Salicylic Acid Improves Tolerance Against Salt Stress Through Boosting Antioxidant Defense System in Black Bean

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ABSTRACT

To evaluate the effect of salicylic acid (SA) on seed germination of black bean plant under saline conditions, seeds were primed with salicylic acid (0, 2, 10, and 20 mM) and germinated under salt stress (0, 50, and 100 mM NaCl). The measured parameters included the percentage and rate of seed germination, seedling length and dry weight, malondialdehyde and hydrogen peroxide levels, and activity of catalase, ascorbate peroxidase and guaiacol peroxidase in the seedlings. Results showed the values of germination indices decreased with increasing the level of salt stress. However, SA priming (10 mM) alleviated the harmful effects of salt stress in black bean. SA increased seed germination percentage by 72% and 45% at 50 and 100 mM NaCl respectively, compared to the control condition. Germination rate augmented by 33% (at 50 mM NaCl) and 60% (at 100 mM NaCl) by SA priming compared to the seeds exposed to salt stress alone. Seedlings dry weight (+ 51% at 50 mM and + 34% at 100 mM) and length (+ 57% at 50 mM and + 29% at 100 mM NaCl) were significantly higher by priming with 10 mM salicylic acid, compared to exclusively salt stress-treated seeds. SA priming increased antioxidant enzymes activities and decreased the levels of lipid peroxidation and hydrogen peroxide in salt stressed black bean seedlings. In conclusion, salicylic acid priming (particularly at 10 mM) enhances salt tolerance in black bean via lessening of oxidative stress.

Introduction

The common bean (Phaseolus vulgaris) is a highly variable species representing a rich source of protein, fiber, vitamins, and minerals. Black bean, as a variety of Phaseolus vulgaris, is an important crop in many Asian countries (such as India, Sri Lanka and China) and an ideal source of high-quality protein with a good balance of amino acids (Jiang et al., 2014). Moreover, the potential health benefits of black beans include: maintaining healthy bones, lowering blood pressure, managing diabetes, decreasing the risk of heart disease, preventing cancer, and healthy digestion. Black bean is well-known for its high amounts of phytochemicals such as polyphenols. Phenolic compounds exhibit high antioxidant capacity that promotes health benefits by reducing oxidative stress. Dong et
al. (2007) have isolated twenty-four compounds (including 12 triterpenoids, 7 flavonoids, and 5 other phytochemicals) from black bean seed coats which have potent antioxidant and antiproliferative activities. Mojica et al. (2015) expressed that black beans have high levels of anthocyanins such as delphinidin and petunidin. These seeds also contain ferulic acid known for its nutraceutical properties. Guajardo-Flores et al. (2013) reported that the extracts obtained from seed coats (containing saponins and flavonoids) of black beans inhibited all cancer cell lines proliferation with no cytotoxicity against control cells. As expressed, the role of genistein was related to its activity against mammary cancer cells but flavonoids and group B saponins were more related to hepatic and colon cancers. However, bean plants are sensitive to salt stress. Salinity, even at quite low levels, markedly reduces bean plant’s growth and yield (Radí et al., 2013).

Environmental stresses, such as salinity, are major limiting factors in agriculture. Salinity is an important factor affecting the growth of crop plants all over the world, particularly in arid and semi-arid region. Furthermore, poor irrigation and/or drainage practices lead to secondary salinization which is an enduring practice in agriculture. As approximated, around 4000 ha of irrigated land in arid and semiarid areas in the world are daily devastated by salinity and become improper for agriculture (Shahala, 2013; Qadir et al., 2014). On the other hand, restoration of salt-affected farming soils is expensive, prolonged and hard process in general. Accordingly, application of simple, inexpensive and convenient methods to enhance salt tolerance in crop plants is important to manage sustainable agriculture (Ondravek et al., 2011).

Seed priming is introduced as an advantageous technique to improve germination characteristics and prompts faster and better germination and emergence and also uniformity of the seedling establishment. In addition, utilization of appropriate seed priming could support seedlings to raise safer under stressful conditions (Jafar et al., 2012).

Salicylic acid (2-hydroxybenzoic acid) is a plant hormone categorized as a type of phenolic acids. First, salicylic acid (SA) was introduced as a defense hormone supporting plants against biotic stresses. However, its role to alleviate harmful effect of abiotic stresses has been increasingly discussed in the recent decade (among many: Ashraf et al., 2010; Hayata et al., 2010; Jayakannan et al., 2015; Abbaspour and Babae, 2017; Shahmoradi and Naderi, 2018; Haghshenas et al., 2020). Application of SA in seed priming to induce salt tolerance has been reported in different plant species such as barley (El-Tayeb, 2005), melon (Basra et al., 2007), hot pepper (Amjad et al., 2007), cucumber (Rehman et al., 2011), wheat (Afzal et al., 2006; Bahrani and Pourreza, 2012), Hedysarum carnosum and Hedysarum coronarium (Dallali et al., 2012), violet (Hussain et al., 2011), tomato (Ghoohestani et al., 2012), maize (Ahmad et al., 2012; Tufail et al., 2013; Bagheri, 2014), rice (Jini and Joseph, 2017), and broad bean (Anaya et al., 2018). Salicylic acid differentially affects physiological processes enhancing some internal mechanisms and inhibiting others depending on its concentration, plant species and environmental conditions (El-Mergawi and Abdel Wahed, 2004). Shi and Zhu (2008) hypothesized that the defensive role of salicylic acid include the regulation of reactive oxygen species and antioxidant enzymes.

The aims of the present study were to evaluate the role of seed priming with salicylic acid on salt tolerance in black bean at seed germination and to find out the possible protective effects of SA to manage oxidative stress (via supporting membrane stability, lessening of H₂O₂ concentration and alternation of antioxidant enzymes) in black bean under saline conditions.
Materials and Methods

Priming and germination conditions
The bean that was used in the present study was a small, black and shiny variety of the common bean (*Phaseolus vulgaris* var. black- JAMARAN–4802) which has a dense and meaty texture. Seeds of black bean were purchased from Yekta Seed Company (Tehran, Iran) and were surface-sterilized with 70% ethanol for 2 min followed by repeated washing with double-distilled water and dried on filter paper. Then, they were primed in 0, 2, 10, and 20 mM of salicylic acid for 24 h at room temperature under dark conditions (Li et al., 2017). Afterwards, seeds were washed with distilled water and then dried at room temperature on filter paper for 24 h. Experiment was conducted in a factorial arrangement based on a completely randomized design with four replicates. The experimental factors included: salt stress at three levels (0, 50, and 100 mM NaCl) and salicylic acid priming at four levels (0, 2, 10, and 20 mM). The treatments were: 1: control (no treatments), 2: seed priming with 0, 2, 10, and 20 mM SA (no salt stress), 3: salt stress containing 50 or 100 mM NaCl (no priming) and, 4: seed priming with SA (as in item 2) along with salt stress (as in item 3). Seeds were placed on a filter paper in Petri-dishes containing distilled water or saline solution. The Petri-dishes were sealed with Parafilm to prevent evaporation and then carefully kept in a germinator at a temperature of 25 ± 1 °C and 12/12 h light/dark cycles (ISTA, 2004). The experiment lasted for 10 days. Seeds with 2 mm emerged radicle were considered as germinated seeds and counted every day. Different parameters of germination were evaluated based on Gharoobi et al. (2012) parameters: a) seed germination percentage (GP, %) = Number of germinated seeds / number of total seeds × 100; b) seed germination rate (GR) = Σ (Gn/Dn), Gn: germinated seeds at the day of n after sowing and Dn: the day of n after sowing; c) seedling length and dry weight.

Measurement of lipid peroxidation
Lipid peroxidation was evaluated in terms of malondialdehyde content (Ksouri et al., 2007). Fresh samples of seedlings (250 mg fresh weight) were homogenized in 5 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10000 × g for 10 min at 4 °C. Supernatant (1 mL) was mixed with 5 mL of 0.5% thiobarbituric acid (TBA) prepared in TCA (20%) and incubated at 95 °C for 30 min. Reaction was stopped by placing the tubes in an ice bath. The samples were centrifuged at 10000 × g for 5 min. The absorbance of supernatant was measured at 532 nm and after subtracting the non-specific absorbance at 600 nm, MDA concentration was determined using the extinction coefficient of 155 mM⁻¹ cm⁻¹.

Estimation of hydrogen peroxide (H₂O₂) content
The concentration of H₂O₂ was assayed by measuring the absorbance of titanium-hydroperoxide complex (Nag et al., 2000). Fresh seedlings (1 g) were homogenized in 12 mL of cold acetone. Then, 4 mL of titanium reagent was added to the mixture followed by 5 mL of concentrated ammonium solution to precipitate hydroperoxide-titanium complex. The mixture was centrifuged in the refrigerated centrifuge for 5 min at 8500 × g. The pellet was washed twice with 5 mL acetone followed by dissolving in 1 M sulphuric acid. The absorbance of orange-yellow H₂O₂-Ti complex was recorded at 410 nm against blank. The concentration of H₂O₂ was determined using standard curve plotted with known concentrations of H₂O₂ (a range of 10-100 μM).

Enzyme extraction and assay
Enzyme extraction procedure was accomplished according to the method of Chen et al. (2000) with some modification. All of the following operations were performed at 4 °C. Fresh seedlings (1 g) were ground in a
mortar with liquid nitrogen and extracted in 100 mM Na-phosphate buffer (pH 6), containing 0.1 mM EDTA. The homogenate was centrifuged at 12000 × g for 20 min. The supernatant was transferred to Eppendorf tubes and kept in the -20 °C freezer. Catalase activity was evaluated spectrophotometrically by determining the consumption of H₂O₂ (ε = 39.4 mM⁻¹ cm⁻¹) at 240 nm in 50 mM phosphate buffer, pH 7.5 and 200 mM H₂O₂ (Nemat-Ala and Hassan, 2006). Total ascorbate peroxidase activity was evaluated spectrophotometrically according to the method of Kato and Shimizu (1985) at 280 nm in 0.2 mM potassium phosphate buffer, pH 7.5, 15 mM ascorbic acid, and 50 mM H₂O₂, as ascorbate (ε = 2.8 mM⁻¹ cm⁻¹) was oxidized. Guaiacol peroxidase activity was assayed in 44 mM H₂O₂ and 45 mM guaiacol. The absorption at 470 nm was recorded and the activity was calculated using the extinction coefficient of 26.6 mM⁻¹ cm⁻¹ (Buchanan and Balmer, 2005).

All enzyme activities were expressed as units per mg of protein. Protein content in all enzyme extracts was determined according to the method of Bradford (1976).

**Statistical Analysis**

The experiments (in a factorial arrangement) were carried out according to a completely randomized design with four replicates. The data was analyzed using the SAS software (V. 9.0) and means values of treatments were compared with the respected control adopting 1% significant level of least significant difference (LSD).

**Results**

The results of variance analysis of the studied germination traits in black bean plant are shown in Table 1 and 2. According to Table 1 and 2, salt stress and salicylic acid priming significantly (p<0.01) affected the germination traits. In addition, a significant interaction was obtained between salt stress and salicylic acid priming (p<0.01).

**Table 1.** Analysis of variance for some seed germination traits of 10-day-old seedling of black bean in response to salicylic acid and salt stress (10 days NaCl application)

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>MS</th>
<th>df</th>
<th>Seed germination percentage</th>
<th>Germination rate</th>
<th>Seedling length</th>
<th>Seedling dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylic acid priming</td>
<td></td>
<td>3</td>
<td>8768**</td>
<td>62.22**</td>
<td>10.87**</td>
<td>2.33**</td>
</tr>
<tr>
<td>Salt stress</td>
<td></td>
<td>2</td>
<td>3646**</td>
<td>141.22**</td>
<td>23.25**</td>
<td>4.21**</td>
</tr>
<tr>
<td>Salicylic acid priming ×</td>
<td></td>
<td>6</td>
<td>3165**</td>
<td>19.16**</td>
<td>14.62**</td>
<td>6.32**</td>
</tr>
<tr>
<td>Salt stress</td>
<td></td>
<td>24</td>
<td>5.22</td>
<td>2.58</td>
<td>8.43</td>
<td>1.02</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>-</td>
<td>9.45</td>
<td>8.76</td>
<td>5.15</td>
<td>8.31</td>
</tr>
</tbody>
</table>

**Table 2.** Analysis of variance for some biochemical traits of 10-day-old seedling of black bean in response to salicylic acid and salt stress (10 days NaCl application)

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>MS</th>
<th>df</th>
<th>H₂O₂ level</th>
<th>MDA content</th>
<th>Catalase activity</th>
<th>Ascorbate peroxidase activity</th>
<th>Guaiacol peroxidase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylic acid priming</td>
<td></td>
<td>3</td>
<td>5.81**</td>
<td>0.0005**</td>
<td>409.84**</td>
<td>35.23**</td>
<td>421.41**</td>
</tr>
<tr>
<td>Salt stress</td>
<td></td>
<td>2</td>
<td>10.88**</td>
<td>0.0004**</td>
<td>581/1**</td>
<td>319.37**</td>
<td>621.01**</td>
</tr>
<tr>
<td>Salicylic acid priming ×</td>
<td></td>
<td>6</td>
<td>18.422**</td>
<td>0.007**</td>
<td>88.58**</td>
<td>17.82**</td>
<td>180.52**</td>
</tr>
<tr>
<td>Salt stress</td>
<td></td>
<td>24</td>
<td>11.87</td>
<td>0.00051</td>
<td>8.44</td>
<td>0.00973</td>
<td>17.47</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>-</td>
<td>18.32</td>
<td>12.96</td>
<td>10.44</td>
<td>9.66</td>
<td>11.96</td>
</tr>
</tbody>
</table>

**:** Significantly at p<0.01
Results showed that salt stress at both levels (50 and 100 mM) significantly (p<0.01) decreased the percentage of seed germination in black bean by 45.5% and 69% respectively, compared to the control (Fig. 1A).

SA priming (10 mM) increased the percentage of seed germination in black bean under salt stress compared to exclusively salt stress (+ 45% at 50 mM and + 43% at 100 mM). Salicylic acid priming at 20 mM did not improve this trait under salinity, but it increased by 16.3% and 21.4% at 50 and 100 mM, respectively.

Germination rate of seeds in black bean significantly reduced by salt stress (p<0.01) (Fig. 2B). At 50 and 100 mM NaCl, germination rate reduced-in turn- by 47% and 76.6% compared to the control. Salicylic acid priming (at 2 and 10 mM) increased this parameter significantly at both concentrations of applied NaCl (p<0.01). However, the positive effect of salicylic acid at 10 mM was significantly more than that of its 2 mM concentration. Salicylic acid priming augmented the germination rate by 33.3% at 50 mM and by 60% at 100 mM NaCl in comparison with the exclusively salt-stressed seedlings. SA priming at 20 mM concentration did not positively affect the rate of seed germination (Fig. 1B).

![Graph A](image1.png)

![Graph B](image2.png)

**Fig. 1.** Germination percentage (A) and germination rate (B) of 10-day-old seedling of black bean in response to salicylic acid and salt stress (10 days NaCl application). Means (four replicates) with the same letter are not significantly different.
Data analysis revealed that salt stress (50 and 100 mM) decreased seedling length of black bean by 50% and 88.5% respectively, compared to the control (Fig. 2A). Salicylic acid priming (2 and 10 mM) significantly increased this parameter under both applied levels of NaCl (p<0.01). But, the effect of 10 mM salicylic acid was more dominant. Salicylic acid priming (at 10 mM) increased seedling length by 70.1% at 50 mM and 3.6-fold at 100 mM NaCl. The level of 20 mM salicylic acid significantly decreased the seedling length in comparison to those treated with salinity alone.

Seedling weight in black bean significantly declined under salt stress (p<0.01). At 50 and 100 mM NaCl, it reduced by 48.5% and 75.5% respectively, compared to the control (Fig. 2B). Priming with salicylic acid (2 and 10 mM) significantly increased the seedling weight under saline conditions. At 50 mM NaCl, priming with salicylic acid (2 and 10 mM) caused 35.3% and 76.5% increase in seedling weight. Besides, salicylic acid at 2 and 10 mM increased seedling weight by 35.3% and 76.5% at 100 mM NaCl. Nevertheless, salicylic acid at 20 mM significantly decreased it compared to the exclusively salt-stressed seedlings (Fig. 2B).

Fig. 2. Seedling length (A) and seedling dry weight (B) of 10-day-old seedling of black bean in response to salicylic acid and salt stress (10 days NaCl application). Means (four replicates) with the same letter are not significantly different.
As shown in Figure 3A, NaCl (at 50 and 100 mM) significantly increased the concentration of malondialdehyde by 97.6% and 2.3 folds compared to the control, respectively, which is indicative of an increase in lipid peroxidation in salinized seedlings of black bean. However, the content of malondialdehyde decreased in the salinized seedlings raised from salicylic acid priming at 2 and 10 mM (p<0.01).

Data showed that SA priming at 10 mM was significantly more efficient than 2 mM in reducing lipid peroxidation in the salt-stressed seedlings. Salicylic acid priming at 10 mM reduced malondialdehyde content in the seedlings grown in the saline culture solution (p<0.01). This decrease was 37% at 50 mM and 39% at 100 mM NaCl. Salicylic acid priming at 20 mM had no positive effect on decreasing malondialdehyde level (Fig. 3B).

Results revealed that salt stress (50 and 100 mM NaCl) increased the level of H$_2$O$_2$ in seedlings of black bean by 2 and 2.7 folds respectively, compared to the control (Fig. 3B).

![Graph A](image1.png)

![Graph B](image2.png)

**Fig. 3.** Malondialdehyde concentration (A) and H$_2$O$_2$ level (B) in the 10-day-old seedling of black bean in response to salicylic acid and salt stress (10 days NaCl application). Means (four replicates) with the same letter are not significantly different.
Fig. 4. Catalase (A), ascorbate peroxidase (B), and guaiacol peroxidase (C) activity in the 10-day-old seedling of black bean in response to salicylic acid and salt stress (10 days NaCl application). Means (four replicates) with the same letter are not significantly different.

However, salicylic acid priming (2 and 10 mM) significantly lessened the level of H$_2$O$_2$ in the seedlings under salt stress (p<0.01). Nevertheless, the effect of 10 mM salicylic acid was the best priming to reduce H$_2$O$_2$ concentration. At 50 and 100 mM NaCl, salicylic acid priming at 10 mM decreased the level of H$_2$O$_2$ by 39.1% and 33.6% respectively, compared to exclusively salt stress treatment (Fig. 3B). However, the injurious effect of salicylic acid priming at 20 mM was much higher than exclusively salt stress as the concentration of H$_2$O$_2$ significantly increased in the seedlings.
Catalase activity significantly decreased by NaCl at both applied concentrations (p<0.01) (Fig. 4A). The impact of 100 mM NaCl on reducing the activity of catalase was greater than that of at 50 mM NaCl. Salicylic acid priming (at 10 mM) significantly increased the activity of this enzyme at 100 mM NaCl. Salicylic acid at 20 mM highly reduced catalase activity even at the absence of NaCl.

Data analysis showed that salt stress (solely) at 50 mM significantly increased the activity of ascorbate peroxidase by 22% compared to the control (p<0.01) (Fig. 4B). Nonetheless, 100 mM NaCl decreased it by 28.5% in comparison with control. Salicylic acid priming (particularly at 10 mM) increased the activity of ascorbate peroxidase under salt stress (Fig. 4B).

Results showed that the pattern of guaiacol peroxidase activity was relatively similar to that of ascorbate peroxidase in the salinized seedlings (Fig. 4C). In other words, the activity of guaiacol peroxidase was increased at 50 mM NaCl compared to the control in the primed and non-primed seedlings. But, the activity of this enzyme decreased at 100 mM NaCl. The best result belonged to the seedlings primed with 10 mM SA (Fig. 4C).

**Discussion**

Crops are commonly damaged by NaCl at low concentrations. Among crop plants, the varieties of common bean are categorized as sensitive plants and have a high tissue salt concentration even at moderate (50 mM NaCl) salt stress (Jebra et al., 2005; Rady and Mohamed et al., 2015; Taibi et al., 2016). Current data showed salt stress reduced the germination parameters in black bean, but salicylic acid priming (particularly at 10 mM) significantly improved the percentage and rate of seed germination, seedling length and dry weight under salt stress. These results were in agreement with the previous reports on diverse plant species (Dolatabadrian et al., 2008; Dallali et al., 2012; Enteshari et al., 2012; Boukraa et al., 2013; Jini and Joseph, 2017; Anaya et al., 2018).

It is already hypothesized (Munns, 2002) that salt stress in plants has two distinct impacts: osmotic stress (the initial phase of growth reduction) and ion toxicity due to progressive NaCl uptake (i.e. salt-specific phase). In the present study, the reduction of seed germination percentage and germination rate in salinized black bean was a consequence of salt osmotic effects due to the reduced water availability. Salt stress reduces the expression of aquaporins which are responsible for water entry into cell (Boursiac et al., 2005). Nevertheless, SA causes to increase water potential tolerance of plants to salt stress (Tari et al., 2002). It is stated that salicylic acid-induced acidification of the cytosol resulted in aquaporin activation and then faster seed imbibition (Boursiac et al., 2005; Verdoucq et al., 2008). Escobar et al. (2010) showed that salicylic acid priming increased osmotic adjustment at the imbibition stage. Sahabutdinova et al. (2003) have suggested SA brings about an increase in the level of abscisic acid which ultimately helps in maintaining better water balance in the plants.

Nonogaki et al. (2010) indicated the reduction of seed germination percentage was due to the specific ionic effects of NaCl which negatively affected the activation of embryo growth using reserve metabolites. They hypothesized salicylic acid priming induced activation of mobilization of reserve metabolites with low molecular weights. As a consequence, much earlier commence of metabolic activities caused earlier seed germination of the primed seeds in comparison to the non-primed seeds. Furthermore, it is proposed that salicylic acid priming enhances the biosynthesis of proteins that are essential for embryo growth at the second stage of germination (Rajjou et al., 2006). SA treatment could stimulate seed germination via biosynthesis of gibberellin and acts as a thermogene inducer (Shah, 2003).
Farooq et al. (2007) pointed out that salicylic acid priming increased cell division within the apical meristem of seedling roots leading to improved seedling length and dry weight. It is hypothesized that salicylic acid prevents the reduction of auxin and cytokinin levels in salt-stressed plants causing an improvement of plant growth. On the other hand, Shaksirova et al. (2003) have reported that seed priming with SA resulted in abscisic acid accumulation which might involve in pre-adaptation of seedlings to salt stress. Apparently, abscisic acid induces the synthesis of an extensive range of anti-stress proteins which protect plants against stress conditions. Szalai et al. (2005) showed an interaction between salt stress and salicylic acid, which up-regulate the genes encoding salt resistance and proceeds seed germination by increasing the physiological activities and mobilization of the reserved material required for the growth.

It is confirmed that salt stress brings about oxidative stress in plants resulting in cell damage or death. The generation of oxidative stress is due to high levels of reactive oxygen species (ROS). Nevertheless, plants use an internal complex defensive system to remove or decrease detrimental effects of oxidative stress (Das and Roychoudhury, 2014; Semida and Rady, 2014; Rady and Mohamed et al., 2015). Antioxidant enzymes can directly manage ROS detoxification in plant cells. Current results showed that antioxidant enzymes exhibited their highest activities at 10 mM salicylic acid priming. This result was in coordinate with the obtained result for seedling dry weight. In line with this result, it has been demonstrated that application of salicylic acid to stressed plants can lower the level of ROS generated by salinity or water stress (Shaksirova et al., 2003; El-Khallal et al., 2009; Hayata et al., 2010; Syeed et al., 2011; Li et al., 2014; Abbaspour and Babaei, 2017; Farhangi-Abriz and Ghassemi-Golezani, 2018; Shahmoradi and Naderi, 2018; Haghshenas et al., 2020). This clarifies that the activities of antioxidant enzymes are directly or indirectly regulated by salicylic acid, which provide an efficient protection against salinity stress. Peroxidases have a crucial function in scavenging H$_2$O$_2$ which is generated during dismutation of O$_2^\cdot$ catalyzed by superoxide dismutase. Catalase, as a major enzyme, eliminates or diminishes H$_2$O$_2$ in the mitochondria and microbodies. Thereby, all mentioned enzymes assist in mitigation of the adverse effects of oxidative stress. It can be concluded that the higher activity of these enzymes would be of the main cause for decreasing H$_2$O$_2$ and subsequently enhancing salt tolerance in black bean.

Current data showed that salicylic acid priming decreased the level of lipid peroxidation in seedlings of black bean plant under saline conditions, which was in coordinate with decreasing the level of H$_2$O$_2$. At the cellular level, the intensity of lipid peroxidation of membranes increases in salinized plants by activation of ROS causing an increase in the level of malondialdehyde. The concentration of malondialdehyde is usually assessed as an indicator of oxidative damage due to accumulation of ROS (such as H$_2$O$_2$). It seems that seed priming with salicylic acid (at 10 mM) improved cellular membrane integration in seedlings of black bean upon salinity, leading to recovery of plant growth under saline condition. Additionally, the reduction of malondialdehyde content was in coordinate with the higher activities of antioxidant enzymes, confirming the positive effects of salicylic acid on reducing the level of H$_2$O$_2$. This finding was in agreement with the previous reports in different plant species (Gunes et al., 2007; Liu et al., 2014; Manaa et al., 2014; Ma et al., 2017; Tahjib-Ul-Arif et al., 2018; Bukhat et al., 2020).

The present data showed salicylic acid is required to boost plant defence mechanism against salt stress. High levels of salicylic acid, by itself, might act as a stress signal and reduce germination much more in comparison to
exclusively salt stress. Therefore, increasing the concentration of salicylic acid is not always helpful (20 mM in this experiment), which is in consistent with the previous reports (Hayata et al., 2010; Anaya et al., 2018).

**Conclusion**
The result of present study showed that seed priming with salicylic acid (10 mM) stimulates salt tolerance in seedlings of black bean plant and improves seed germination under saline condition. Salicylic acid signal transduction pathway altered the activation of enzymatic antioxidant system in the seeds to reduce the level of H$_2$O$_2$. As a result, the cellular component and normal metabolism sustain robust in response to salinity. Therefore, more vigorous seedlings are established under salt stress. This means destructive effects of oxidative stress due to salinity could be decreased by seed priming with salicylic acid.

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**Conflict of interest**
The authors indicate no conflict of interest for this work.

**References**


