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# SALICYLIC ACID INDUCED AMELIORATION IN GROWTH, BIOCHEMICAL METABOLITES AND YIELD OF MUNGBEAN (*VIGNA RADIATA* L.) UNDER SALINITY STRESS

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## SUMMARY

**The study was conducted in green house to determine whether exogenous application of salicylic acid (SA) through foliar spray could modulate the plant growth, biochemical attributes, mineral nutrition and yield in mungbean (*Vigna radiata* L.) under two levels of salinity. The chloride dominated (Cl: $SO_4$ ratio 7:3) salinity was maintained in earthen pots at 4 and 6 dS $m^{-1}$ levels after seed germination. At flower initiation, two concentration of salicylic acid viz 0.25 and 0.50 mM were applied as foliar spray on both control and salinity treated plants and observations were recorded at 2 and 6 days after spray of salicylic acid. Increasing salt stress reduced the dry matter accumulation in leaves, stem and roots alongwith leaf area. The salicylic acid application at 0.5 mM improved the growth parameters and the response was more at 6 days stage. Biochemical metabolites i.e. total soluble protein declined under salinity stress, however, free proline, total soluble carbohydrates, and free amino acids content showed sharp rise under salinity stress. Foliar SA application increased all the above biochemical metabolites under salinity. The leaf ionic composition of $Na^+$, $Cl^-$ and $SO_4^-$ increased but that of $K^+$ declined under salinity stress. Salicylic acid reversed the accumulation trend of ions under salt stressed condition. Significant reduction in seed yield and its attributes were recorded under both levels of salinity. Salicylic acid application protected against salinity induced decline in yield components. The recovery was more pronounced at 6 dS $m^{-1}$ salinity as compared to 4 dS $m^{-1}$ salinity level particularly with 0.5 mM salicylic acid.**

***Key words*: Free amino acids, proline, ionic composition, mungbean, salicylic acid, salinity, yield**

## INTRODUCTION

Salinity is the major abiotic stress that can affect physiological and biochemical processes of plant development, resulting in reduced growth and yield (Ashraf 2004). Around the world, 100 million ha, or 5% of arable land is adversely affected by high salt concentration. Accumulation of soluble salts in the soil leads to osmotic stress, biochemical imbalance, specific ion toxicity and ionic imbalance in the plants caused deficiency of nutrient ion. In order to overcome the unfavourable saline conditions, plant developed various mechanism of adaptation which include accumulation of compatible solutes, like glycinebetaine, proline and total soluble carbohydrates (Munns 2003).

Salicylic acid (SA), a plant hormone, plays an important role in abiotic stress tolerance (Raskin 1992). Salicylic acid may act as endogenous signal molecule responsible for inducing abiotic stress tolerance by improving plant growth, stimulate the synthesis of protein, retard or enhance the accumulation of proline content. Convincing data have been obtained concerning the salicylic acid increase the resistance in bean, tomato,

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desert pea, corn, wheat, maize and rice to salinity (Arfan *et al*. 2007, Gunes *et al*. 2007, Senaratna *et al*. 2007). The salicylic acid is used in small concentration because the higher concentration of SA (1.0 mM or more) proved inhibitory under nonsaline and saline conditions. Nemeth *et al.* (2002) provided the evidence that inhibition of APX with the higher concentration of SA blocks the $H_2O_2$ degrading pathway in the plant cell leading to increased levels of endogenous $H_2O_2$. The SA alleviates the negative effect of salt stress on photosynthesis depending on the concentration of SA used. The application of 1.0 mM SA under salt stress resulted in inhibition in growth, photosynthesis and yield (Khan *et al*. 2010).

To further clarify the action of salicylic acid in plant tissue under salt stress, the present study was conducted to assess the efficiency of foliar application of low concentration of salicylic acid (≤ 0.5 mM) in alleviating the adverse effect of salt stress on growth and yield of mungbean.

## MATERIALS & METHODS

Mungbean (*Vigna radiate* L. Wilczek) cv. Asha was raised in earthen pots with 5 kg dune sand under screen house conditions in the Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar (situated at 29°10' N latitude, 75°48' E longitude, 215 m altitude) India. Mungbean seeds of uniform size were surface sterilized by treating with 95% ethanol for 10 seconds followed by treatment with 2.5% sodium hypochlorite for 10 minutes. The seeds were thoroughly washed with sterile water and then inoculated with *Rhizobium* culture (S-24) obtained from Department of Microbiology, CCS Haryana Agricultural University, Hisar. Five seeds per pot were sown at uniform depth and distance. Finally, after thinning two healthy seedlings per pot were retained. Plants were nourished by nutrient solution of Wilson and Reisenaur (1963) at 10 days intervals. The saline solution was prepared for chloride ($Cl^-$) dominated salinity using mixture of different salts like NaCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$, where, Na: Ca + Mg was in the ratio of 1:1, Ca: Mg in the ratio of 1:3 and Cl: $SO_4$ ratio was 7:3 on meq basis. After germination, the desired salinity was applied to saturate each pot so as to maintain two levels i.e. 4 and 6 $dSm^{-1}$ of chloride ($Cl^-$) dominated salinity. The pots of non-saline (control) were irrigated to field capacity with tap water (EC 0.25 $dSm^{-1}$). After the salinity treatments, the pots were irrigated 50 per cent from the surface and 50 per cent (of the total) from the subsurface (through a slightly inclined embedded plastic feeder tube having a pad of glass wool at its lower end). In this way uniformly required ECe was maintained throughout the dune sand. At the initiation of flowering (40 DAS), two concentrations of salicylic acid i.e. 0.25 and 0.50 mM were applied as foliar spray on control as well as 4 and 6 $dSm^{-1}$ stressed plants. For each parameter, three pots having two plants per pot were sampled at a time which comprises of three replicates. The plants were sampled for following observations after 2 and 6 days of salicylic acid treatments.

The different plant parts were separated into root, stem and leaves and were dried in labeled paper envelops in an oven at 70°C until constant weight and their dry weights were determined and expressed as g $plant^{-1}$. Leaf area was measured with the help of leaf area meter (Model LI 3000, LI COR Ltd., Nebraska, USA) and expressed as sq. cm $plant^{-1}$. The proline and total soluble carbohydrates (TSC) content were estimated by the method of Bates *et al*. (1973) and Yemm and Willis (1954), respectively. The total soluble protein content was measured with Folin-Ciocalteau reagent (Lowry *et al*., 1951). The values were calculated as mg $g^{-1}$ dry weight. Free amino acids content was estimated by Yemm and Cocking (1955) and values were calculated as μ mole $g^{-1}$ dry weight. Sodium and potassium contents were determined using flame photometer (Elico, India). Chloride and sulphate content were measured by the method of Chhabra (1973) and Tabatobai and Bremner (1970), respectively and the values were calculated in terms of m mole $g^{-1}$ dry weight.

The seed yield and its attributes were recorded after final harvest at physiological maturity (63 DAS). Data were analyzed using complete randomized design (CRD) for two factors. Data were subjected to analysis of variance (ANOVA) using Online Statistical Analysis Package (OPSTAT, Computer Section, CCS Haryana Agricultural University, Hisar, India) and mean separated by Duncan's Multiple Range Test at $P<0.05$. Correlation and regression were also calculated.

## RESULTS & DISCUSSION

Increasing level of salinity reduced the plant height and dry mass of root, stem and leaves and leaf area (Table 1). The decrease was more under 6 $dSm^{-1}$ over the irrigated control plants. Application of salicylic acid (both concentrations) through foliar spray increased the plant dry matter and leaf area of both control as well as

**Table 1.** Effect of salinity and salicylic acid on dry weight of different plant parts and leaf area in mungbean.

| Salinity Levels | Salicylic Acid (mM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 Days after spray | | | | 6 Days after spray | | | |
| | Control | 0.25 | 0.50 | Mean | Control | 0.25 | 0.50 | Mean |
| **Leaves dry weight (g $plant^{-1}$)** | | | | | | | | |
| Control | 2.22 | 2.24 | 2.26 | 2.24 | 2.27 | 2.33 | 2.42 | 2.34 |
| 4dS $m^{-1}$ | 1.81 | 1.83 | 1.86 | 1.83 | 1.95 | 2.09 | 2.17 | 2.07 |
| 6 dS $m^{-1}$ | 1.42 | 1.44 | 1.46 | 1.44 | 1.47 | 1.59 | 1.71 | 1.59 |
| Mean | 1.82 | 1.84 | 1.86 | | 1.90 | 2.00 | 2.10 | |
| CD at 5% | A | 0.25 | B | N.S | A | 0.24 | B | 0.04 |
| | AxB | N.S. | | | AxB | N.S. | | |
| **Stem dry weight (g $plant^{-1}$)** | | | | | | | | |
| Control | 1.63 | 1.64 | 1.65 | 1.64 | 1.81 | 1.86 | 1.91 | 1.86 |
| 4dS $m^{-1}$ | 1.44 | 1.46 | 1.47 | 1.46 | 1.48 | 1.53 | 1.65 | 1.55 |
| 6 dS $m^{-1}$ | 1.12 | 1.15 | 1.16 | 1.14 | 1.18 | 1.29 | 1.34 | 1.27 |
| Mean | 1.40 | 1.42 | 1.43 | | 1.49 | 1.56 | 1.63 | |
| CD at 5% | A | 0.15 | B | 0.05 | A | 0.26 | B | 0.06 |
| | AxB | N.S. | | | AxB | N.S. | | |
| **Roots dry weight (g $plant^{-1}$)** | | | | | | | | |
| Control | 2.53 | 2.54 | 2.55 | 2.54 | 2.7 | 2.78 | 2.85 | 2.78 |
| 4dS $m^{-1}$ | 1.89 | 2.92 | 2.93 | 2.58 | 2.08 | 2.34 | 2.46 | 2.29 |
| 6 dS $m^{-1}$ | 1.38 | 1.41 | 1.43 | 1.41 | 1.41 | 1.62 | 1.71 | 1.58 |
| Mean | 1.93 | 2.29 | 2.30 | | 2.06 | 2.25 | 2.34 | |
| CD at 5% | A | 0.47 | B | 0.06 | A | 0.40 | B | 0.08 |
| | AxB | N.S. | | | AxB | N.S. | | |
| **Plant dry weight (g $plant^{-1}$)** | | | | | | | | |
| Control | 6.38 | 6.42 | 6.46 | 6.42 | 6.78 | 6.97 | 7.18 | 6.98 |
| 4dS $m^{-1}$ | 5.14 | 5.21 | 5.26 | 5.20 | 5.51 | 5.96 | 6.28 | 5.92 |
| 6 dS $m^{-1}$ | 3.92 | 4.00 | 4.05 | 3.99 | 4.06 | 4.5 | 4.76 | 4.44 |
| Mean | 5.15 | 5.21 | 5.26 | | 5.45 | 5.81 | 6.07 | |
| CD at 5% | A | 0.84 | B | 0.12 | A | 0.90 | B | 0.08 |
| | AxB | N.S. | | | AxB | N.S. | | |
| **Leaf area ($cm^2$ $plant^{-1}$)** | | | | | | | | |
| Control | 422.5 | 426.5 | 428.4 | 425.8 | 430.9 | 438.1 | 458.4 | 442.5 |
| 4dS $m^{-1}$ | 318.4 | 322.3 | 326.5 | 322.4 | 327.9 | 371.9 | 389.6 | 363.1 |
| 6 dS $m^{-1}$ | 208 | 211.8 | 214.3 | 211.4 | 215.2 | 263.1 | 284.2 | 254.2 |
| Mean | 316.3 | 320.2 | 323.1 | | 324.7 | 357.7 | 377.4 | |
| CD at 5% | A | 15.4 | B | 5.58 | A | 13.9 | B | 3.01 |
| | AxB | NS | | | AxB | NS | | |

A = Salinity; B = Salicylic acid; AxB = Interaction stressed plants. Effect of salicylic acid was more pronounced at 6 days after spray. However, the interaction effect was non significant in all growth parameters at both stages of observations. Kaydan *et al*. (2007) also reported that dry weight of plants and leaf area was decreased due to salinity but SA application improved the dry weight of the plant and green leaf area. The stimulating effect of salicylic acid on dry matter accumulation and leaf area may be due to increase in relative water content and photosynthetic capacity (Khan *et al*. 2003).

Salinity caused reduction in biochemical parameter i.e. free amino acid, total soluble protein and consequently affected the growth and development. The ameliorative effects of salicylic acid on physiological and biochemical process under salinity stress in a range of crops were observed by Szepesi *et al.* (2005), Genus *et al.* (2007) and Noreen *et al*. (2009). Total soluble protein content decreased significantly in both levels of salinity (Fig. 1a). Spray on stressed plant with salicylic acid increased the protein content. Increase in protein content was considerably more in 0.5 mM than in 0.25 mM salicylic acid. Effect of SA was almost similar at both 2 and 6 days after spray. Total free amino acid and total soluble carbohydrate content increased significantly from 18.4 to 43.5 per cent with increasing level of salinity (Fig. 1b, c). Foliar application salicylic acid further increased the above biochemical constituents significantly in control as well as in salinity stressed plant and the effect of 0.5 mM salicylic acid was more pronounced. Sharp increase in amino acid content could either be due to disruption in protein synthesis or slowing down of flow of amino acids to physiological active sink (Garg *et al*. 2004). The enhancement in free amino aids content with salicylic acid might be due to the stimulation of haxose and valine uptake resulting from an increase in activity of the plasma membrane $H^+$-ATPase and corresponding haxose and amino acid transporter (Yang *et al*. 2004). Salicylic acid caused a decrease in total soluble carbohydrates suggesting that SA may be activating the metabolic consumption of soluble sugar to form new cell constituents as a mechanism to stimulate the growth of the plants. Our assumption could be supported by the results that SA increased polysaccharide levels on the sake of soluble sugar (Khodary 2004). Increasing level of salinity caused increase in proline content (Fig. 1d). Deficiency of $K^+$ induced by salinity may increase the level of free amino acid especially the proline (Hussein *et al.* 2007). Salicylic acid treatment further increase proline content and the increase was more with 0.5 mM salicylic acid. Our findings are supported by Tammam (2003) who observed that salicylic acid induced ABA mediated protective reactions of plants in response to salinity in *Vicia faba*.

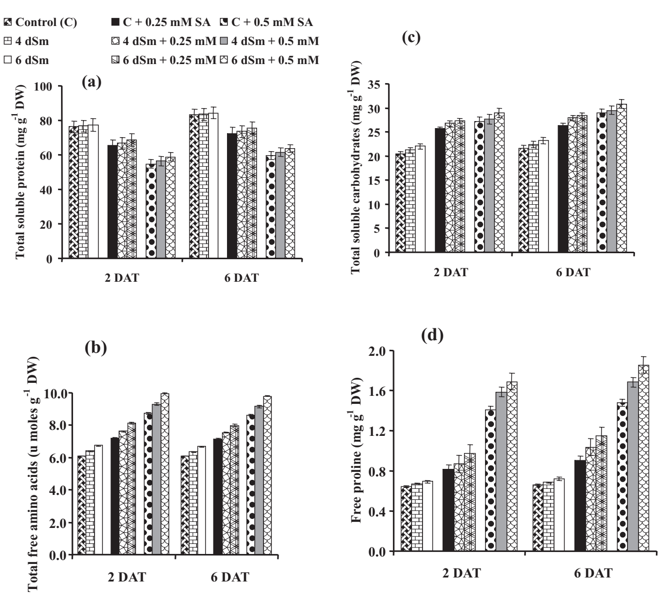


**Fig. 1. Effect of salinity and salicylic acid on (a) Total soluble proteins (b) Free amino acids (c) Total soluble carbohydrates (d) Free proline content of leaf in mungbean. DAT: Days after Salicylic Acid Treatment.**

Salinity caused increased content of $Na^+$, $Cl^-$ and $SO_4^-$ and a decrease in $K^+$ (Table 2). The positive correlation between increase in salinity and increase in $Na^+$, $Cl^-$ and $SO_4^-$ has been well documented by Kukreja *et al.* (2006). The application of salicylic acid significantly reduced the $Na^+$, $Cl^-$ and $SO_4^-$ and increased $K^+$ under salinity. The decreasing level of these ions explain the ameliorating effect of salicylic acid likely due to enhance accumulation of compatible solute i.e. proline, sugar and also $K^+$ which protects plants during salinity stress. Genus *et al*. (2007) also suggested that salicylic acid induced a decline in $Na^+$ and $Cl^-$ content and an increase in $K^+$ due to an increase in antioxidant activity of salicylic acid in salinity stressed maize plants. This indicates that salicylic acid application induced a reduction in $Na^+$, $Cl^-$

**Table 2.** Effect of salinity and salicylic acid on different ionic content in leaves of mungbean.

| Salinity Levels | Salicylic Acid (mM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 Days after spray | | | | 6 Days after spray | | | |
| | Control | 0.25 | 0.50 | Mean | Control | 0.25 | 0.50 | Mean |
| **$K^+$ (m moles $g^{-1}$ DW)** | | | | | | | | |
| Control | 2.86 | 2.88 | 2.94 | 2.89 | 2.82 | 2.87 | 2.9 | 2.86 |
| 4dS $m^{-1}$ | 1.80 | 1.94 | 1.99 | 1.91 | 1.99 | 2.12 | 2.3 | 2.14 |
| 6 dS $m^{-1}$ | 1.32 | 1.44 | 1.59 | 1.45 | 1.41 | 1.59 | 1.7 | 1.57 |
| Mean | 1.99 | 2.09 | 2.17 | 2.08 | 2.07 | 2.19 | 2.30 | |
| CD at 5% | A | 0.36 | B | 0.06 | A | 0.53 | B | 0.04 |
| | AxB | 0.61 | | | AxB | N.S. | | |
| **$Na^+$ (m moles $g^{-1}$ DW)** | | | | | | | | |
| Control | 2.34 | 2.31 | 2.20 | 2.28 | 2.31 | 2.24 | 2.08 | 2.21 |
| 4dS $m^{-1}$ | 4.12 | 3.75 | 3.25 | 3.71 | 3.89 | 3.67 | 3.43 | 3.66 |
| 6 dS $m^{-1}$ | 4.19 | 3.78 | 3.67 | 3.88 | 4.24 | 3.63 | 3.50 | 3.79 |
| Mean | 3.55 | 3.28 | 3.04 | | 3.48 | 3.18 | 3.00 | |
| CD at 5% | A | 0.06 | B | 0.08 | A | 0.05 | B | 0.07 |
| | AxB | 0.10 | | | AxB | 0.11 | | |
| **$Cl^-$ (m moles $g^{-1}$ DW)** | | | | | | | | |
| Control | 2.62 | 2.56 | 2.29 | 2.49 | 3.19 | 3.11 | 2.74 | 3.01 |
| 4dS $m^{-1}$ | 3.86 | 3.56 | 3.17 | 3.53 | 4.71 | 4.34 | 3.94 | 4.33 |
| 6 dS $m^{-1}$ | 4.53 | 4.23 | 3.92 | 4.23 | 5.46 | 4.89 | 4.42 | 4.92 |
| Mean | 3.67 | 3.45 | 3.13 | | 4.45 | 4.11 | 3.70 | |
| CD at 5% | A | 0.66 | B | 0.08 | A | 0.58 | B | 0.18 |
| | AxB | 0.71 | | | AxB | N.S. | | |
| **$SO_4^{-2}$ (m moles $g^{-1}$ DW)** | | | | | | | | |
| Control | 1.43 | 1.35 | 1.24 | 1.34 | 1.64 | 1.53 | 1.41 | 1.53 |
| 4dS $m^{-1}$ | 1.96 | 1.76 | 1.64 | 1.79 | 2.16 | 1.91 | 1.78 | 1.95 |
| 6 dS $m^{-1}$ | 2.58 | 2.34 | 2.13 | 2.35 | 2.95 | 2.68 | 2.33 | 2.65 |
| Mean | 1.99 | 1.82 | 1.67 | | 2.25 | 2.04 | 1.84 | |
| CD at 5% | A | 0.42 | B | 0.06 | A | 0.48 | B | 0.08 |
| | AxB | 0.47 | | | AxB | N.S. | | |

A = Salinity; B = Salicylic acid; AxB = Interaction

and $SO_4^-$ absorption and harmful effect and increased $K^+$ which is reflected in low membrane injury and dry matter production (El-Tayeb 2005).

The yield attributes viz number of pods, pod weight and 100 seed weight decreased significantly at both level of salinity (Table 3). The reduction in number of pods, pod weight and 100 seed weight ranged from 25.0 to 41.7, 21.4 to 35.8 and 18.8 to 13.0 per cent under 4 and 6 $dSm^{-1,}$ respectively. The foliar application of salicylic acid (0.25 mM and 0.5 mM) increased the yield attributes in control as well as in salinity stressed plants. However, the interaction effect of salinity and salicylic acid was significant in pod weight only. Seed yield significantly decreased up to 37.2 per cent with increasing level of salinity as compared to control plants; however, biomass yield reduced from 15.0 to 23.4 per cent under 4 and 6 dSm-1 salinity levels, respectively. Exogenous application of salicylic acid enhanced the seed and biomass yield in stressed and control plants. Higher concentration of salicylic acid (0.5 mM) was more effective under salinity stress than lower concentration. The per cent increase

**Table 3.** Effect of salinity and salicylic acid on yield and its attributes in mungbean.

| Salinity Levels | Salicylic Acid (mM) | | | |
|---|---|---|---|---|
| | Control | 0.25 | 0.50 | Mean |
| **Number of pods plant$^{-1}$** | | | | |
| Control | 24.0 | 24.7 | 26.0 | 24.9 |
| 4dS m$^{-1}$ | 18.0 | 18.7 | 19.8 | 18.8 |
| 6 dS m$^{-1}$ | 14.0 | 15.2 | 16.1 | 15.1 |
| Mean | 18.7 | 19.5 | 20.6 | 19.6 |
| CD at 5% | A 3.02 | B 0.71 | AxB NS | |
| **Pods weight plant$^{-1}$ (g)** | | | | |
| Control | 13.29 | 13.48 | 14.41 | 13.73 |
| 4dS m$^{-1}$ | 10.44 | 10.92 | 11.48 | 10.95 |
| 6 dS m$^{-1}$ | 8.53 | 9.15 | 9.64 | 9.11 |
| Mean | 10.75 | 11.18 | 11.84 | 11.26 |
| CD at 5% | A 1.38 | B 0.32 | AxB 1.43 | |
| **100 seed weight (g)** | | | | |
| Control | 6.18 | 6.26 | 6.35 | 6.26 |
| 4dS m$^{-1}$ | 5.02 | 5.19 | 5.27 | 5.16 |
| 6 dS m$^{-1}$ | 4.26 | 4.41 | 4.56 | 4.41 |
| Mean | 5.15 | 5.29 | 5.39 | 5.28 |
| CD at 5% | A 0.57 | B 0.09 | AxB NS | |
| **Seed yield plant$^{-1}$(g)** | | | | |
| Control | 10.96 | 11.14 | 11.93 | 11.34 |
| 4dS m$^{-1}$ | 8.42 | 8.83 | 9.35 | 8.87 |
| 6 dS m$^{-1}$ | 6.88 | 7.46 | 7.92 | 7.42 |
| Mean | 8.75 | 9.14 | 9.73 | 9.21 |
| CD at 5% | A 1.05 | B 0.25 | AxB 1.09 | |
| **Biomass yield plant$^{-1}$(g)** | | | | |
| Control | 32.1 | 32.8 | 34.9 | 33.3 |
| 4dS m$^{-1}$ | 27.8 | 28.7 | 29.7 | 28.7 |
| 6 dS m$^{-1}$ | 24.1 | 25.5 | 26.7 | 25.4 |
| Mean | 28.0 | 29.0 | 30.4 | 29.1 |
| CD at 5% | A 2.08 | B 0.88 | AxB 2.21 | |

A = Salinity; B = Salicylic acid; AxB = Interaction

in seed and biomass yield was observed to be 11.0 and 10.8 per cent at 4 dSm$^{-1}$ and 15.1 and 6.5 per cent at 6 dSm$^{-1}$ respectively. The reduction at higher salinity level in most of the yield attributing characters may be because of adverse affect on growth and dry matter accumulation. With increase in salicylic acid concentration, there was corresponding increase in yield due to improved water status (data not shown) at both levels of salinities. The response of applied salicylic acid in terms of yield and its attributes was relatively more at 0.5 mM of salicylic acid and 6 dSm$^{-1}$ salinity level. Salicylic acid induced yield improvement was also reported by Arfan *et al.* (2007). The seed yield of mungbean showed significant and positive association with total soluble protein ($R^2$ = 0.95), number of pods per plant ($R^2$ = 0.99) and biomass across ($R^2$ = 0.98) at both levels of salinity. From the above results, it can be concluded that salinity caused reduction in yield could be improved by foliar application of salicylic acid. The improvement in yield is associated with increase or decrease in biochemical metabolites and a significant balance in ionic composition.

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