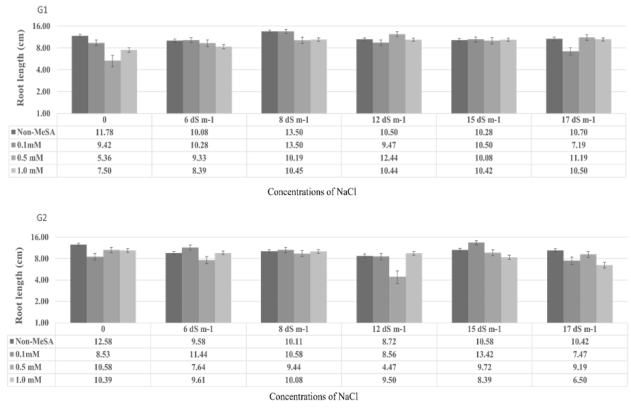


Fig. 5. Effects of MeSA on root length of rice under different saline conditions at the seedling stage.

reported by Kalaivani et al. (2016) and Ha et al. (2020) in rice and Yusuf et al. (2013) and Ha et al. (2019) in mustard brassica where it showed significant variation in plant height and root length based on the concentration of MeSA treatment under different saline conditions.

Some researchers showed that the impact of salt stress has been correlated with some physiological traits like reduction in fresh and dry weight (Abdollahi et al. 2011; Syed et al. 2015). Total dry matter is an important trait to evaluate the performance of rice genotypes for salt tolerance. The data effect of MeSA seeds treatment on the fresh weight and dry weight of shoots under salt stress are given in Table 2. Several studies have shown the impact of MeSA seed treatment on early seedling growth (Kalaivani et al. 2016). In the current study, the maximum fresh weight was observed in T7 (0.5 mM MeSA + 0 dS m^{-1}) (59.67 mg) and minimum fresh weight was observed in T4 (0 MeSA + 15 dS m^{-1}) (27 mg) in the G1 seedling ($P < 0.05$). At 6, 12, and 17 dS m^{-1}, the results for the 0.1, 0.5, and 1 mM of MeSA was a negative effect ($P < 0.05$) from the controls (non-MeSA) in G1 seedlings. In the MeSA-treated G1 seedling, the fresh weight increased ($P < 0.05$) for T13, T14 (41 mg), and T19 (39.67 mg) at 8 and 15 dS m^{-1} compared to the controls (T2: 28 mg and T4: 35.67 mg) (non-MeSA), respectively. The fresh weight of shoots in G2 controls (T0) was 34 mg (Table 2). It increased with an increase in the 0.1, 0.5, and 1 mM MeSA and was highest (52.67 mg) in T11 (1.0 mM MeSA + 6 dS m^{-1}). A gradual increase in dry weight of shoots was observed with 0.1 mM (T12: 40.67 mg) and 0.5 mM (T16: 32.67 mg and T19: 36.67 mg) and 1 mM MeSA (T22, 23: 37.33 mg) under 8 (T2: 35.67), 12 (T3: 31 mg), 15 dS m^{-1} (T4: 35.67 mg), and 17 dS m^{-1} (T5: 35.33 mg) ($P < 0.05$) of G2 seedling, respectively.

The dry weight of shoots in G1 control (T0) was 8.33 mg (Table 2). The dry weight increase was observed in T7 (0.5 mM MeSA + 0 dS m^{-1}) (6.67 mg) and highest in T8 (0.1 mM MeSA + 0 dS m^{-1}) (10.67 mg) ($P < 0.05$), while the dry weight of shoots in G1 non- MeSA (T1 and T2) was 7.67 mg and 6.67 mg. It was increased in the treatment T 9 (0.1 mM MeSA + 6 dS m^{-1}) and T14 (1.0 mM MeSA + 8 dS m^{-1}) (8.33 mg). An insignificant difference was observed between treatments of 0.1, 1.00, and 0.5 mM MeSA (6.67 mg) was lower than T3 (7.33 mg) under 12 dS m^{-1} ($P < 0.05$). The greater effect on dry weight trait was observed from G1 seedling treatments of 0.1, 0.5, and 1 mM MeSA (from 7.33 to 8.33 mg) when compared to T4 (6.67 mg) under 15 dS m^{-1} ($P < 0.05$). In the case of G2

Table 2. Effect of MeSA on fresh weight and dry weight of shoots of rice in different salt conditions at the seedling stage.

Treatments		Fresh Weight (mg)		Dry Weight (mg)	
		G1	G2	G1	G2
0 MeSA control under non-salt condition		41.00 ± 1.00^{d}	34.00 ± 1.00^{kl}	8.33 ± 0.58^{bc}	4.00 ± 0.00^{hi}
	6 dS/m^{-1}	43.33 ± 1.53^{b}	45.33 ± 0.58^{c}	7.67 ± 0.58^{bc}	5.67 ± 0.58^{def}
	8 dS m^{-1}	28.00 ± 1.00^{mn}	35.67 ± 0.58^{hij}	6.67 ± 0.58^{de}	6.67 ± 0.58^{cd}
0 MeSA	12 dS m^{-1}	41.33 ± 1.53^{cd}	31.00 ± 1.00^{no}	7.33 ± 0.58^{cd}	6.67 ± 0.58^{cd}
	15 dS m^{-1}	27.00 ± 1.00^{n}	35.67 ± 0.58^{hij}	6.67 ± 0.58^{de}	6.67 ± 0.58^{cd}
	17 dS m^{-1}	31.33 ± 0.58^{jk}	35.33 ± 0.58^{ijk}	7.33 ± 0.58^{cd}	12.00 ± 1.00^{a}
	0.1mM MeSA	35.67 ± 0.58^{fg}	36.00 ± 0.00^{g-j}	7.33 ± 0.58^{cd}	6.00 ± 0.00^{de}
Non- salt	0.5mM MeSA	59.67 ± 0.58^{a}	38.00 ± 0.00^{f}	8.67 ± 0.58^{b}	3.67 ± 0.58^{i}
	1.0mM MeSA	43.00 ± 1.73^{bc}	35.67 ± 0.58^{hij}	10.67 ± 0.58^{a}	4.67 ± 0.58^{f-i}
	0.1mM MeSA	37.00 ± 0.00^{ef}	42.33 ± 0.58^{d}	8.67 ± 0.58^{b}	4.67 ± 0.58^{f-i}
6 dS m^{-1}	0.5mM MeSA	37.00 ± 1.00^{ef}	47.33 ± 1.16^{b}	6.00 ± 0.00^{f}	4.33 ± 0.58^{ghi}
	1.0mM MeSA	34.33 ± 1.16^{hi}	52.67 ± 0.58^{a}	7.33 ± 1.53^{cd}	5.67 ± 1.16^{def}
	0.1mM MeSA	36.67 ± 1.16^{ef}	40.67 ± 0.58^{e}	6.67 ± 0.58^{de}	8.00 ± 1.00^{b}
8 dS m^{-1}	0.5mM MeSA	41.00 ± 2.65^{d}	37.00 ± 1.00^{fgh}	6.67 ± 0.58^{de}	5.67 ± 0.58^{def}
	1.0mM MeSA	41.00 ± 1.00^{d}	27.67 ± 0.58^{p}	8.33 ± 0.58^{bc}	7.33 ± 0.58^{bc}
	0.1mM MeSA	35.67 ± 0.58^{fg}	30.33 ± 0.58^{o}	7.33 ± 0.58^{cd}	6.00 ± 1.00^{de}
12 dS m^{-1}	0.5mM MeSA	36.67 ± 0.58^{ef}	32.67 ± 0.58^{lm}	6.67 ± 0.58^{de}	6.00 ± 0.00^{de}
	1.0mM MeSA	35.00 ± 1.00^{gh}	32.00 ± 1.00^{mn}	7.33 ± 0.58^{cd}	7.67 ± 0.58^{bc}
	0.1mM MeSA	33.00 ± 1.00^{ij}	35.33 ± 1.16^{ijk}	7.33 ± 1.16^{cd}	5.33 ± 0.58^{efg}
15 dS m^{-1}	0.5mM MeSA	39.67 ± 0.58^{d}	36.67 ± 1.53^{f-i}	7.33 ± 0.58^{cd}	6.67 ± 0.58^{cd}
	1.0mM MeSA	37.67 ± 0.58^{e}	36.33 ± 1.16^{g-j}	8.33 ± 0.58^{bc}	5.00 ± 0.00^{e-h}
	0.1mM MeSA	30.67 ± 0.58^{kl}	35.00 ± 1.00^{jk}	6.67 ± 0.58^{de}	7.33 ± 0.58^{bc}
17 dS m^{-1}	0.5mM MeSA	29.00 ± 1.00^{lm}	36.67 ± 1.53^{f-i}	6.33 ± 0.58^{ef}	8.33 ± 0.58^{b}
	1.0mM MeSA	30.00 ± 1.00^{kl}	37.33 ± 0.58^{fg}	6.67 ± 0.58^{de}	7.33 ± 0.58^{bc}

Each value represents the mean ± standard error (P<0.05). Mean with column similar letters do not differ significantly.

Table 3. Effect of MeSA on fresh weight and dry weight of roots of rice in different salt conditions at the seedling stage.

Treatments		Fresh Weight (mg)		Dry Weight (mg)	
		G1	G2	G1	G2
0 MeSA control under non-salt condition		90.53 ± 0.15^{g}	101.33 ± 1.53^{k}	13.57 ± 0.51^{abc}	6.67 ± 0.58^{m}
	6 dS/m^{-1}	92.47 ± 0.21^{f}	102.33 ± 1.53^{k}	11.33 ± 0.58^{fgh}	10.33 ± 0.58^{ij}
	8 dS m^{-1}	57.23 ± 0.15^{s}	101.67 ± 1.53^{k}	12.00 ± 1.00^{def}	17.33 ± 0.58^{b}
0 MeSA	12 dS m^{-1}	86.30 ± 0.17^{i}	137.00 ± 2.00^{fg}	11.67 ± 1.53^{efg}	14.00 ± 1.00^{d}
	15 dS m^{-1}	93.77 ± 0.15^{e}	112.33 ± 1.53^{j}	12.67 ± 0.58^{cde}	9.33 ± 0.58^{jk}
	17 dS m^{-1}	89.3 ± 0.10^{h}	138.00 ± 1.00^{f}	14.00 ± 1.00^{ab}	18.33 ± 0.58^{ab}
	0.1mM MeSA	92.33 ± 0.21^{f}	153.00 ± 1.00^{c}	10.67 ± 0.58^{gh}	12.33 ± 0.58^{fg}
Non- salt	0.5mM MeSA	70.87 ± 0.15^{o}	157.33 ± 1.53^{b}	14.33 ± 0.58^{a}	7.33 ± 0.58^{lm}
	1.0mM MeSA	54.03 ± 0.21^{t}	115.00 ± 1.00^{i}	9.33 ± 0.58^{i}	8.00 ± 1.00^{l}
	0.1mM MeSA	95.57 ± 0.15^{d}	151.33 ± 1.53^{c}	11.00 ± 1.00^{fgh}	8.33 ± 0.58^{kl}
6 dS m^{-1}	0.5mM MeSA	92.53 ± 0.06^{f}	155.67 ± 1.53^{b}	11.00 ± 1.00^{fgh}	13.33 ± 0.58^{def}
	1.0mM MeSA	43.27 ± 0.21^{u}	101.33 ± 0.58^{k}	11.33 ± 0.58^{fgh}	11.00 ± 1.00^{hi}
	0.1mM MeSA	97.47 ± 0.12^{c}	151.33 ± 1.15^{c}	13.67 ± 0.58^{abc}	13.33 ± 0.58^{def}
8 dS m^{-1}	0.5mM MeSA	81.83 ± 0.12^{l}	156.33 ± 1.53^{b}	10.33 ± 0.58^{hi}	17.67 ± 0.58^{ab}
	1.0mM MeSA	81.13 ± 0.23^{m}	101.33 ± 1.15^{k}	12.67 ± 0.58^{cde}	18.67 ± 0.58^{a}
	0.1mM MeSA	84.23 ± 0.15^{k}	175.33 ± 2.52^{a}	13.00 ± 1.00^{bcd}	9.33 ± 0.58^{jk}
12 dS m^{-1}	0.5mM MeSA	85.77 ± 0.15^{j}	146.33 ± 2.31^{d}	11.33 ± 0.58^{fgh}	14.33 ± 0.58^{d}
	1.0mM MeSA	84.33 ± 0.15^{k}	138.00 ± 1.00^{f}	10.33 ± 0.58^{hi}	11.67 ± 0.58^{gh}
	0.1mM MeSA	74.30 ± 0.27^{n}	133.33 ± 1.53^{h}	11.33 ± 1.16^{fgh}	16.00 ± 1.00^{c}
15 dS m^{-1}	0.5mM MeSA	99.20 ± 0.20^{a}	135.33 ± 1.53^{gh}	10.33 ± 0.58^{hi}	9.33 ± 0.58^{jk}
	1.0mM MeSA	98.03 ± 0.06^{b}	103.00 ± 1.00^{k}	13.67 ± 0.58^{abc}	10.33 ± 0.58^{ij}
	0.1mM MeSA	64.90 ± 0.10^{q}	139.00 ± 1.00^{f}	9.33 ± 0.58^{i}	13.67 ± 0.58^{de}
17 dS m^{-1}	0.5mM MeSA	66.53 ± 0.15^{p}	142.33 ± 1.53^{e}	11.67 ± 0.58^{efg}	12.67 ± 0.58^{efg}
	1.0mM MeSA	64.03 ± 0.15^{r}	139.00 ± 0.58^{f}	12.00 ± 1.00^{def}	10.67 ± 0.58^{hi}

Each value represents the mean ± standard error (P<0.05). Mean with column similar letters do not differ significantly.

seedling, the shoots dry weight in control plants (T0) was 4.0 mg (Table 3). The highest shoot dry weight (16 mg) was observed in T5 (0 MeSA + 17 dS m^{-1}). The 0.1, 0.5 and1.0 mM MeSA treatments decreased the shoot dry weight in T9, T10, T11 when compared with control non-MeSA (T1) under 6 dS m^{-1} and T18, T19, and T20 when compared with control non-MeSA (T4) under 15 dS m^{-1}, and T21, T22, and T23 when compared with control non-MeSA (T5) under 17 dS m^{-1}. At 8 m^{-1}, the shoot dry weight in T12 (8 mg) treated with 0.1 mM of MeSA was higher ($P < 0.05$) than the control T2 non-MeSA (6.67 mg).

The data effect of MeSA seeds treatment on the fresh weight and dry weight of roots under salt stress are given in Table 3. Results showed that the highest value (99.20 mg) was observed in G1 seedlings treated with 0.5 mM MeSA + 15 dS m^{-1} salinity and G2 seedlings treated with 0.1 mM MeSA + 12 dS m^{-1} salinity (175.33 mg). However, the root fresh weight reduction induced by MeSA concentrations (0.1, 0.5, and 1.0 mM) was observed in G1 seedlings under 12 dS m^{-1} and 17 dS m^{-1} salinity but it was recorded with an increase under 8 dS m^{-1} when compared to T2 (0 MeSA + 8 dS m^{-1}). Talebi et al. (2012) had shown treating the borage seeds with salicylic acid has increased the root's fresh weight. In this study, results showed that the greater values (95.57 and 98.03 mg were observed in G1 seedlings treated with 0.1 mM MeSA + 6 dS m^{-1} salinity and 1.0 mM MeSA + 15 dS m^{-1} salinity, respectively when compared to T1 (0 MeSA + 6 dS m^{-1}) and T4 (0 MeSA + 15 dS m^{-1}). For G2 seedling, the root fresh weight showed a positive effect on treatments treated with 0.1 and 0.5 mM MeSA as compared with control non-MeSA under 6, 8, and 15 dS m^{-1} ($P < 0.05$). In comparison with control non-MeSA under 12 and 17 dS m^{-1}, results showed also that a higher value was observed in G2 seedlings treated with 0.1, 0.5, and 1.0 mM MeSA ($P < 0.05$).

In this study, among all treatments of G1 seedling, the highest value of roots dry weight (14.33 mg) was shown in T7 (0.5 mM MeSA) under the control condition of non-salt (Table 3). There was a negative effect on dry root weight of G1 seedling treated MeSA under 6 and 17 dS m^{-1} as compared to the control non-MesA T1 and T5 ($P < 0.05$). However, results showed that the greater values were observed in G1 seedlings treated with 0.1

mM MeSA under 8 (13.67 mg), 12 dS m^{-1} salinity (13 mg) and 1.0 mM MeSA under 15 dS m^{-1} salinity (13.67 mg) as compared to T2 (0 MeSA + 8 dS m^{-1}), T3 (0 MeSA + 12 dS m^{-1}) and T4 (0 MeSA + 15 dS m^{-1}). In G2 seedling, treatment with concentration (1.0 mM) of MeSA under 8 dS m^{-1} salinity was more effective in increasing roots dry weight (18.67 mg) than higher other treatments ($P < 0.05$) (Table 3). While treated G2 seedlings with MeSA (0.1, 0.5, and 1.0 mM) had a negative lower roots dry weight than those in control T4 (0 mM+ 12 dS m^{-1}) and T5 (0 mM+ 17 dS m^{-1}). However, results was also showed that treatments of T10 (0.5 mM MeSA + 6 dS m^{-1}), T11 (1.0 mM MeSA + 6 dS m^{-1}), T13 (0.5 mM MeSA + 8 dS m^{-1}), T18 (0.1 mM MeSA + 15 dS m^{-1}), and T20 (1.0 mM MeSA + 15 dS m^{-1}) with MeSA increased the roots dry weight as compared to controls non-MeSA (T1, T2, T4, and T5) (Table 3).

This indicates that the symptoms frequently observed in the low-or high-dosage treated plants are enhancement or inhibition of seedling growth responses (Wi et al. 2007). The effect of exogenous SA on growth depends on concentration and plant species (Jayakannan et al. 2015). Considering the results from conducted experiments, it can be concluded that the increase of growth traits in rice is due to genetic change as a result of the MeSA treatment. This demonstrated that with different concentrations of MeSA and salinity, there was a significant increase or decrease in the fresh and dry mass of plants. Further investigations, field experiments, and molecular studies will give more information about the level of salinity tolerance.

Effects of MeSA on Chlorophyll a and b Contents

Chlorophyll content varies due to salinity, which ultimately affects plant growth and development (Acosta -Motos et al. 2017). Some physiological pathways such as photosynthesis, respiration, nitrogen fixation, and carbohydrate metabolism are greatly affected by high salinity, with the chlorophyll content in rice decreasing under salt pressure (Chandramohanan et al. 2015). The toxic effects observed on the leaves were mainly necrosis and loss of chlorophyll mostly in higher leaves NaCl concentration (Rahneshan et al. 2018). In the present study, the highest chlorophyll-a and chlorophyll-b contents were observed in G1 treated with 0.1 mM MeSA + 17 dS m^{-1} (1.83 and 2.418 µg/mL) (Fig. 6 and Fig. 7). The result also showed that chlorophyll a content increased for G1 treated MeSA under normal conditions but decreased under 6 and 8 dS m^{-1} when compared with that control no-MeSA ($p < 0.05$). Aside from this, the higher chlorophyll reduction was observed in G1 seedlings treated with 0.1 mM MeSA (chlorophyll a: 1.476 µg/mL and chlorophyll b: 2.215 µg/mL) and 1 mM MeSA (chlorophyll a: 1.158 µg/mL) when compared to non-MeSA treatment under 15 dS m^{-1}. On the other hand, chlorophyll a content G1 seedling treated MeSA increased under 17 salinity, and statistically significant differences between controls and other treatments treated MeSA (Fig. 6). A significant decrease in chlorophyll b was observed in 6 dS m^{-1} salinity of G1 seedling treated MeSA (Figure 6). Salinity had no significant effect on the chlorophyll b of G1 seedling treated with 0.1 and 0.5 MeSA under 8 and 12 dS m^{-1}.

In the case of G2 seedlings, the control plants not treated with MeSA showed significant change in chlorophyll a content under different salinity conditions (Fig. 6). Compared to the control plants non-MeSA under salt stress, G2 seedling treated different concentrations of 0.5 and 0.1 mM MeSA were significantly enhanced chlorophyll a under 6 and 17 dS m^{-1}, and another under 8, 12, and 15 dS m^{-1} were detected at 1 mM MeSA (Fig. 6).

Chlorophyll b content in G2 seedling treated with MeSA had a significantly negative effect by 6 and 17 dS m^{-1} salinity when compared to those without MeSA. However, chlorophyll b in G2 seedling treated with 0.1-1.0 mM of MeSA (8 dS m^{-1}), 0.1 mM MeSA (12 dS m^{-1}),

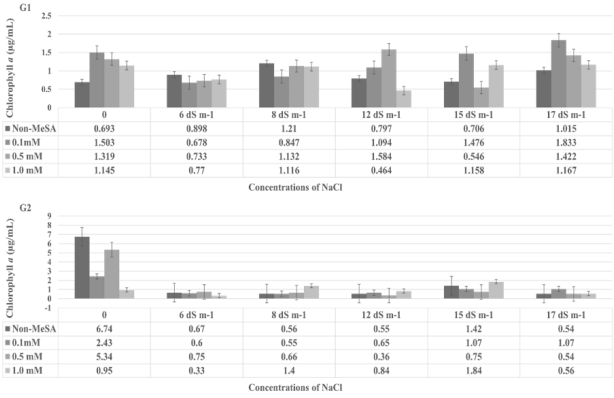


Fig. 6. Effect of MeSA on chlorophyll a of rice in different salt conditions at the seedling stage.

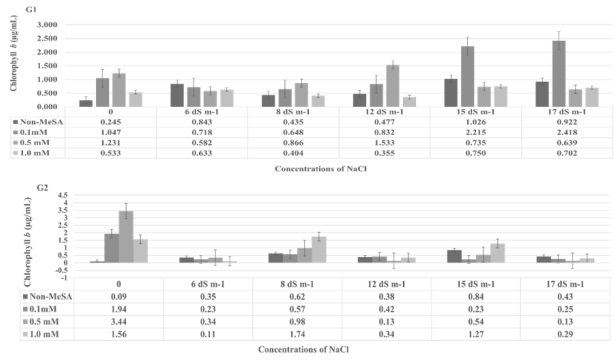


Fig. 7. Effect of MeSA on chlorophyll b of rice in different salt conditions at the seedling stage.

and 1 mM MeSA (15 dS m^{-1}) accumulated at a higher level than in plants non-treated with MeSA treatment (Fig. 7). Several studies suggest chlorophyll content as a biochemical marker of salt tolerance in plants. Our results are in agreement with the previously reported for mustard brassica was observed in the 1 mM MeSA + 50 mM NaCl treatment (Ha et al. 2019). Therefore, depending on the MeSA concentration, salinity and different rice varieties can significantly increase the chlorophyll content in rice. Thus, more studies are needed to decipher the exact role of MeSA in influencing the photosynthetic parameters during salt stress.

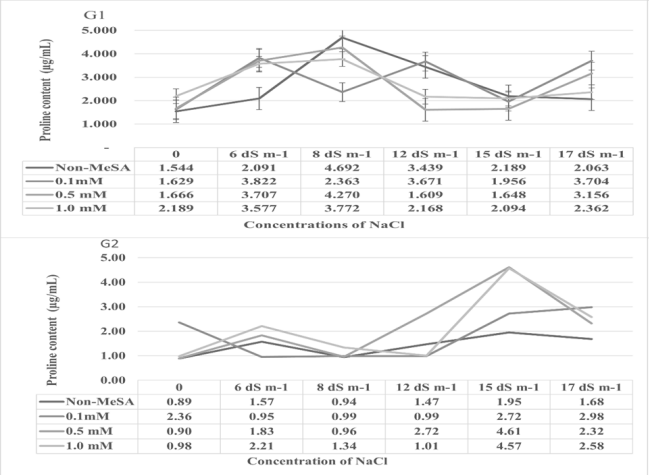
Effects of MeSA on Proline Content

Proline is the most common endogenous osmolyte accumulated under various abiotic stresses including salinity (Ashraf and Foolad 2007; Szabados and Savouré 2010; Hayat et al. 2012). Nakamura et al. (2002) reported that there was proline accumulation in response to salinity stress of rice. In this study, for G1 seedling treated with concentrations of MeSA, there was positive increased proline content under the control condition (Fig. 7). However, for the MeSA treatment of G1 seedling, there was a negative effect on proline content for the treatments under 8 and 15 dS m^{-1}, with only the MeSA treatments for the positive effect at 6 and 17 dS m^{-1} compared to treatments non-treated with MeSA and grown the same saline condition (Fig. 8). Even though the effect of salinity stress is significant in inducing a drastic change in the proline parameter , the 0.1 mM MeSA of G1 seedling treatment improved proline content, especially at salinity 12 dS m^{-1} (Fig. 8).

In the case of proline in G2 seedling treated with 0.1-1.0 mM of MeSA under 15 and 17 dS m^{-1}, accumulation was at a higher level than in plants with no MeSA treatment at the same saline condition, and the highest was observed at 0.5 mM MeSA + 15 dS m^{-1} (Fig. 8). In addition, proline content in the G2 treated with 0.5 mM MeSA (under 6 and 12 dS m^{-1}) and 1 mM MeSA (under 6 and 8 dS m^{-1}) has positively increased when compared to those without non-MeSA treatment at the same salinity (Fig. 8). These results were suggested that MeSA improved the proline content under salt stress levels. These results are in agreement with the previous study of Ha et al. (2019) who showed the increase in proline by the application of MeSA in mustard brassica. The increased accumulation of proline in plants was correlated with improved salinity tolerance (Hien et al. 2003; Hasanuzzaman et al. 2014). These results are also in agreement with those of Sultana et al. (1999) on rice and Soussi et al. (1999) on green beans, which showed that the plants treated with MeSA increased proline content under salt stress compared to those grown without MeSA treatment. The results of the present study demonstrated that depending on the MeSA concentration, salinity, and different rice varieties, the proline content in rice can improve salinity tolerance.

Effects of MeSA on Total Phenolic and Flavonoid Contents

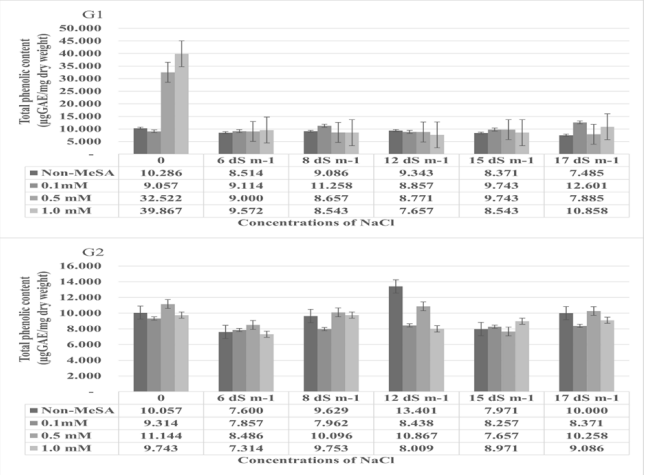
Many recent studies have shown that sensitivity to salt stress is primarily related to oxidative stress (Omer et al. 2017). To avoid damage caused by salt stress, plants produce polyphenolic compounds (such as phenolic acid, flavonoid, proanthocyanidin, and anthocyanin) known as antioxidant metabolites to prevent the spread of oxidative chain reactions. These polyphenolic compounds play an important role in reducing the adverse effects caused by salinity (Hichem et al. 2009). Park et al. (2007) reported that MeSA is also proposed as a mobile signal for systemic salt resistance. In the current



	0	6 dS m-1	8 dS m-1	12 dS m-1	15 dS m-1	17 dS m-1
Non-MeSA	1.544	2.091	4.692	3.439	2.189	2.063
0.1mM	1.629	3.822	2.363	3.671	1.956	3.704
0.5 mM	1.666	3.707	4.270	1.609	1.648	3.156
1.0 mM	2.189	3.577	3.772	2.168	2.094	2.362

	0	6 dS m-1	8 dS m-1	12 dS m-1	15 dS m-1	17 dS m-1
Non-MeSA	0.89	1.57	0.94	1.47	1.95	1.68
0.1mM	2.36	0.95	0.99	0.99	2.72	2.98
0.5 mM	0.90	1.83	0.96	2.72	4.61	2.32
1.0 mM	0.98	2.21	1.34	1.01	4.57	2.58

Fig. 8. Effect of MeSA on proline content of rice in different salt conditions at the seedling stage.



	0	6 dS m-1	8 dS m-1	12 dS m-1	15 dS m-1	17 dS m-1
Non-MeSA	10.286	8.514	9.086	9.343	8.371	7.485
0.1mM	9.057	9.114	11.258	8.857	9.743	12.601
0.5 mM	32.522	9.000	8.657	8.771	9.743	7.885
1.0 mM	39.867	9.572	8.543	7.657	8.543	10.858

	0	6 dS m-1	8 dS m-1	12 dS m-1	15 dS m-1	17 dS m-1
Non-MeSA	10.057	7.600	9.629	13.401	7.971	10.000
0.1mM	9.314	7.857	7.962	8.438	8.257	8.371
0.5 mM	11.144	8.486	10.096	10.867	7.657	10.258
1.0 mM	9.743	7.314	9.753	8.009	8.971	9.086

Fig. 9. Effect of MeSA on total phenolic content of rice in different salt conditions at the seedling stage.

study, the application of MeSA to the 12 dS m^{-1} salinity did not increase the phenolic content in both two varieties ($P < 0.05$) (Fig. 9). However, MeSA application to the 6, 8, 15, and 17 dS m^{-1} salinity significantly ($P < 0.05$) increased the phenolic content from 0.1-1.0mM MeSA, except for the concentration of 0.5 and 1.0 mM MeSA (8 dS m^{-1}), 0.1 mM MeSA (17 dS m^{-1}) in G1 seedling (Fig. 8). Moreover, the phenolic content was significantly ($P < 0.01$) enhanced in G2 seedling by the application of MeSA to the different salt concentrations as 0.1 and 0.5 (6 dS m^{-1}), 0.5 and 1mM (8 dS m^{-1}), 0.1 and 1mM (15 dS m^{-1}) and 0.5 mM (17 dS m^{-1}) when compared to control no-MeSA treatment at the same saline condition (Fig. 9). Li et al. (2017) and Ha et al. (2020) concluded that MeSA led to increased phenolic and flavonoid content on germination of rice depending on the MeSA concentration applied. Li et al. (2019) reported that the application of methyl salicylate enhanced the flavonoid content at 1 mM and flavonoid content at 5 mM in tea leaves. In the current study, the application of MeSA to the 12 dS m^{-1} salinity decreased the flavonoid content in both two varieties ($P < 0.05$) and other salinity concentrations in G2 seedling except 0.5 mM MeSA under 6 dS m^{-1} as compared to control no-MeSA in the same conditions (Fig. 10). However, flavonoid content in G1 seedlings treated with MeSA was a significantly positive effect by 6 dS m^{-1} salinity when compared to those without MeSA. Also, the flavonoid content was significantly ($P < 0.01$) higher in G1 seedling by the application of MeSA to the different salt concentrations as 0.1 mM (8 and 15 dS m^{-1}), 0.5 mM (15 and 17 dS m^{-1}) when compared to control no-MeSA treatment at the same saline condition (Fig. 10). This indicates that the positive effect of MeSA on the flavonoids depends on different concentrations and rice varieties under various salinity stress. This study agrees with our previous study. These results are in agreement with those of our study on rice during germination which showed that the seed rice treated with MeSA and subsequently subjected to NaCl possessed phenolic and flavonoid contents compared to those grown without MeSA treatment (Ha et al. 2020).

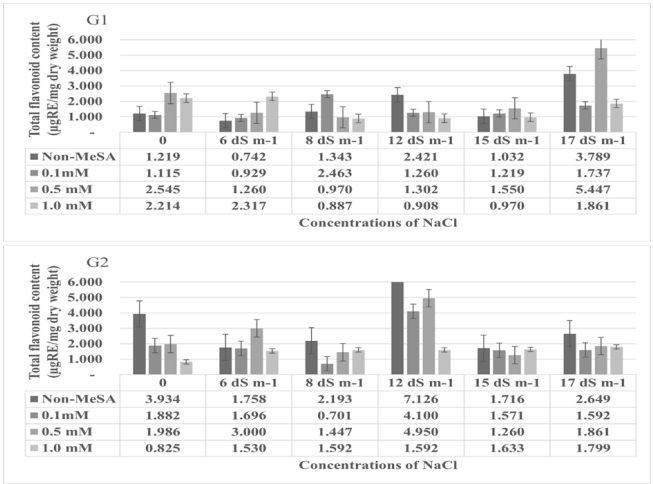


Fig. 10. Effect of MeSA on total flavonoid content of rice in different salt conditions at the seedling stage.

In our performed experiment, we show how both growth and biochemical characteristics can cause positive and negative effects which can be for the application of different MeSA concentrations in improving salinity tolerance of rice at the seedling stage. The results by Ding et al. (2002) on tomatoes also showed that the increased salt stress tolerance by application to the concentration of 0.01 mM MeSA. In the current study, the application of MeSA also contributed to the reduction of the inhibitory effect of salinity but with different responses from the two cultivars. It can be inferred that the range of growth and biochemical characteristics through induced MeSA is random, bi-directional, and the direction of the mutagenesis depends on the genotype/salinity condition/traits under study and the dose applied. The results of the present study provide useful information regarding the effects of MeSA on rice during the plating stage under salt stress to develop new methods to improve the salinity tolerance of rice.

CONCLUSION

The results showed that the effect of MeSA on the physiology and biochemical characteristics was dependent on concentration and varieties by changes in different salinities. For G1 seedling, physiology parameters (root length, the shoot dry weight, and roots dry weight) under 6 dS m^{-1}, 8 dS m^{-1}, respectively, and biochemical characteristics (chlorophyll a, chlorophyll b, phenolic content) under 17 dS m^{-1} were positively affected by the concentration of 0.1 mM MeSA. The physiology parameters (survival rate, seedling height, the shoot fresh weight, the root fresh weight) under 6 and 8 dS m^{-1} and biochemical characteristics (proline content and flavonoid content) under 17 dS m^{-1} were positively affected by the concentration of 0.5 mM MeSA. For G2 seedlings, the root length under 15 dS m^{-1} was positively affected by the concentration of 0.1mM MeSA while at 0.5 increased the shoot dry weight (17 dS m^{-1}), the root fresh weight (8 dS m^{-1}), proline content (15 dS m^{-1}), phenolic and flavonoid contents (12 dS m^{-1}). Moreover, MeSA at 1.0 mM increased the survival rate (6 dS m^{-1}), seedling height (15 dS m^{-1}), the shoot fresh weight (6 dS m^{-1}), roots dry weight (8 dS m^{-1}),

chlorophyll a (15 dS m^{-1}), and chlorophyll b (8 dS m^{-1}). Further research should be conducted to create new methods that can help improve rice production and grow rice under the pressure of salt. These seedlings will be more analyzed for screening for the salt tolerance improvement of rice.

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