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EXOGENOUSLY APPLIED SALICYLIC ACID IMPROVED GROWTH, PHOTOSYNTHETIC PIGMENTS AND OXIDATIVE STABILITY IN MUNGBEAN SEEDLINGS (*VIGNA RADIATA*) AT SALT STRESS

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Abstract

Application of salicylic acid is known to resist the adverse effect of salinity on crop species. This study was aimed to assess the effect of salicylic acid (50 µM) as a soaking agent in (*Vigna radiate*) mungbean (NM19-19) before and after induction of 50, 100 and 150 mM NaCl stress. The indicators of crop performance such as seedling length, fresh & dry biomass, relative water content, chlorophyll *a*, chlorophyll *b*, carotenoids, total pigments, malonaldehyde content, total protein, ascorbate peroxidase, guaiacol peroxidase, catalase and superoxidase dismutase enzymes were studied. Salt stress caused a reduction in seedling growth and also modulated physio-biochemical attributes over untreated controls and SA treated seedlings. In contrast, the soaking of seedsin SA after salt stress; physiological changes such as growth, total proteins, pigments and oxidative stability were increased and led to improve biomass as compared to the exposure of salt alone. While the application of SA prior to salt stress (SA+NaCl) showed remarkably higher mean values for studied growth parameters and antioxidant enzymes activities compared to NaCl+SA soaking treatment. Therefore, it was concluded that application of SA prior to salt stress increased the capacity of mungbean seedlings in acclimating to salt stress and thus increased growth. Therefore, application of SA is an effective method of managing ROS at high salinity and therefore can alleviate salinity stress in mungbean seedlings that might enhance food supply and conservation of saline resources.

Key words: Malondialdehyde content, Total proteins, Peroxidase enzymes, Relative water content, Salinity, Superoxidase dismutase.

Introduction

Salinity is an imminent abiotic stress that impairs the composition of soil and seedlings performance (Sairam *et al*., 2002; Khan *et al*., 2017). It reduces seedling growth, water content and photosynthetic pigments by disturbing chloroplast function (Miller *et al*., 2010; Jiang *et al*., 2017; Stavridou *et al*., 2017). Salinity dependent changes in the metabolism cause seedling death, which leads to compromise productivity that ultimately, reduces economic return (Munns & Gilliham, 2015). At present, 2-9% earth (Zhang & Cai, 2011), and 26% irrigated lands in Pakistan have been harmed by saline irrigation (Anon., 2010). This damage will be increased by the passage of time in the coming years and becomes the biggest threat to an agricultural and industrial sector. The majority of edible crops cannot be well grown on saline lands, especially Mungbean (Sehrawat *et al*., 2013), Maize (Jiang *et al*., 2017), Onion (Çavuşoğlu *et al*., 2016) and wheat (Mahboob *et al*., 2016). Mungbean is very important and second major legume crop cultivated in Pakistan (Rasul *et al*., 2012). It is rich in protein (24.2%), carbohydrates (60.4%), dietary fiber (14.7%), but very low in fat (0.7%); and has an important place in the vegetarian diet (Lee *et al*., 1997; Hussain *et al*., 2011a). This valuable nutritive pulse has a low yield on the farm by the shortage of irrigated land available. The yield of bean can be achieved by utilizing saline lands and brackish water, therefore research towards optimization of biomass production and improvement in salinity resistance of mungbean is the prerequisite for sustainable agriculture.

Low stomatal conductance inhibits photosynthesis (Abideen *et al*., 2014) which results in over-reduction of the photosynthetic electron transport chain leading to increased formation of reactive oxidative species (ROS) (Qasim *et al*., 2017). Reactive oxygen species are produced in an excessive amount by salt stress which brings about an oxidative injury to seedlings development (Ghosh *et al*., 2015). Hydrogen peroxide (H_2O_2), superoxide (O^-_2), singlet oxygen ($^1O_2$), and hydroxyl radical (·OH) (Abogadallah, 2010), could create disturbance in an equilibrium of vital mineral nutrients as Na^+ and Cl^- ions (Munns & Tester, 2008). ROS destruct cell membrane (Abbas *et al*., 2013), protein content, DNA (Tuna *et al*., 2008), and diminish photosynthesis rate (Roychoudhury & Ghosh, 2013). The disturbed photosynthesis will influence seedling survival that alters yield performance of crops. Therefore, photosynthetic organisms need strategies to maintain a balance between ROS production and itsquenching. Such a strategy is needed for survival but also is of economic importance because it has an impact on the productivity and suitability for an industrial purpose (Wijffels & Barbosa, 2010). Hence, on exposure to stress, seedlings can increase non-enzymatic and enzymatic antioxidants for their defense mechanisms, which encounter the deleterious effects of ROS (Shahid *et al*., 2011; Arias-Moreno *et al*., 2017). The enhanced level of antioxidants can be produced in seedlings by exogenous application of salicylic acid that can maintain better growth (Khan *et al*., 2010).

Salicylic acid (SA) performs a crucial role in plant growth processes, fruit ripening, pathogen attacks and abiotic stress acclimation (Miura & Tada, 2014). It has been reported that SA application alleviates the adverse effects of sodium chloride stress (NaCl) by enhancing water content and photosynthetic pigments on crops (Yusuf *et al*., 2008; Misra & Saxena, 2009; Habibi, 2012; Fayez & Bazaid, 2014). SA acts as major secondary signals activator that activates antioxidant enzymes i.e., superoxide dismutase (SOD), catalase (CAT), and

peroxidase (POX) (Khan *et al*., 2010; Xia *et al*., 2011). The SOD gene activation is the primary step in detoxifying ROS which displaces superoxide ions into H_2O_2 and O_2. Moreover, the hydrogen peroxide deposition is inhibited by CAT whereas; peroxidase converted it into water molecule (Khan *et al*., 2010). Hence, seedlings have the elevated level of antioxidant enzymes activities can resist better to the oxidative damage, activated by ROS (Abbas *et al*., 2013). Therefore, this study is designed to evaluate the impact of pre-and post-soaking treatments of salicylic acid on morpho-physiological properties of mungbean seedlings at various levels of salinity.

Materials and Methods

Seeds of mungbean (*Vigna radiata*) variety NM 19-19 were collected from National Agricultural Research Center (NARC), Islamabad. The experiment was conducted in Petri dishes during October to November 2014 in the lab of Department of Genetics, University of Karachi. In this study, three levels of sodium chloride (50 mM, 100 mM, and 150 mM) were used. An already optimized concentration (50 µM) of salicylic acid (Shakeel & Mansoor, 2012a) was supplied as soaking dose to mungbean. Twenty uniform sized seeds per treatment and total two hundred and forty seeds per replication were sterilized in 10% of sodium hypochlorite solution for 5 minutes, washed several times with distilled water (d/w). The twelve treatments were divided into four sets. Each set had three treatments.

Set-1: the control group that had three controls i.e. 0mM SA/NaCl control, SA pre-control and SA post-control. The 0mM SA/NaCl was imbibed in distilled water for 24 h. While, SA pre-control was imbibed in 50 µM salicylic acid (Sigma-Aldrich, Germany) solution for 24 h. Thereafter, both air-dried controls were sown in distilled water for 72 h. In the case of SA post-control; twenty seeds were first imbibed in distilled water (24 h) and then germinated in Petri dishes for 24 h in d/w. These seedlings were further soaked in 50 µM salicylic acid solution (24 h) and shifted over distilled water lined petri dishes for 24 h.

Set-2: salt stressed group; 60 seeds were imbibed in distilled water for 24 h and air dried. The seedlings were divided into three groups according to NaCl (Fisher Scientific, UK) concentrations. Seedlings were treated with 50, 100, and 150 mM NaCl stress separately for 24 h. Further, stressed seedlings were placed in distilled water moistened filter paper holding petri dishes for 48 h.

Set-3: Pre-soaked group with salt stress; 60 seeds were imbibed in 50 µM salicylic acid solution for 24 h. These sixty air dried seedlings were split into three distinctive groups. Each group had twenty healthy uniform size seedlings that were exposed to 50, 100 and 150 mM NaCl for 24 h individually, afterward grown in distilled water for 48 h.

Set-4: Post-soaked group with salt stress; the same number of seeds like set-3 were imbibed in distilled water for 24 h and grouped. Each group consisted of twenty seedlings were subjected to 50, 100, and 150 mM NaCl solution (24 h) and soaked in 50 µM SA (24 h). These seedlings were allowed to grow further in distilled water for 24 h. All treated seedlings were harvested on the same day after 96 h and saved for subsequent analysis.The twelve treatments in four sets were also listed in Table 1.

Morphological parameters: The seedling length was measured from the apex of leaf till the root tip by measuring tape in centimeter. The twenty seedlings of each treatment from all replication were weighted for seedlings total fresh biomass. Fresh weight was noted by randomly taking 3 seedlings from each treatment immediately after the harvest. The seedlings dry weight was recorded after 72 h of drying at 65˚C (Heraeus D-63450, Germany). Total fresh biomass, fresh and dry biomass was taken in grams by using Sartorius electronic balance (Model: TE214S, Germany). Relative water content (RWC) was calculated (Misra & Dwivedi, 2004) and expressed in fresh weight percentage by using following formula;

$$\text{RWC\%} = \frac{(\text{fresh weight} - \text{dry weight})}{\text{fresh weight}} \times 100$$

Physiological attributes: Photosynthetic pigments were estimated by the protocol of Arnon (1949) with minor alterations. The 0.05g of seedling tissues were homogenized with 5mL of ice-cold 80% acetone solution (Sigma-Aldrich, Germany) by an ice chilled pestle and mortar. These homogenized samples were centrifuged at 4000 RPM for 15 min in the clinical centrifuge (Dragon Lab DM 0412, China). The supernatant was collected into the capped centrifuged tube and the absorbance was taken at 663 nm, 646 nm and 470 nm against 80% acetone, which was used as a blank. Photosynthetic pigments expressed as milligram per gram fresh weight (Metzner *et al*., 1965; Arnon, 1949; Wellburn & Lichtenthaler, 1984) using the following formulae;

$$\text{Chlorophyll } a = [(12.21 \times \text{absorbance at 663 nm}) - (2.81 \times \text{absorbance at 646 nm})]$$

$$\text{Chlorophyll } b = [(5.03 \times \text{absorbance at 663 nm}) - (20.13 \times \text{absorbance at 646 nm})]$$

$$\text{Total chlorophyll} = \text{chlorophyll } a + \text{chlorophyll } b$$

$$\text{Carotenoids content} = \frac{1000 \times \text{absorbance at 470 nm} - [(3.27 \times \text{chlorophyll } a) + (104 \times \text{chlorophyll } b)]}{229}$$

$$\text{Total pigments} = \text{chlorophyll } a + \text{chlorophyll } b + \text{Carotenoids}$$

Fig. 1. Impact of 50 µM salicylic acid pre-imbibition and post-soaking for 24 h on the growth in NM19-19 variety of mung bean seedling, subjected to three sodium chloride concentrations i.e., 50, 100 & 150 mM NaCl. Numbers in the figures indicated the treatments which start from left hand side 1 =0mM SA/NaCl control, 2 =SA+0mM NaCl, 3 =0mM NaCl+SA, 4 =NaCl, 5 =SA+NaCl, 6 =NaCl+SA.

Table 1. The twelve treatments of three salt concentrations i.e. 50, 100 and 150 mM NaCl with post and pre-applications of salicylic acid along their controls.

Labels	Imbibed (24 h) at 30°C in beaker	Treatments at 30°C		
		In NaCl (24 h) at 30°C in beaker	In SA (24 h) at 30°C	Sown in d/w at 30°C
0mM SA/NaCl	d/w	0 mM (d/w)	-	d/w
SA+0mM NaCl	SA	0 mM (d/w)	-	d/w
0mM NaCl+SA	d/w	0 mM (d/w)	SA	d/w
NaCl (50)	d/w	50 mM	-	d/w
NaCl (100)	d/w	100 mM	-	d/w
NaCl (150)	d/w	150 mM	-	d/w
SA+NaCl (50)	SA	50 mM	-	d/w
SA+NaCl (100)	SA	100 mM	-	d/w
SA+NaCl (150)	SA	150 mM	-	d/w
NaCl (50) +SA	d/w	50 mM	SA	d/w
NaCl (100) +SA	d/w	100 mM	SA	d/w
NaCl (150) +SA	d/w	150 mM	SA	d/w

Biochemical assessment: Harvested seedlings were used for the assessment of antioxidant enzymes, MDA, and total proteins. Antioxidant enzymes were extracted (Jiang & Bingru, 2001). Ascorbate peroxidase [EC 1.11.1.11] enzyme activity was estimated by the method of Nakano & Asada (1981). Catalase [EC 1.11.1.6] activity was determined by the procedure of Aebi (1984). Superoxide dismutase [EC 1.15.1.1] activity was measured, according to Sairam *et al*., (2002). Guaiacol peroxidase [EC 1.11.1.7] activity was noted as Evers *et al*., (1994) described. The Malondialdehyde was extracted and assessed as Carmak & Horst (1991) done and calculated through 156 mM^{-1} cm^{-1} extinction coefficient, given by Ashraf *et al*., (2013). Total protein was quantitatively tested by Lowry *et al*., (1951) protocol. Absorption was recorded by spectrophotometer (Shimadzu UV-1601, Germany).

Statistical analysis: An experiment was conducted in Complete Randomized Design (CRD) with six replications. Data of morpho-physiological parameters were calculated on Excel 2013 and statistically analyzed through IBM SPSS Statistics software version 19. The Duncan Multiple Range test (DMRT) was used to compare means at $p \leq 0.05$ significance level.

Results

Morphological parameters: Mungbeanseedling length, biomass, fresh and dry weights and relative water content were negatively affected by salt concentrations (Figs. 2-6). Sodium chloride stress significantly led to the suppression of bean seedling length; 16% inhibition at 50 mM NaCl, 25% at 100 mM NaCl and 29% at 150 mM NaCl concentration when compared with 0mM SA/NaCl control. However, application of SA stimulated the length of seedlings under saline and non-saline conditions. Interestingly, this effect was manifested more prominently by pre-imbibition of salicylic acid as compared to post-soaking of SA (Figs. 1-2).

Biomass of mung seedlings was decreased significantly (4%) when exposed to 50 mM, (15%) 100 mM and (28%) 150 mM stress of NaCl. Exogenous application of salicylic acid increased biomass significantly both under non-saline and saline conditions (Fig. 3). However, the effect of salicylic acid was most pronounced at SA+0mM NaCl and SA+NaCl 50 mM treatments (Fig. 3). Likewise, sodium chloride concentration caused a significant reduction in seedlings fresh weight in a concentration-dependent manner. Whereas, pre-imbibition of salicylic acid under all experimental condition and post-soaking following by salt stress had significantly improved seedlings fresh weight (Fig. 4).

SA application significantly mitigated the adverse effects of salt by ameliorating the dry weight of salt-stressed beans seedlings (Fig. 5). Dry weight was highly affected, about 27 % reduced by 150 mM solution of salt. While it was promoted 54, 44, and 34 times by pre-treatment of salicylic acid under 50, 100 and 150 mM saline condition, respectively over 0mM SA/NaCl control (Fig. 5).

Physiological attributes: Salinity alone and post-SA treatment with salt negatively affect relative water content in mungbean seedlings. Furthermore, RWC was lowest in salt-stressed seedlings without SA treatment. In pre-treatment of salicylic acid, under highest concentration of applied sodium chloride (150 mM), the relative water content was statistically similar with untreated non-saline control (Fig. 6).

Salt stress adversely degraded chlorophyll *a*, *b*, carotenoids and total pigments (Figs. 7-10). The maximum reduction (54%) in chlorophyll *a* was seen in 150 mM NaCl stressed seedlings. While SA pre-imbibition treatment was found to be effective in promoting chlorophyll *a* content (Fig. 7). The pre-stressed (SA+NaCl) treatment increased chlorophyll *a* about 26% at 50 mM salt level comparing with 0mM SA/NaCl control. In contrast, NaCl+SA treatment failed to ameliorate chlorophyll *a* at 100 and 150 mM salt stress relative to untreated non-saline control (Fig. 7). Similarly, 150 mM concentration of sodium chloride inhibited 56% synthesis of chlorophyll *b* (Fig. 8). However, chlorophyll *b* showed maximum (100%) stability in seedlings raised from imbibition of SA. The pre-imbibition of SA promoted 56% chlorophyll *b* content whereas, post-soaking of SA decreased 13% chlorophyll *b* in beans seedlings at 150 mM salt level over non-soaked distilled water control.

Salt stress also affected carotenoids content and showed up to 50% reduction under higher NaCl saltiness (Fig. 9). However, pre-treatment of SA enhanced carotenoids by 41% under 150 mM NaCl stress. The SA+0mM NaCl showed maximum carotenoids content (82%). Where SA post-soaked seedlings exhibited 14% decrease in carotenoids comparing with 0mM SA/NaCl control. In the case of total pigments, a decline was observed by 28 folds at 50 mM, 38 folds at 100 mM and 53 folds at 150 mM NaCl stress with respect to 0mM SA/NaCl control (Fig. 10). On the other hand, SA+NaCl treatment produced better photosynthetic pigment by demonstrating 48, 36, and 23 times more pigments at 50, 100 and 150 mM NaCl respectively over untreated control (Fig. 10).

Biochemical assessment: Oxidative stress on mung bean seedlings was measured in terms of malondialdehyde content. Sodium chloride stress significantly enhanced the production of malondialdehyde and total protein content. There was 5%, 11%, and 17% increase in MDA content under 50, 100 and 150 mM NaCl stress, respectively. However, MDA content was reduced to 28%, 24%, and 15% under 50, 100 and 150 mM salt concentration with pre-imbibition of salicylic acid in comparison to 0mM SA/NaCl control (Fig. 11).

An increase in total proteins was not revealed by NaCl stress as compare to SA+NaCl and NaCl+SA treatments (Fig. 12). Seedlings raised from pre-imbibed SA exhibited a significant accumulation of total proteins by 60 (50 mM), 66 (100 mM) and 76 folds (150 mM) over untreated control. Similarly, 35, 47 and 62 folds promotion was also noted in post-soaked seedlings (under 50, 100 and 150 concentrations of salt, respectively) over 0mM SA/NaCl control (Fig. 12).

The APX enzyme activity exhibited gradual decrease by 63, 34 and 20-fold at 50, 100, and 150 mM of sodium chloride concentrations alone (Fig. 13). The SA post-treated seedlings showed an increase of 98, 90 and 62% in ascorbate peroxidase enzyme activity at 50, 100 and 150 salinity stress, respectively. Nevertheless, imbibition of salicylic acid before 50, 100 and 150 mM NaCl stress restored seedlings about 204, 172 and 162 times more than post application of salicylic acid (Fig. 13).

The activity of catalase showed a marked decrease under NaCl stress but significantly increased by the salicylic acid application (Fig. 14). SA imbibition prior to salt stress, raised 226, 201 and 139 times more activity of catalase than SA post-treated seedlings at 50, 100 and 150 concentrations of salt, respectively as compared to 0mM SA/NaCl control.

Seedlings treated with 50, 100 and 150 mM NaCl showed 99, 70 and the 26-fold increase in GPX activity, respectively (Fig. 15). While it was significantly increased to 172, 161 and 117% by the post soaking of mungbean in SA over 0mM SA/NaCl control. With pre-imbibition of SA, the GPX enzyme activity elevated appreciably in mung seedlings by about 291, 227 and 197 folds higher under 50, 100 and 150 mM saline conditions, respectively as compared to 0mM SA/NaCl control (Fig. 15).

In the case of superoxide dismutase, enzymatic activity elevated by 43% at 50 mM, 23% at 100 mM and 15% at 150 mM NaCl over 0mM SA/NaCl control (Fig. 16). It was observed that the activity of SOD was enhanced significantly with alleviation of salt stress by about 201, 174 and 132% at post-soaking of SA and 230, 207 and 201 at pre-imbibition of SA under 50, 100 & 150 concentrations of salt over 0mM SA/NaCl control, respectively (Fig. 16).

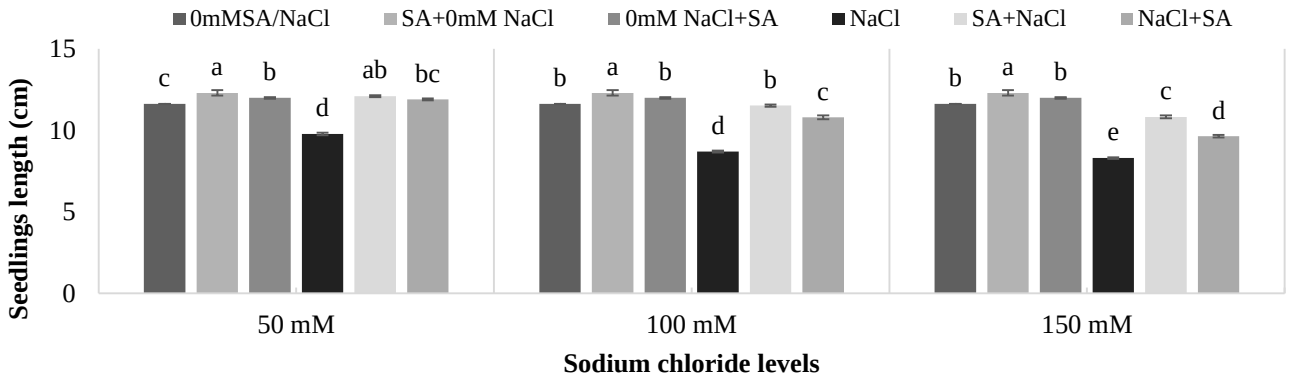

Fig. 2. Impact of exogenously applied salicylic acid (50 µM) on seedlings length in mungbean subjected to salt stress. The Duncan multiple range test values with different letters are differed significantly at $p \leq 0.05$.

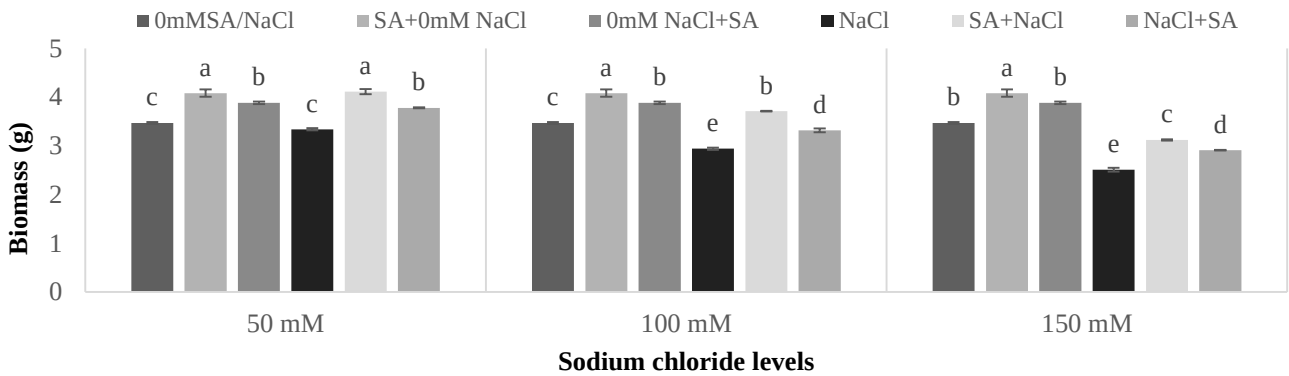

Fig. 3. Impact of exogenously applied salicylic acid (50 µM) on Biomass or total fresh weight in mungbean subjected to salt stress. The Duncan multiple range test values with different letters are differed significantly at $p \leq 0.05$.

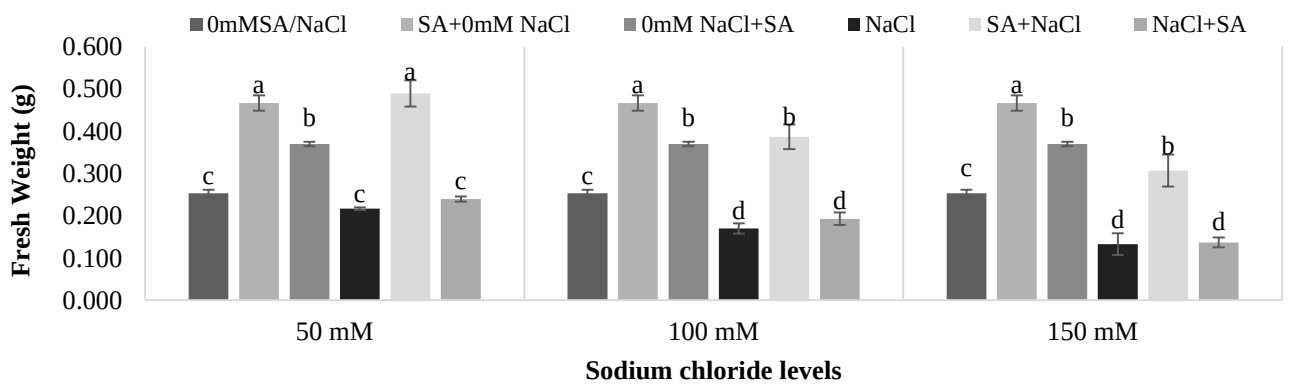

Fig. 4. Impact of exogenously applied salicylic acid (50 µM) on Fresh weight (g) in mungbean subjected to salt stress. The Duncan multiple range test values with different letters are differed significantly at $p \leq 0.05$.

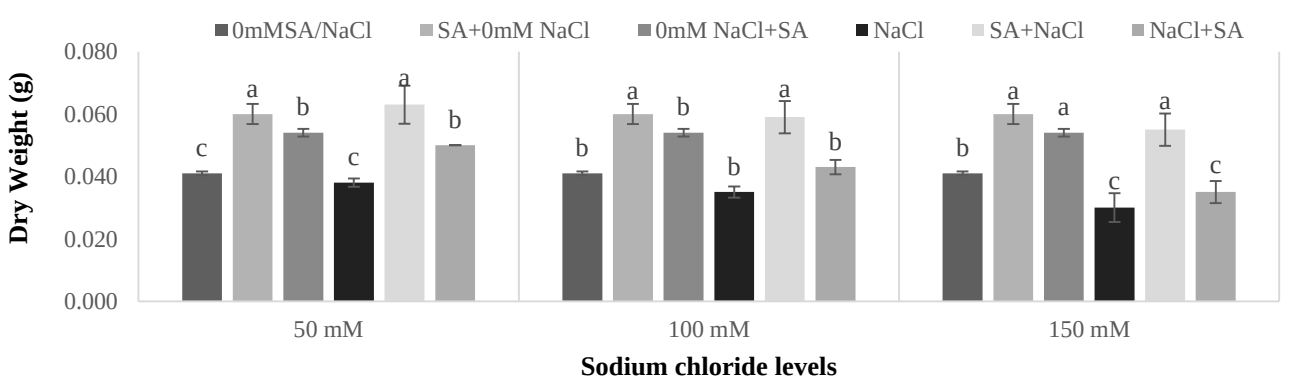

Fig. 5. Impact of exogenously applied salicylic acid (50 µM) on Dry weight (g) in mungbean subjected to salt stress. The Duncan multiple range test values with different letters are differed significantly at $p \leq 0.05$.

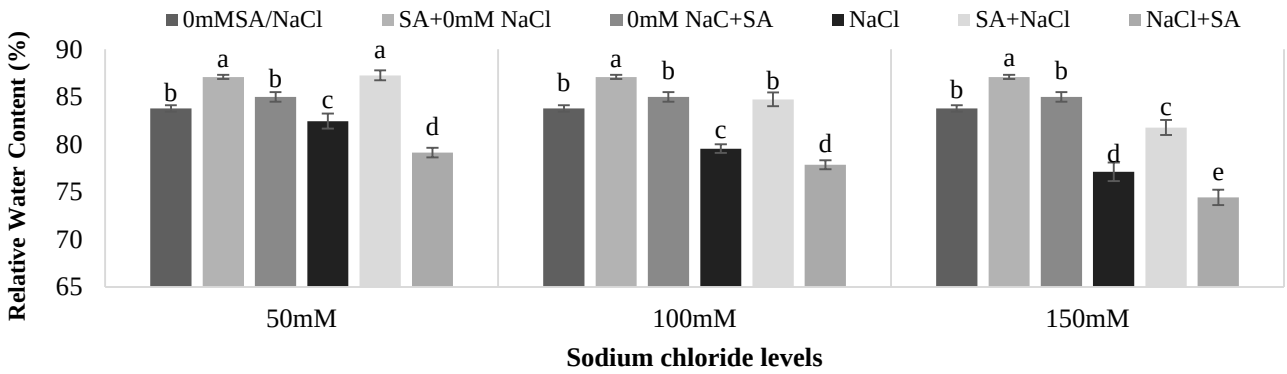

Fig. 6. Impact of exogenously applied salicylic acid (50 µM) on Relative Water content (RWC) (%) in mungbean subjected to salt stress. The Duncan multiple range test values with different letters are differed significantly at $p \leq 0.05$.

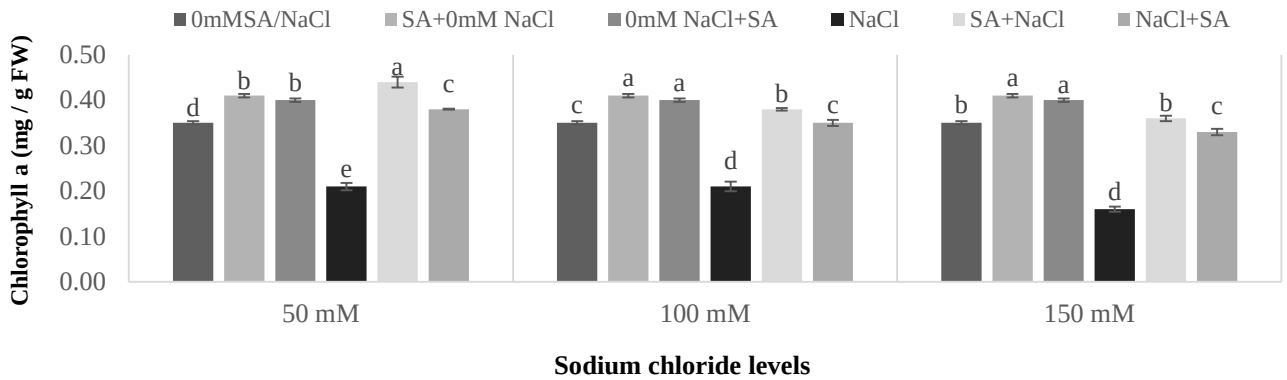

Fig. 7. Impact of exogenously applied salicylic acid (50 µM) on Chlorophyll a (mg / g fresh weight) in mungbean subjected to salt stress. The Duncan multiple range test values with different letters are differed significantly at $p \leq 0.05$.

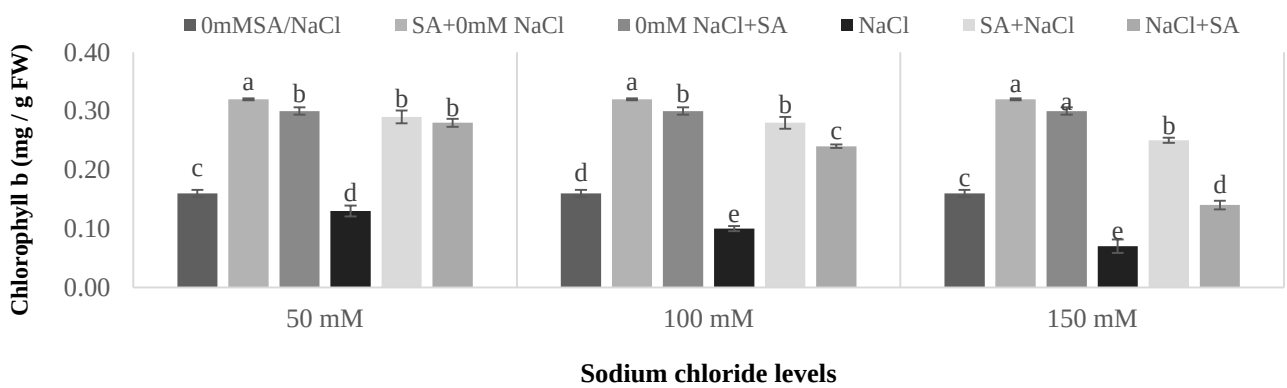

Fig. 8. Impact of exogenously applied salicylic acid (50 µM) on Chlorophyll b (mg / g fresh weight) in mungbean subjected to salt stress. The Duncan multiple range test values with different letters are differed significantly at $p \leq 0.05$.

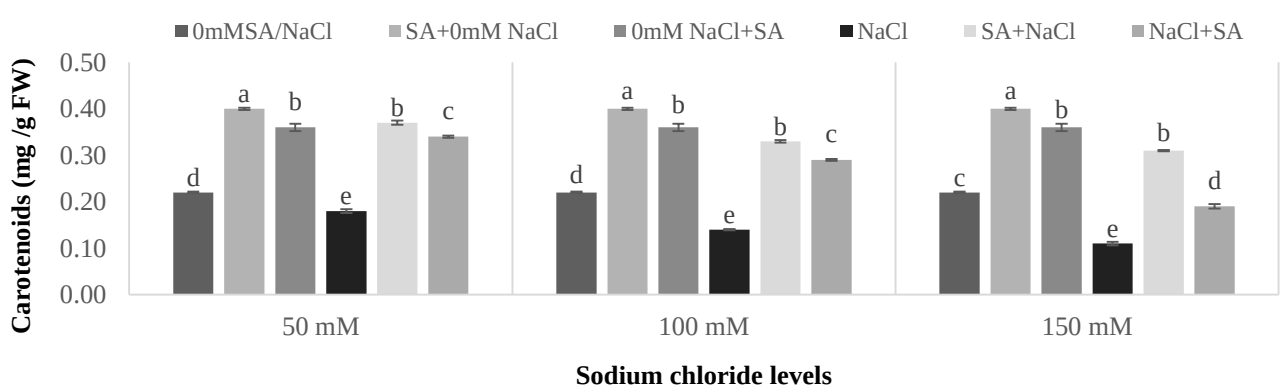

Fig. 9. Impact of exogenously applied salicylic acid (50 µM) on Carotenoids (mg / g fresh weight) in mungbean subjected to salt stress. The Duncan multiple range test values with different letters are differed significantly at $p \leq 0.05$.

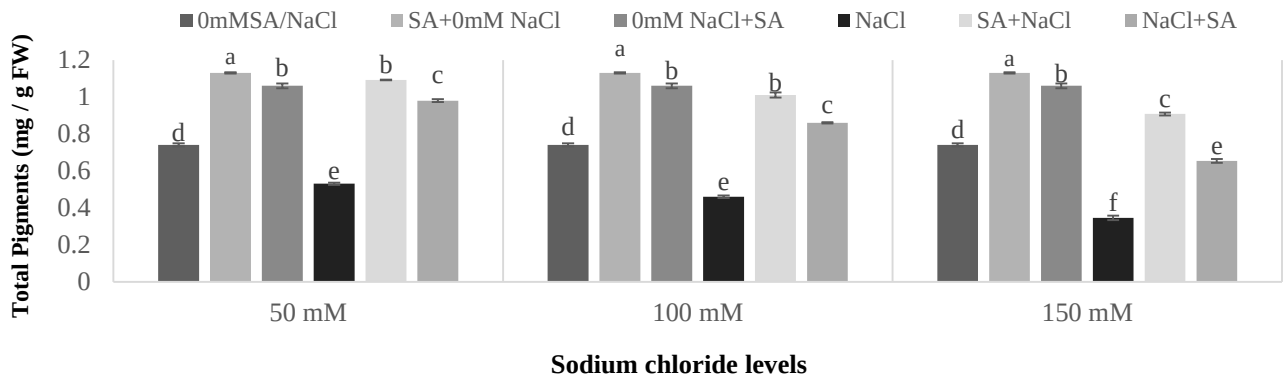

Fig. 10. Impact of exogenously applied salicylic acid (50 µM) on Total pigments (mg / g fresh weight) in mungbean subjected to salt stress. The Duncan multiple range test values with different letters are differed significantly at $p \leq 0.05$.

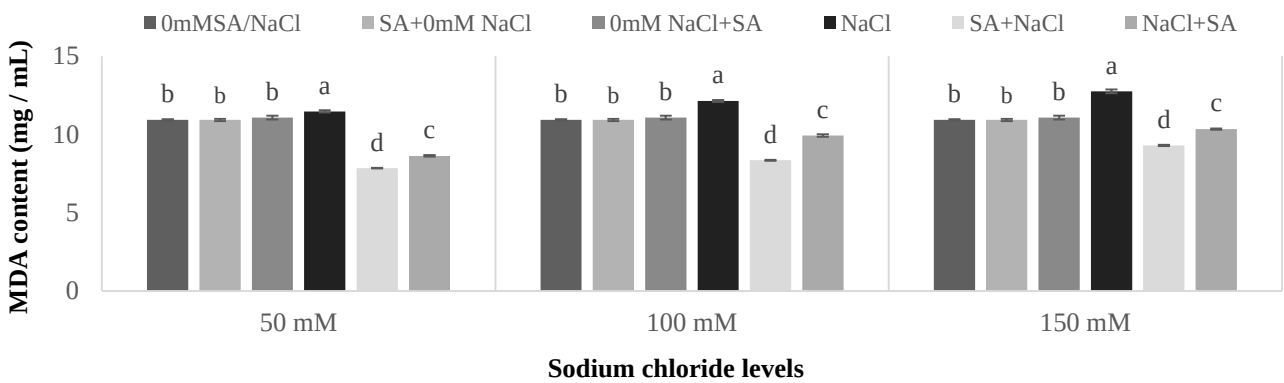

Fig. 11. Impact of exogenously applied salicylic acid (50 µM) on Malondialdehyde content (MDA) (mg / mL) in mungbean subjected to salt stress. The Duncan multiple range test values with different letters are differed significantly at $p \leq 0.05$.

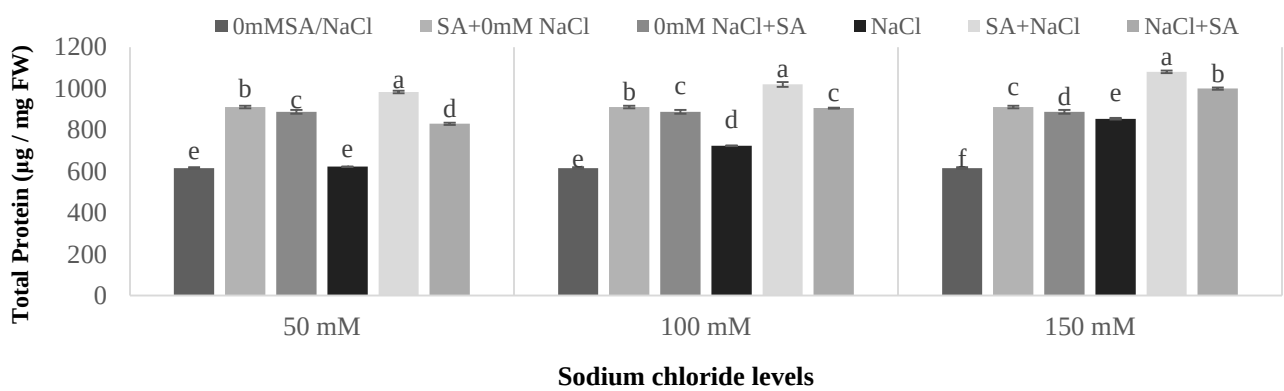

Fig.12. Impact of exogenously applied salicylic acid (50 µM) on total protein content (µg / mg fresh weight) in mungbean subjected to salt stress. The Duncan multiple range test values with different letters are differed significantly at $p \leq 0.05$.

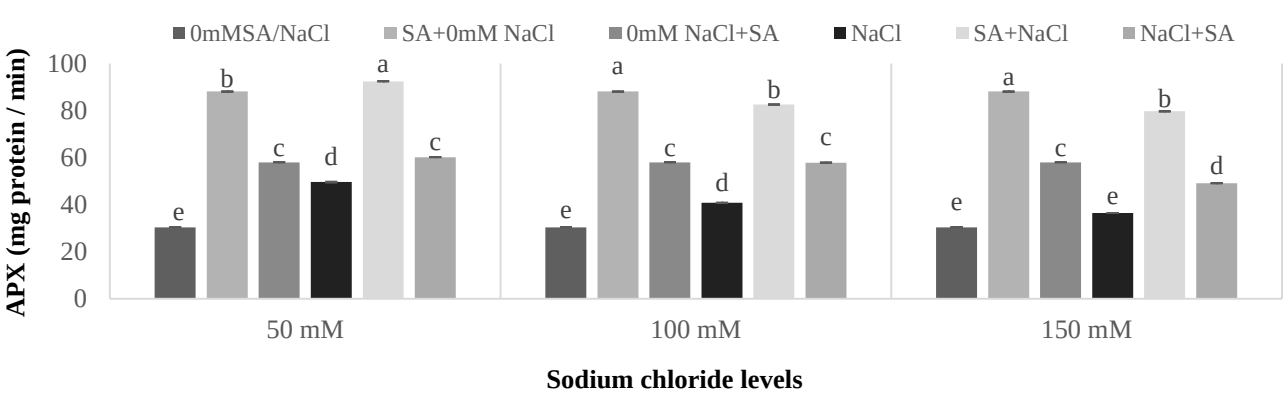

Fig. 13. Impact of exogenously applied salicylic acid (50 µM) on Ascorbate peroxidase (APX) enzyme activity (mg ascorbate protein / min) in mungbean subjected to salt stress. The Duncan multiple range test values with different letters are differed significantly at $p \leq 0.05$.

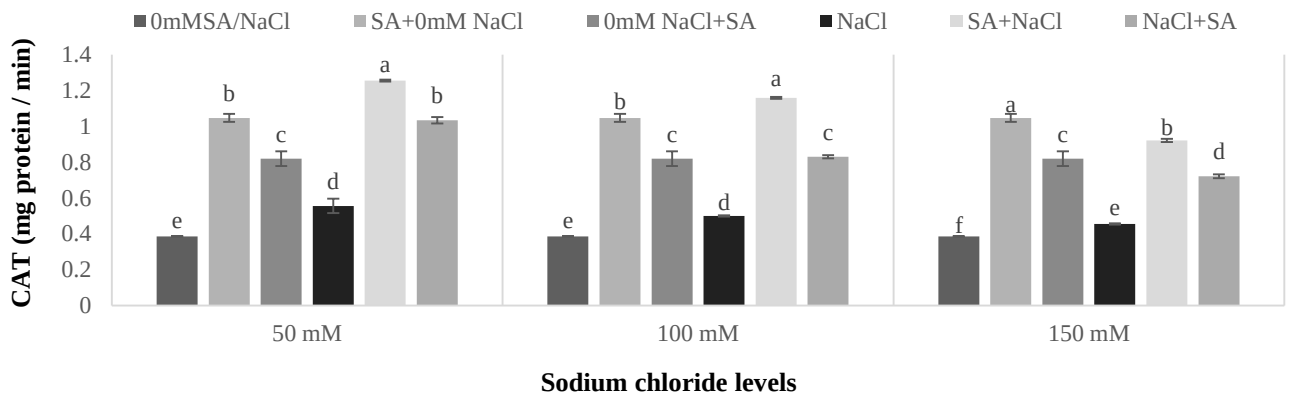

Fig. 14. Impact of exogenously applied salicylic acid (50 µM) on Catalase (CAT) enzyme activity (mg protein / min) in mungbean subjected to salt stress. The Duncan multiple range test values with different letters are differed significantly at $p \leq 0.05$.

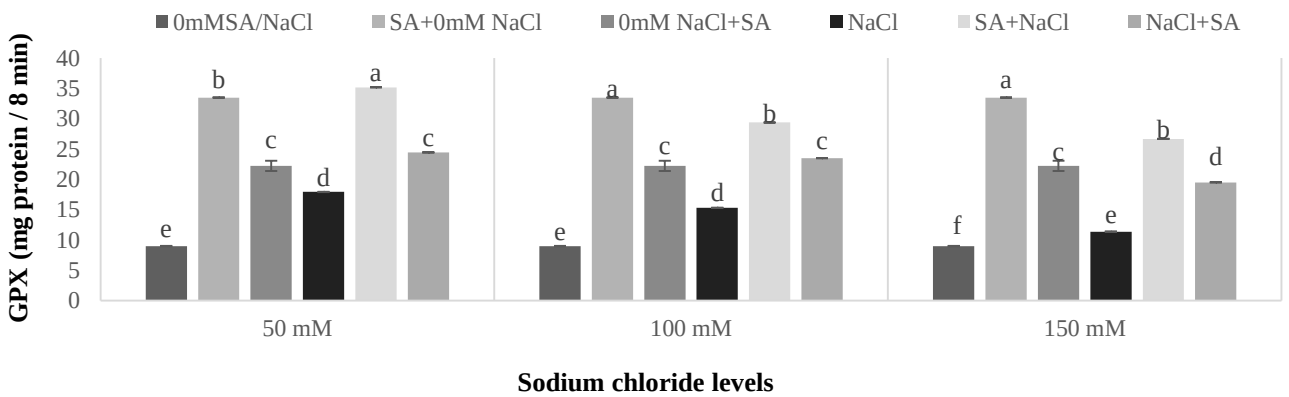

Fig. 15. Impact of exogenously applied salicylic acid (50 µM) on Guaiacol peroxidase (GPX) enzyme activity (mg guaiacol protein / min) in mungbean subjected to salt stress. The Duncan multiple range test values with different letters are differed significantly at $p \leq 0.05$.

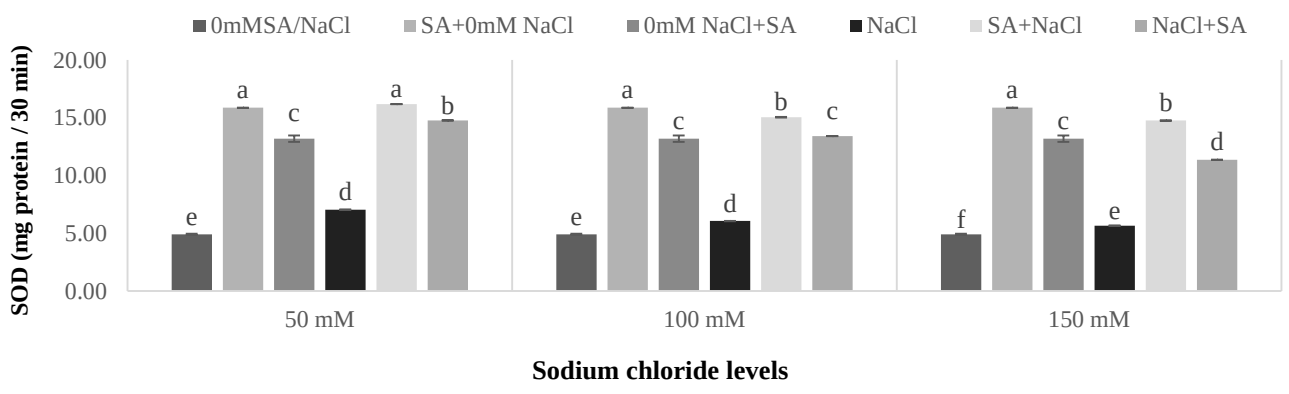

Fig. 16. Impact of exogenously applied salicylic acid (50 µM) on Superoxide dismutase (SOD) enzyme activity (mg / 30 min) in mungbean subjected to salt stress. The Duncan multiple range test values with different letters are differed significantly at $p \leq 0.05$.

Salinity is not only limiting factor for plant propagation but also destroy land fertility by accumulating an excessive amount of chloride and sodium ions in the soil (Pandolfi *et al*., 2012). The higher quantity of toxic ions damaged irrigated lands that ultimately affect vigor, growth, biomass, water content, photosynthesis, and biochemistry of seedling (Chunthaburee *et al*., 2016; Mahboob *et al*., 2017). Reduction in growth and its related traits were gradually preceded as NaCl stress increased from 50 mM to 150 mM in mungbean (Figs. 2-5). It was observed that highest level of NaCl stress produced more severe destruction at morphological, physiological and biochemical traits in various crop species such as mungbean (El-Kafafi *et al*., 2015), potato (Faried *et al*., 2016), oat (Chauhan *et al*., 2016), maize (Jiang *et al*., 2017), pepper (Abbas *et al*., 2013), and wheat (Mahboob *et al*., 2016). In the present study, salinity induced reduction in growth was alleviated by pre-and post-application of salicylic acid on mungbean seedlings. Salicylic acid is the well-documented chemical which increases crop acclimation against salt which leads to building resistance against salinity (Jayakannan *et al*., 2013). The earlier study revealed that pre-treatment of salicylic acid induced resistance to salt stress (Shakeel & Mansoor, 2012a; Jayakannan *et al*., 2013).

Discussion

A common response of plants to salinity is a shift of relative water content to improve moisture uptake that positively effects germination, growth and physiological performance. Under salinity, inhibition of water uptake decreased the hydrolysis and translocation of food that caused a reduction in growth and seedling vigor (Misra & Dwivedi, 2004; Noreen & Ashraf, 2010; Parveen *et al*., 2016). The findings of present experiment with mungbean seedlings supported the previous work; where beans seedlings were not able to maintain significantly relative water content at salinity (Fig. 6). Similarly, earlier researchers found a decrease in relative water content in corn plants (Kaya *et al*., 2013; Abbasi *et al*., 2014 & 2015), and *Limonium bicolor* (Wang *et al*., 2016). Abideen *et al.,* (2014) suggested that the reduction in growth with an increase of salinity contributed to minimizing water loss. However, when seedlings provided with SA application contributed to maintaining higher relative water content without the subsequent reduction in biomass production which is the prerequisite for survival and biomass production in stress condition. These findings are confirmed by earlier reports (Azooz *et al*., 2011; Hussain *et al*., 2011b; Habibi, 2012; Abedinpour, 2016).

Seedlings raised under salinity showed a decreasing effect on photosynthetic pigments. While, chlorophyll *a*, *b* and carotenoids were significantly increased with the application of SA (Fig. 7-10). These findings are in accordance to Fayez & Bazaid (2014), who reported that an exogenous application of SA significantly enhanced carotenoids and chlorophyll content of barley plant under salinization stress. That increase in chlorophyll content probably indicates changes in the size of an antenna with respect to reaction centers (Ashraf & Harris, 2013). In contrast, Bota *et al.,* (2004) suggested the active involvement of rubisco species enhance the synthesis of photosynthetic pigments. Hence, further improvement in both rubisco activity and chlorophyll content were restored by SA under severe stress (Idrees *et al*., 2010). The reduced leaf area in response to an elevated level of salinity results in lower transpiration rate to conserve water as well as inhibited photosynthesis due to limiting the surface area for the synthesis of pigments (Ahmed *et al*., 2013). Therefore, chlorophyll reduction in mungbean might be due to restricted chlorophyll biosynthesis, degradation of existing chlorophyll or lower relative water content in this experiment. These assumptions are strengthened by the findings of the previous researcher (Ashraf & Harris, 2013; Moinuddin *et al.,* 2017).

The NaCl toxicity increases in Malondialdehyde content which is the product of lipid peroxidation process which degrades cell membrane lipid and as well as enhances membrane permeability (Ghosh *et al*., 2015). It was noted that MDA production was lowest at lower (50 mM) NaCl stress while its accumulation subsequently increased at higher concentration of NaCl (150 mM) (Fig. 11). Therefore, it is suggested that Malondialdehyde content has a direct relationship with salt toxicity. These findings agreed with Wang *et al.,* (2016), Butt *et al*., (2016), and Fayez & Bazaid (2014) studies. It has been reported that greatest reduction in MDA content was observed under salt stress when exposed to exogenous salicylic acid (Fig. 11). The findings of Horvath *et al*., (2007) and Fayez and Bazaid (2014), are also in accordance with the results of a current study. The salicylic acid plays a defensive role in the subsequent integrity of plasma membrane (Khan *et al*., 2010). However, stress hormone production might be limited under sub-optimal circumstances (El-Khallal *et al*., 2009; Hussain *et al*., 2011b). Therefore, exogenously applied salicylic acid increases the synthesis of secondary metabolites such as lipid, phenolics, alkaloids, anthocyanins, glucosinolate, glycine betaine, soluble sugar, amines, etc. (Khan *et al*., 2015) and contribute to induce stress tolerance.

In the current analysis, the total proteins synthesized in less concentration at low NaCl stress while it is accumulated more in seedlings under severe salt stress. Similar results have been reported earlier in *Arabidopsis thaliana* (Quintero *et al*., 1996), *Camarosa* species (El-Baz *et al*., 2003) and *Zea mays* (Arora *et al*., 2008). The maximum storage of proteins in the vegetative part of the seedlings revealed the function of late-embryogenesis-abundant (LEA) protein and stress tolerant proteins (Hundertmark & Hincha, 2008). It is suggested that accumulation of these proteins might be involved in protecting embryo by the post-dehydration process (Ingram & Barlds, 1996; Tolleter *et al*., 2010). In contrast, El-Khallal *et al.,* (2009), Shahid *et al.,* (2011) and Shakeel & Mansoor (2012b), were observed the significant decrease of protein content at higher concentration of NaCl. However, an exogenous application of salicylic acid enhanced the accumulation of proteins under salt stress as compared to NaCl alone (Fig. 12). This might be due to the role of salicylic acid as signaling molecule under stress condition (Lorenzo & Solano, 2005; Xia *et al*., 2011). Similar results were reported in past studies that, SA enhanced the expression of proteins and regulates the transcription of many genes (El-Khallal *et al*., 2009; Shakeel & Mansoor, 2012b). These genes stimulate defense mechanism against stress (Khan *et al*., 2015), ultimately refine the overall growth of the crop (El-Khallal *et al*., 2009).

The salt stress ameliorative response is generally associated with antioxidant enzyme system triggered by ROS (Raza *et al*., 2013; Fayez & Bazaid, 2014). It was noted that the over-production of H_2O_2 cause deleterious impact on seedling physiology that was restored by SA application (El-Khallal *et al*., 2009). It was also observed that CAT, APX, GPX and SOD enzymes activity was subsequently decreased by each successive concentration of salt in both with and without SA treatments (Fig. 16). However, salt-stressed without SA treated seedlings had lower enzymes activity, indicated the unwanted higher deposition of ROS in various cell compartments (McCord, 2000). CAT and APX remove the H_2O_2 found in chloroplasts, peroxisomes, and cytosol while CAT is known to dismutate H_2O_2 into H_2O and O_2 (Khan *et al*., 2010; Erdal *et al*., 2011). In addition, SOD is one of a several important antioxidant enzyme with the ability to repair oxidative damage caused by ROS (Sakhabutdinova

et al., 2003; El-Khallal *et al*., 2009). The enhancement of POX and SOD activities by SA in beans has been well documented to encounter and repair oxidative damage caused by ROS (Khan *et al*., 2010), as observed in mungbean seedlings in this study. Furthermore, increased activity of antioxidant metabolism and simultaneous reduction in MDA level in mung seedling would be the ideal indicator of stress alleviation. Hence, salt resistance could be associated with elevated activities of antioxidant enzymes (Gill & Tuteja, 2010) in plant species regulated by SA application. These findings were consistent with earlier work, carried on *Vigna radiata* (Khan *et al*., 2010), *Abelmoschus esculentus* (Raza *et al*., 2013), and *Hordeum vulgare* (Habibi, 2012), exposed to salt stress.

In conclusion, mungbean growth was increased on exposure to exogenous SA application, particularly before imposition of salt stress (SA+NaCl). SA imbibition improved photosynthetic pigments total proteins and relative water content as well as caused a reduction in MDA content by maintaining adequate levels of antioxidant activity that favors increased photosynthesis and higher biomass production. Growth inhibition under higher salinity without SA application could be attributed to low leaf tissue hydration and degradation of chlorophyll, leading to higher production of ROS and an antioxidant enzyme. The present study revealed that mungbean seedlings were sensitive to salt stress, but when the seeds were imbibed in salicylic acid for 24 h prior to salinity stress, exhibited better physiological and biochemical parameters. Thus, the exogenous application of salicylic acidcan be considered as a cheap and an effective method to alleviate stress on *Vigna radiata* seedlings.

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