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Mitigation of salt stress in rice plant at germination stage by using methyl jasmonate

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Abstract: To explore the possibility of using methyl jasmonate (MeJA) for alleviation of salt stress, the present study was conducted where six rice varieties (BRRI dhan31, BRRI dhan46, Gota, Kajalsail, Pokkali and Pengek) were grown in non saline (0 dSm⁻¹), saline (12 dSm⁻¹), saline (12 dSm⁻¹) + 10 μM MeJA and saline (12 dSm⁻¹) + 20 μM MeJA conditions at germination stage (till the 9 days). MeJA was applied by imbibing seeds in it for 24 hours. To evaluate the effect of MeJA on saline stressed plant at germination stage, change in growth parameters namely germination percentage, shoot and root length, fresh and dry wt. of shoot, fresh and dry wt. of root and biochemical component- activity of alpha-amylase were monitored. Salinity had a minimum effect on final germination percentage (FGP) of rice varieties, but delayed in attaining it. MeJA had least positive effect. Salinity significantly reduced the shoot and root length, fresh and dry weight of all the varieties. MeJA had more decreasing effect in susceptible varieties while increasing effect in moderately tolerant and tolerant. Activity of alpha-amylase in germinated seed varied from 0.051 mg to 0.111 mg mal./mg tissue. At 12dSm⁻¹ salinity, susceptible and moderately tolerant varieties showed significant reduction but tolerant showed significant increase. MeJA intensified the negative effect further in susceptible varieties. In tolerant and moderately tolerant varieties, increment in activity took place and 10 μM had a better effect over 20 μM MeJA.

Keywords: methyl jasmonate; rice; growth parameter; alpha- amylase

1. Introduction

Salinity is one of the most severe problems of agriculture worldwide. In the global context approximately 20% of the irrigated lands in the world are presumably affected by soil salinization (Yeo 1999). Coastal salinity and accumulation of salts in the irrigated land primarily decrease crop yield. In Bangladesh, coastal area covers about 20% of the country and over thirty percent of the net cultivable area. Out of 2.85 million hectares of the coastal and offshore areas, about 1.5 million hectares coastal and offshore land is affected by different degrees of salinity. So, for growing crops in those areas, innovative and commercially feasible alternative need to be explored.

Rice (*Oryza sativa*) is the most important and extensively grown food crop and leading cereal in the world including Bangladesh. It is one of the most widely grown crops in coastal areas as well and frequently inundated with saline seawater during high tidal period. Sudden up-surfing of saline water demands necessary measures to counteract the saline stress for a short period. Post-application with exogenous jasmonic acid (JA) can ameliorate salt-stressed rice seedlings (Kang *et al.*, 2005).

Abiotic stress leads to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity. Rice varieties show a great variation in germination due to salinity effect (Sharmin 2012; Khan *et al.*, 1997). Several reports suggest that saline condition causes delayed germination (Prado *et al.*, 1995) by reducing hydrolytic activities (Ashraf *et al.*, 2002). Mobilization of seed

reserves, which occurs during early seed germination, is crucial. Alpha amylase plays an important role in starch degradation in germinating seeds and mobilization of reserved material required for the growth of young seedling (Chen *et al.*, 2006).

Methyl jasmonate (MeJA) and jasmonic acid (JA) are collectively referred to as jasmonate and are important cellular regulators which are involved in diverse developmental process such as germination, root growth, fertility, fruit ripening and senescence (Creelman and Rao 2002; Westernack and House 2002).

The response of plants to salt stress depends on multiple factors, but phytohormones are thought to be among the most important endogenous substances involved in the tolerance or susceptibility. Exogenous JA application after salt treatment may change the balance of endogenous hormones, such as ABA, which contribute in protection mechanisms against salt stress (Kang *et al.*, 2005). Pre-treatment with JA reduced the inhibitory effect of high salt concentrations on growth and photosynthesis (Tsonev *et al.*, 1998; Yoon *et al.*, 2009). Exogenously applied jasmonates elicit several different physiological responses to stress and therefore increase plant resistance (Walia *et al.*, 2007; Ding *et al.*, 2002).

It should be noted that there is a lack of studies on the ameliorative effect of jasmonate on crop production under saline conditions and additional studies on this topic are warranted. The aim of the current study was to evaluate the role of MeJA in counteracting salinity stress in rice plant at germination stage. The effect of MeJA on alpha-amylase activity as well as on growth parameters such as germination percentage, shoot and root length, fresh and dry wt. of shoot and fresh and dry wt. of root in salt stressed rice plant at their germination stage was monitored.

2. Materials and Methods

The experiment was conducted at the laboratory of the Department of Biochemistry and Molecular Biology, Bangladesh Agricultural University, Mymensingh, during the period from January to November, 2014.

2.1. Plant material

Two high yielding varieties (BRRI dhan31 and BRRI dhan46) and four local varieties (Pokkali, Pengek, Kajalsail, Gota) were used in the experiment. Among the varieties, BRRI dhan31 and BRRI dhan46 are susceptible, Kajalsail and Gota are moderately tolerant and Pokkali and Pengek are tolerant to salt stress as reported by Bangladesh Rice Research Institute (BRRI).

2.2. Treatments application

Salinity (12 dSm^{-1}) combined with two levels of methyl jasmonate ($10 \mu\text{M}$ and $20 \mu\text{M}$) were applied. Rice (*Oryza sativa*) seeds were sterilized with 2.5% sodium hypochlorite for 15 min and washed thoroughly with distilled water. These seeds were then placed in deep petri dishes containing filter papers. Each petri dish contained 25 rice seeds. In case of control (T_0), 10 ml of distilled water was added to the petri dish. To develop salinity stress T_1 ($\text{EC}=12 \text{ dSm}^{-1}$), filter papers were moistened with 10 ml of salt solutions. In case of treatments, T_2 and T_3 , seeds were imbibed in presence of methyl jasmonate for 24 h before placement to the petri dishes. Properly imbibed seeds were placed in petri dishes containing moistened filter papers to allow them to germinate. Each treatment was performed three times. The seeds were allowed to germinate at around 30°C in the dark for 9 days.

2.3. Determination of growth parameters

2.3.1. Germination percentage

The number of sprouted and germinated seeds was counted daily commencing from 4th day till the 9th. After 9 days, final count was done and germination percentage of each day was calculated by the following formula:

Germination Percentage (%GE) = (Total number of seeds germinated/Total number of seeds taken for germination) $\times 100$.

2.3.2. Shoot and root length

Shoot and root length of five randomly selected seedlings from each replication were measured daily from 5th to 9th days withdrawing the seedlings from the petri dish.

2.3.3. Fresh and dry weight of shoot and root

Fresh weight of shoot and root were determined immediately after harvest. Dry weight was recorded by drying the sample in an oven at 70°C till attained a constant weight.

2.3.4. Determination of alpha-amylase activity

Alpha-amylase activity was determined spectrophotometrically by using 3, 5 dinitrosalicylic acid (DNSA) following the procedure of Bernfeld (1955). After 9 days of salt treatment, germinating seeds were used for the determination of amylase activity. Growing embryonic tissues (scutellum, roots, and shoots) and seed coat were carefully removed from rice seeds. Endosperm tissues were used for amylase extraction. Five hundred milligrams endosperm tissue was crushed with 10 ml of 0.02 M phosphate buffer at pH 6.0. The homogenate was centrifuged at 2500 rpm for 20 minutes. The supernatant was used to determine the enzyme activity.

2.4. Statistical analysis

The experiment were arranged in a completely randomized design with three replications and the data collected and calculated were analyzed using MSTAT – C computer programs and comparison of means were tested for significance using least significant difference (LSD) test, at 0.01 level of probability.

3. Results

3.1. Germination percentage

Salinity imposed a negligible reduction in all rice varieties from 4th to 9th days (Table 1). In this study, the effect of salinity on final germination percentage (FGP) of rice seeds was not pronounced. Under control condition, two susceptible varieties showed 100% and 96% germination at 4th day respectively. In each treatment, the maximum germination rate attained by a variety was considered as final germination percentage (FGP) and it was 100% in case of both BRRI dhan31 and BRRI dhan46. Under saline condition, BRRI dhan31 showed a slight reduction (96%) whereas BRRI dhan46 had the same value compared to the control plants.

Exogenously applied MeJA had negligible effect. When the rice seeds of BRRI dhan31 was treated with 10 μ M and 20 μ M MeJA and were grown in saline condition, the observed germination percentage (%GE) were 98% and 96% respectively. Under similar condition, BRRI dhan46 had 100% and 96% respectively. Moderately tolerant varieties, Gota and Kajalsail showed similar FGP (100) at 9th day under control condition. Salinity caused slight reduction in FGP (96 and 98). MeJA treatment had positive effect and FGP reached to 100% on MeJA treatment in case of both the varieties as well as treatments (10 μ M and 20 μ M). Tolerant varieties, Pokkali and Pengek had 100% FGP under control condition. However, under saline condition, Pokkali showed a reduction (96%) compared to that of control at 9th day. Both varieties attained a maximum germination percentage (100%) at 10 μ M MeJA condition. At 20 μ M MeJA treatment, Pokkali maintained its optimum germination rate but reduction (98%) was noticed in Pengek.

3.2. Shoot length

It appeared that saline conditions significantly reduced the shoot length of all varieties (Figure 1). At the salt concentration, reduction was ranged from 25.96% to 49.14%. In applying MeJA, reduction was further enhanced in the susceptible varieties and the maximum reduction (3 times) was shown by BRRI dhan46 with the application of 20 μ M MeJA. In case of moderately tolerant and tolerant varieties, positive effect was monitored and increase rate ranged from 5.61% to 19.52% in moderately tolerant while 0.929% to 12.21% in tolerant varieties.

3.3. Root length

The root length of all the rice varieties was significantly reduced in saline condition and the reduction rate ranged from 37.51% to 52.63% (Figure 2). On applying MeJA, further reduction was noticed in the susceptible varieties and the reduction rate ranged from 8.36% to 41.16%. In case of moderately tolerant and tolerant varieties, reduction due to salinity was mitigated and root length increase rate ranged from 4.51% to 6.13% in moderately tolerant while 2.94% to 18.91% in tolerant varieties. Better performance was observed in Pokkali on applying 20 μ M MeJA.

3.4. Fresh and dry weight of shoot

Shoot fresh wt. and dry wt. was inversely proportional to salinity i.e. decrease in saline condition (Table 2). Reduction rate ranged from 23.81% to 59.09% in case of fresh wt. while 16.67% to 61.11% of dry wt. On applying MeJA, both fresh and dry wt. were decreased in susceptible varieties which ranged from 4.80% to 37.63% in case of fresh wt. while 12.5% to 38.10% of dry wt. compared to saline condition. In case of moderately tolerant and tolerant varieties, both fresh and dry wt. was further enhanced upon applying MeJA. Maximum increase of fresh wt. was observed in Pengek upon 20 μ M MeJA application while in case of dry wt., both tolerant varieties got maximum increase.

Table 1. Changes in germination percentage of rice seed under salt stress and MeJA application.

	Variety	Days of germination	Germination percent (%GE)			
			T ₀	T ₁	T ₂	T ₃
Susceptible variety	BRR1 dhan31	4 th day	100	88	96	96
		5 th day	100	92	98	96
		6 th day	100	96	98	96
		7 th day	100	96	98	96
		8 th day	100	96	98	96
		9 th day	100	96	98	96
	BRR1 dhan46	4 th day	96	96	92	88
		5 th day	100	100	96	96
		6 th day	100	100	100	96
		7 th day	100	100	100	96
		8 th day	100	100	100	96
		9 th day	100	100	100	96
Moderately Tolerant variety	Kajalsail	4 th day	96	92	100	100
		5 th day	100	96	100	100
		6 th day	100	96	100	100
		7 th day	100	96	100	100
		8 th day	100	96	100	100
		9 th day	100	96	100	100
	Gota	4 th day	100	92	100	100
		5 th day	100	98	100	100
		6 th day	100	98	100	100
		7 th day	100	98	100	100
		8 th day	100	98	100	100
		9 th day	100	98	100	100
Tolerant variety	Pokkali	4 th day	100	96	100	96
		5 th day	100	96	100	100
		6 th day	100	96	100	100
		7 th day	100	96	100	100
		8 th day	100	96	100	100
		9 th day	100	96	100	100
	Pengek	4 th day	100	96	100	98
		5 th day	100	100	100	98
		6 th day	100	100	100	98
		7 th day	100	100	100	98
		8 th day	100	100	100	98
		9 th day	100	100	100	98

Table 2. Changes in fresh weight and dry weight of shoot of rice seedlings under salt stress and MeJA application.

Variety	Fresh weight of shoot (g / 5 plants)			
	T ₀	T ₁	T ₂	T ₃
BRR1 dhan31	0.192 ^a	0.104 ^b (-45.83)	0.099 ^b ^c (-4.80)	0.065 ^c (-37.5)
BRR1 dhan46	0.132 ^a	0.093 ^b (-29.55)	0.062 ^c (-33.33)	0.058 ^c (-37.63)
Kajalsail	0.119 ^a	0.060 ^b (-49.58)	0.062 ^b (+3.33)	0.061 ^b (+1.67)
Gota	0.147 ^a	0.112 ^b (-23.81)	0.126 ^{ab} (+12.5)	0.122 ^b (+8.93)
Pokkali	0.184 ^a	0.086 ^c (-53.26)	0.107 ^{bc} (+24.42)	0.130 ^b (+51.16)
Pengek	0.198 ^a	0.081 ^c (-59.09)	0.124 ^b (+53.08)	0.134 ^b (+65.43)
Dry weight of shoot (g / 5 plants)				
BRR1 dhan31	0.0165 ^a	0.0105 ^b (-36.36)	0.0065 ^b (-38.10)	0.0085 ^b (-19.05)
BRR1 dhan46	0.020 ^a	0.012 ^b (-40)	0.010 ^b (-16.67)	0.0105 ^b (-12.5)
Kajalsail	0.018 ^a	0.007 ^b (-61.11)	0.008 ^b (+14.29)	0.009 ^b (+28.57)
Gota	0.018 ^a	0.015 ^a (-16.67)	0.016 ^a (+6.67)	0.017 ^a (+13.33)
Pokkali	0.026 ^a	0.014 ^c (-46.15)	0.018 ^{bc} (+28.57)	0.019 ^b (+35.71)
Pengek	0.029 ^a	0.014 ^c (-51.72)	0.018 ^b (+28.57)	0.019 ^b (+35.71)

Figures in parenthesis of T₁ were calculated as percentage based on the values in control (T₀) and figures in parenthesis of T₂ and T₃ were computed as percentage based on the values obtained from saline stress (T₁).

(+) indicates “%increase”, (-) indicates “%decrease”. Significant differences in the treatments are shown with different letters (p <0.01).

Table 3. Changes in fresh weight and dry weight of root of rice seedlings under salt stress and MeJA application.

Variety	Fresh weight of root (g / 5 plants)			
	T ₀	T ₁	T ₂	T ₃
BRR1 dhan31	0.133 ^a	0.083 ^b (-37.59)	0.074 ^b (-10.84)	0.067 ^b (-19.27)
BRR1 dhan46	0.142 ^a	0.101 ^b (-28.87)	0.067 ^c (-33.66)	0.058 ^c (-42.57)
Kajalsail	0.154 ^a	0.068 ^b (-55.84)	0.078 ^b (+14.71)	0.072 ^b (+5.88)
Gota	0.103 ^a	0.056 ^b (-45.63)	0.103 ^a (+83.93)	0.101 ^a (+80.36)
Pokkali	0.11 ^a	0.066 ^b (-40)	0.066 ^b (0)	0.071 ^b (+7.58)
Pengek	0.126 ^a	0.026 ^c (-79.37)	0.054 ^b (+107.7)	0.049 ^b (+88.46)
Dry weight of root (g / 5 plants)				
BRR1 dhan31	0.016 ^a	0.009 ^b (-43.75)	0.006 ^b (-33.33)	0.005 ^b (-44.44)
BRR1 dhan46	0.0165 ^a	0.0085 ^b (-48.48)	0.006 ^b (-29.41)	0.007 ^b (-17.65)
Kajalsail	0.0185 ^a	0.007 ^c (-62.16)	0.013 ^b (+92.86)	0.008 ^c (+14.29)
Gota	0.020 ^a	0.011 ^b (-45)	0.014 ^b (+27.27)	0.012 ^b (+9.09)
Pokkali	0.0145 ^a	0.0075 ^b (-48.27)	0.0089 ^b (+18.67)	0.0095 ^b (+26.67)
Pengek	0.0195 ^a	0.005 ^b (-74.36)	0.009 ^b (+80)	0.0095 ^b (+90)

Figures in parenthesis of T₁ were calculated as percentage based on the values in control (T₀) and figures in parenthesis of T₂ and T₃ were computed as percentage based on the values obtained from saline stress (T₁).

(+) indicates “%increase”, (-) indicates “%decrease”. Significant differences in the treatments are shown with different letters (p <0.01).

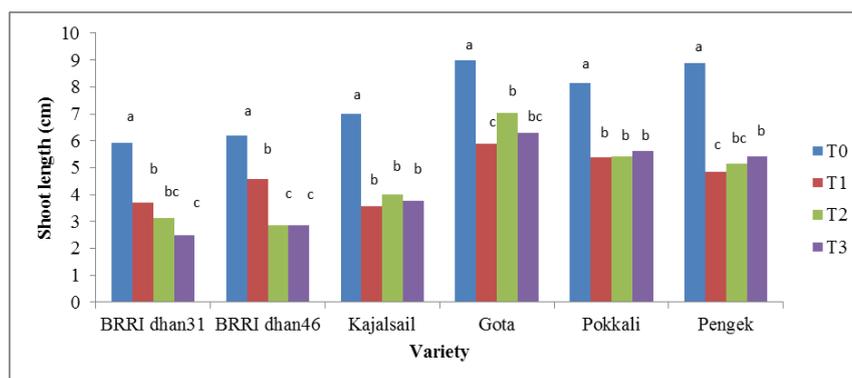


Figure 1. MeJA-induced changes in shoot length of salt stressed rice varieties at 9th day. Significant differences in the treatments are shown with different letters (p <0.01).

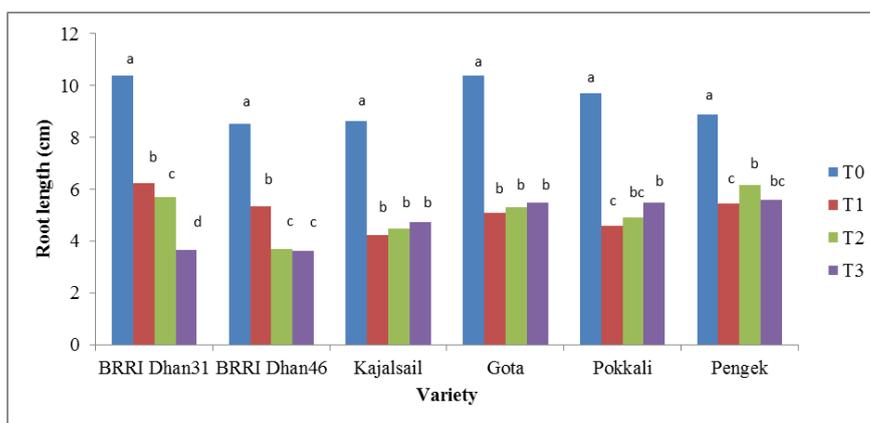


Figure 2. MeJA-induced changes in root length of salt stressed rice varieties at 9th day. Significant differences in the treatments are shown with different letters (p <0.01).

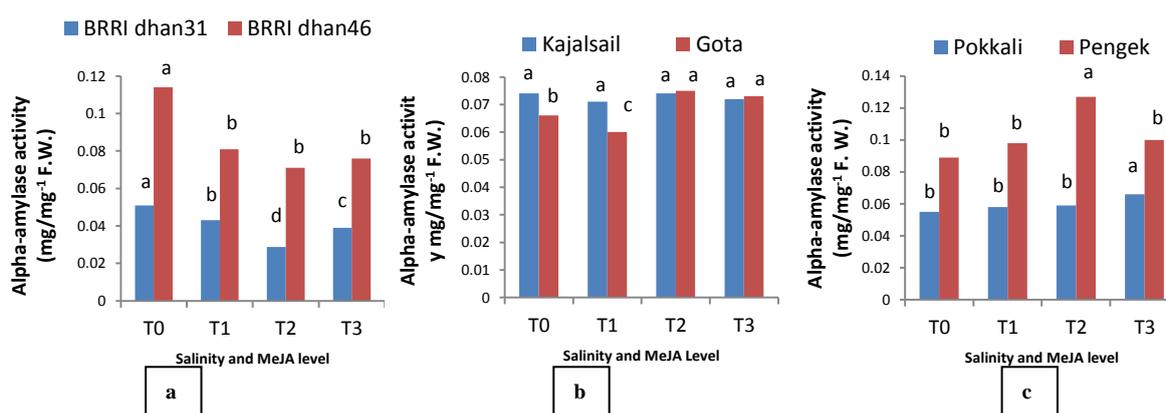


Figure 3. MeJA-induced changes in alpha-amylase activity of salt stressed a) susceptible, b) moderately tolerant, c) tolerant varieties. Significant differences in the treatments are shown with different letters (p <0.01).

3.5. Fresh and dry weight of root

Like shoot wt., all the varieties had significant reduction of both fresh and dry wt. of root on applying salt stress (Table 3). The reduction rate ranged from 28.87% to 79.37% in case of fresh wt. while 43.75% to 74.36% of dry wt. On applying MeJA, except susceptible varieties both moderately tolerant and tolerant varieties had increased and this rate ranged from 0% to 107.7% in case of fresh wt. while 9.09% to 92.84% of dry wt. compared to saline condition. Better performance was shown by Pengek in case of fresh wt. on applying 10 μM MeJA whereas 20 μM in case of dry wt.

3.6. Alpha-amylase activity

In germinated rice seed (control, T₀), alpha-amylase activity varied from 0.051 to 0.114 mg mal. /mg tissue and under saline stress (T₁), the activity ranged from 0.043 to 0.098 mg mal. / mg tissue (Figure 3). At 12 dSm⁻¹ salinity, susceptible and moderately tolerant varieties showed significant reduction while tolerant varieties showed increase in alpha amylase activity.

Upon applying MeJA, negative effect on alpha-amylase activity was observed in the susceptible varieties. In BRRi dhan31, reduction of 33.26% and 9.30% were observed under 10 μM and 20 μM MeJA treated plants respectively while 12.35% and 6.17% reduction occurred in BRRi dhan46 plants. In moderately tolerant varieties, both the varieties showed positive effects upon applying MeJA. In Kajalsail, 4.22% and 1.41% while in Gota 25.0% and 21.67% increase in alpha-amylase activity was observed when 10 μM and 20 μM MeJA were applied in saline stressed rice seedlings. In tolerant varieties, salt stress increased the alpha-amylase activity which was further enhanced by MeJA application. However, no dose dependent enhancement in alpha-amylase activity was found. In Pokkali, an increment of 1.72% and 13.79% while in Pengek 29.59% and 2.04% increment was observed in 10 μM and 20 μM MeJA treated plants respectively.

4. Discussion

Salinity did not produce any noticeable change in the %GE. As a result a substantial positive effect of MeJA against saline stress was hard to ascertain. Nevertheless, MeJA showed a positive effect on %GE at 10 μ M MeJA on 4th day except in BRR1 dhan46. Application of 20 μ M MeJA often demonstrated negative effect. The present study shows that the germination percentage slightly decreased in most of the varieties due to induced salinity. The finding is in line with those of Anbumalaramathi and Mehta (2013); Mondal *et al.* (2015) and Kumar *et al.* (2012). Kapoor (2011) also reported that *Oryza sativa* showed significant reduction in seed germination with 100 mM NaCl concentration in comparison to control. Upon applying MeJA, dual effect occurred in present study which is also supported by Jin-Hua *et al.* (2007). In susceptible varieties, increased level of MeJA reduces the %GE which is also supported by Hasibi *et al.* (2008) and Norastehnia *et al.* (2007). The present study also revealed that low concentration of MeJA increases %GE which is also supported by Jin-Hua *et al.* (2007) and Enteshar and Jafari (2013).

The present study shows that applying salt stress at germination stage reduced the shoot and root length of all the varieties which is also supported by Mondal *et al.* (2015); Ologundudu *et al.* (2014) and Abbas *et al.* (2013). Pre-soaking maize grains with MeJA led to increase in shoot and root length and counteracted the negative effects of NaCl stress on plant (Rezai *et al.*, 2013; Abdelgawad *et al.*, 2014; Yoon *et al.*, 2009). Inhibitory effect also showed by some varieties upon MeJA application which also supported by Enteshar and Jafari (2013). The findings are matching well with result of present study where external use of MeJA counteracted the inhibitory effect of salt stress on shoot length. However, this was true for moderately tolerant and tolerant varieties. In susceptible varieties, MeJA couldn't restrain the inhibitory effect of salt stress.

Hussain *et al.* (2013); Mondal *et al.* (2015) and Shereen *et al.* (2007) reported that applying salinity resulted in reduction in fresh and dry weight of both shoot and root of rice seedlings which are in line with the present work as in susceptible varieties. Upon applying methyl jasmonate, fresh and dry weight increased in moderately tolerant and tolerant varieties which were also mentioned by Rezai *et al.* (2013) and Abdelgawad *et al.* (2014).

The present study shows that alpha-amylase activity in the germinating seeds was reduced significantly due to salt stress which is also mentioned by Ben Dkhil and Denden (2010). Singh (2004) stated that higher activities of amylase was observed in tolerant varieties than in susceptible varieties which is also in line with the present work where tolerant varieties showed increase in alpha-amylase activity. Upon applying MeJA, decreasing activity of alpha amylase in susceptible varieties was recorded which was also supported by Bialecka and Kepczynski (2003) and Jin-Hua *et al.* (2007). Positive effect of MeJA to increase the alpha-amylase activity in salt stressed tolerant plants might be a plausible reason to survive those plants in salinity.

5. Conclusions

Facts on alpha-amylase activity and growth parameters support the positive effect of MeJA in alleviating saline stress. External use of MeJA in seedling and reproductive stages deserves due attention to counteract the threat from salinity.

Conflict of interest

None to declare.

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