Influence of Foliar Application of Salicylic acid on mitigating the deleterious effect of salt stress on Morpho -physio biochemical and yield traits of Mungbean (*Vigna radiata* L.)

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Abstract

The study was conducted to evaluate the effect of Salicylic acid (SA) as a foliar spray to mitigate the deleterious effects of salt stress on mungbean plants. Two mungbean genotypes viz; Shikha and Heera were grown under induced saline conditions (100, 150 and 200 mM NaCl) and compared with untreated control. Salt exposure decreased the morpho-physiological, biochemical and yield traits of both the genotypes, which was responded in the same way under induced salt stress. SA found to be essential in mitigating the adverse effects of salt stress in both tested mungbean genotypes. At the threshold combination of SA and NaCl (100 ppm SA + 100 mM NaCl), SA led to maximum plant height, number of leaves per plant, relative water content (RWC), chlorophyll content, number of pods and seed yield. Its foliar application was also expressed positive response in modulating the total proline content under different salt stress conditions.

It was also concluded that foliar application of SA @100 ppm was highly effective in reducing the deleterious effect of salt at the combination with 100 mM NaCl and 150 mM NaCl and strengthened the ability of survival of both the mungbean genotypes under salt stress.

Key words: Salicylic acid, Salt stress, Relative water content, Total Chlorophyll content, Proline content,

INTRODUCTION

Changing the environmental scenario, yield losses might be the result of salt stress during the different plant growth stages, leading to increased substantial reduction in its potential yield. Soil salt is a major abiotic production constraint that has the potential to significantly reduce crop growth, development, and productivity in the world's pulse-growing regions. Mungbean (*Vigna radiata* L.) is a great source of high-quality protein (25%), high digestibility, good source of vitamin C, thiamine, and riboflavin. It is also utilized as cattle feed and good source of nitrogen as green manure crop. Enough salts, particularly sodium chloride, are present in the root zone of saline soils to considerably impede plant growth. The detrimental effects of salt stress on plant activity are linked to osmotic effects that reduces the soil water supply, which can result in direct toxicity, physiological drought, nutritional deficiency (Ashraf *et al*.,2005). It is also explained by the fact that plants cannot grow and develop when exposed to salt and can also lead to physiological water limitation due to severe salt deposition in rhizosphere causing a low osmotic potential and ion imbalance (Zhu, 2002, Munns & Tester, 2008). Salt stress adversely affected the photosynthetic pigment because imposition of salt stress causes deterioration in the structure of chloroplast, e.g., thylakoid membranes and plastids due to direct Na^+ toxicity and cellular oxidative damage (Mittler, 2002). Numerous plant species respond to salt stress by accumulating proline, which may help them defend against salinity stress. Proline is one of the most vital compatible compounds, it has been found that the concentration of proline increases when plants are exposed to salt stress (Wang et al., 2007; Pagter et al*.,* 2009). Pre-treatment with Salicylic acid @ 1.0 mM, decreased proline per cent in both genotypes at different concentrations of NaCl as compared to individual salt treated plants. The mode of action and mechanism may be defined as reduction in proline content by Salicylic acid may be enhanced sugar accumulation and water uptake in salt stress seedlings.

In this regard, one of the capable measures is exogenous foliar applications of Salicylic acid (SA) which may be effectively used as supportive and capable to combat the deleterious effect of salt stress.

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Keeping these facts in view, the current investigation was carried out against two varieties of mung bean by inducing salt stress using NaCl @ 100, 150, and 200 mM and the harmful effect of salt stress were mitigated through foliar application of SA @100 ppm in experimental pots.

MATERIALS AND METHODS

The pot experiment was carried out at the College of Agriculture experimental field in Rewa, Madhya Pradesh, during the summer of 2023. Shikha and Heera, the two most common and promising mungbean genotypes, served as the experimental plant materials.

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The design of the experiment was factorial CRD, and data was analyzed for analysis of variance (Gomez and Gomez ,1984). Both mungbean genotype seeds were surface sterilized for five minutes with frequent shaking using a 0.2% $HgCl_2$ solution, and the $HgCl_2$ was removed by repeatedly washing the seeds with de-ionized water. The application of NaCl (at 100, 150, and 200 mM) was then used to induce salt stress in soil in single shock to attain electrical conductivity (EC) 8, 13, and 19 dSm^{-1}, respectively. Ten carefully chosen seeds of each mungbean genotype were placed in 20 cm x 20 cm plastic pots filled with 5 kg of farm soil having a moisture content of 12–14% at the time of planting. At the time of sowing, a single dose of each fertilizer was applied as the basal dose. The individual and combined treatments were taken as T0 (control: without NaCl and SA), T1(100 ppm SA), T2 (100 mM NaCl), T3(150 mM NaCl), T4 (200 mM NaCl), T5 (100 mM NaCl + 100 ppm SA), T6 (150 mM NaCl + 100 ppm

SA), T7 (200 mM NaCl + 100 ppm SA). The treatments were replicated three times. The exogenous foliar application of SA (@100 ppm) was applied on 20, 30 and 40 days after sowing. Morpho-physiological, biochemical, yield and yield attributes related observations were recorded after maturity.

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Measurement of various morpho-physiological, biochemical and yield parameters

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Plant height was measured with the help of scale from the base of the plant to the top of the main shoot. The average height of five plants was considered. leaf area index was measured by leaf area meter (CI-203). Total dry matter was measured and average weight was calculated from the replicated data and represented in gram (g). The plants were harvested carefully and oven dried for killing at 105°C for 1 h followed by keeping the plant materials at 65-70°C up to 72 h; weighed with the help of electronic balance at every 24 h till the constant weight was obtained. Chlorophyll content was recorded with the help of chlorophyll meter (CCM-200) and the average of five readings was reported as SPAD value. The proline content (μg g^{-1} fresh weight) was estimated by the method of Bates et al.., (1973). Plant material was homogenized with 3% aqueous sulfosalicylic acid and centrifuged it at 10,000 rpm. The supernatant was used for reaction mixture consisted of 2 mL of acid ninhydrin and 2 mL of glacial acetic acid. The content was boiled at 100ºC for 1 h. After termination of reaction in the ice bath, the reaction mixture was extracted with 4 mL of toluene and its absorbance was recorded at 520 nm. Data of yield and yield related parameters including number of pods plant^{-1} and seed yield plant^{-1} (g) were also recorded after maturity.

RESULTS AND DISCUSSION

Morpho-physiological parameters

Plant height (cm)

In both genotypes, Shikha and Heera, plant height declined when NaCl concentrations increased to 100, 150, and 200 mM. Both genotypes showed a significant decrease in plant height at 200 mM NaCl, with Shikha and Heera showing reductions of 47.3% and 46.4%, respectively, in comparison to the control.

Salicylic acid (SA) foliar spray considerably increased plant height in saline environments in both the mungbean genotypes. Salicylic acid (SA) @ 100 ppm increased plant height at the threshold levels of NaCl However, reduction percentage in plant height decreased significantly in both the genotypes in combination with SA (@ 100 ppm) and NaCl (@ 100 mM and 150 mM as compared to salt treated ones, i.e., 16.3 % and 3.5 % in Shikha and 17.5 % and 7.2 % in Heera.

On the other hand, foliar salicylic acid spray had no effect on greater salt concentrations, such as 200 mM NaCl.

These results align with those of Chinnusamy and Zhu (2003), who discovered that salinity reduced osmotic potential relative to the untreated control, which would have resulted in less water accessible and roots that couldn't absorb enough nutrients and water for healthy plant growth. In conditions of salt stress, salicylic acid (SA) is crucial for maintaining the equilibrium of osmotic and ionic potential. Salicylic acid effectively raised the osmotic potential under salinity, which is required to restore the turgor pressure, according to studies by Akhtar et al. (2013) and Aslam *et al*. (2021).

Leaf area index (LAI)

Leaf area index represents a measure of plant growth which can be influenced by different levels of salt. LAI was reduced significantly in both Mungbean genotypes under salt stress at different NaCl 100, 150 and 200 mM) concentrations. The significant reduction percentage in LAI were recorded @ 200 mM NaCl, i.e., 22.58 % in Shikha and 24.29% in Heera as compared to control. In many plant species, salt stress reduces the number of leaves per plant and the intermodal distance; this decrease may be caused by a decrease in turgor potential, which is essential for cell elongation (Iqbal and Ashraf, 2005). Remarkably, Salicylic acid (@100 ppm) increased LAI with NaCl @ 100, 150 mM NaCl as compared to salt treated plant. In comparison to plants treated with salt, a significant drop in the reduction percentage in LAI was seen in both genotypes treated with SA @ 100 ppm in combination with NaCl @ 100 mM, i.e., 18.12% in Shikha and 9.63% in Heera. This supports the current findings and is in line with studies by Syeed et al. (2011) and Hanif et al. (2024) which found that foliar application of SA to salt-grown plants enhanced maximum leaf area and dry mass.

Total dry weight plant^{-1} (g)

In both mungbean genotypes, salinity stress reduced the total dry matter plant^{-1}. Table 2 explains and illustrates the greatest reduction percentage that was seen in both genotypes @ 200 mM NaCl, which was 11.6% in Shikha and 12.8% in Heera, in comparison to control plants. Salicylic acid (SA) treated plants (@ 100 ppm) significantly decreased the reduction percentage in total dry weight at the two levels of NaCl (100, 150 mM). in both the genotypes, significant decrease in reduction percentage was noted in combination with SA (@ 100 ppm) and NaCl (@ 100 mM), i.e., 3.5% in Shikha, and 4.1% in Heera, and combination with SA (@ 100 ppm) and NaCl (@ 150 mM) i.e., 0.4% in Shikha and 10.1% in Heera as compared with salt treated plants. This finding was in agreement with findings of Khodary, (2004), who stated that SA improved the production characteristics, shoot dry weight and root dry weight of salt-stressed plants.

Relative water content (RWC)

Relative water content (RWC) was harmed by salt stress because it increased salt buildup. Maintaining the integrity of cellular membranes under stress is thought to be a crucial component of mechanisms for salt tolerance since biological membranes' integrity and functions are extremely vulnerable to environmental stress and stress-induced membrane degradation (Nishida and Murata 1996). In the current experiment, both tested genotypes' relative water content (RWC) under salt stress dramatically dropped as compared to the control. Shikha has the maximum RWC than Heera at different concentration of NaCl (i.e.,100, 150, 200 mM). RWC decreased significantly in both the genotypes @ 200 mM NaCl as compared to control in both the mungbean genotypes. Additionally, Shahid et al. (2011) noted that increased H_2O_2 buildup and lipid peroxidation under salt stress were likely the causes of the higher solute leakage. In

Szepsi *et al*. (2005) found similar results, stating that SA decreased the quantity of ion leakage in plants under salt stress. Exogenous application of SA on leaves may improve their diffusive resistance, reduce transpiration rates, preserve their relative water content, and make it easier for membrane functions to be maintained under stressful conditions**.**

Biochemical parameters

Chlorophyll content (SPAD value)

Total chlorophyll content in mungbean leaf revealed a decline with the increasing NaCl concentrations (i.e.,100, 150, 200 mM) as compared to the control. Total chlorophyll content significantly decreased by 15.2 % in Shikha and 16.8 % in Heera were recorded @ 200 mM NaCl. However, the application of SA resulted in significantly increase in total chlorophyll content @ 100 and 150 mM NaCl as compared to salt treated plants leaf i.e., 9.0% and 10.8% in Shikha and 12.5% and 15.6% in Heera respectively. The reduction in chlorophyll contents in both mungbean genotypes may occur due to acceleration in chlorophyll degradation or reduction in chlorophyll synthesis. However, it has been reported that imposition of salt stress causes deterioration in the structure of chloroplast, e.g., thylakoid membranes and plastids due to direct Na^+ toxicity and cellular oxidative damage. Similar findings were reported by Zhao et al. (1995) and Zahra et al. (2010). The enhancing effects of Salicylic acid on photosynthetic capacity could be attributed to its stimulatory effects on *rubisco* activity and net assimilation rate. Salicylic acid treated plants exhibited higher values of pigment concentration than salt treated plants.

Proline content (µg g^{-1} fresh weight)

Proline plays a protective role in cellular structures under salt stress exposure in mungbean plants. Proline content in mungbean increased as the salinity level increased and maximum increase were observed in 100 and 150 mM NaCl treated mungbean plants i.e., 6.3% and 9.5% in Shikha and 11.3% and 6.7% in Heera respectively in comparison to control. Accumulation of proline under salinity stress in plants has been interrelated with stress tolerance. Similar results were reported by Farkhondeh et al*.,* (2012). The foliar application of SA @ 100 ppm significantly increased the proline content in both the mungbean genotypes @ 100 and 150 mM NaCl i.e., 4.5% and 2.9% in Shikha and 1.8% and 5.8% in Heera respectively as compared to salt treated ones. These results are consistent with El-Tayeb (2005) observations, who reported that greater water intake and preserving cell turgidity through osmotic adjustment to protect plants in adverse environments may be linked to higher proline content accumulation under stress conditions.

Yield Parameters

Number of pods $plant^{-1}$

In both mungbean genotypes, salt stress had a negative impact on the quantity of pods produced per plant. As the quantity of salt treatment increased, the number of pods per plant dropped in comparison to the control. In comparison to the control, the 200 mM NaCl-treated plants showed the highest reduction percentage in pods per plant, which was 50.2% in Shikha and 39.5% in Heera. These results are consistent with those of Hamayun et al. (2010), who found that higher NaCl levels dramatically decreased the number of pods and pod dry weight. In contrast to plants treated with just salt, foliar application of SA @100 ppm raised these values at 100 and 150 mM NaCl, i.e., 18.4% and 8.2% in Shikha and 24.3% and 24.0% in Heera, respectively. Khan et al. (2003) and Karlidag et al. (2009) reported similar results, indicating that the application of salicylic acid to salt-treated mung bean plants was beneficial. This could be because it affected the translocation of CO_2 assimilation into the seeds and increased the rate of photosynthetic activity.

Seed yield $plant^{-1}$ (g)

Plant production is intimately correlated with the detrimental effects of salt stress on plant growth and development. Salt stress significantly decreased seed production and yield contributing factors in the current experiment. Maximum concentration of salt (@ 200mM NaCl) significantly reduced the seed yield per plant in both the mungbean genotypes i.e., 66.3% in

Shikha and 71.7% in Heera as compared to control. These findings concur with those of Navya et al. (2021) and Farouk et al. (2011). They proposed that presoaking seeds and spraying plants with the exact amounts of salicylic acid could mitigate the growth and yield reduction caused by sodium chloride salt stress. Higher photosynthesis was achieved by plants treated with salicylic acid (SA), which naturally manifested as healthier plant development and higher dry mass output. Salicylic acid enhanced plant growth and metabolism under salt stress and stopped alterations in the cell membrane architecture (Uzunova and Popova, 2000).

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Further discussion needs more citations to justify reason.

Table 1: Influence of foliar spray of Salicylic acid (SA) on mungbean plant on plant height (cm) and leaf area index (LAI) under induced salt stress

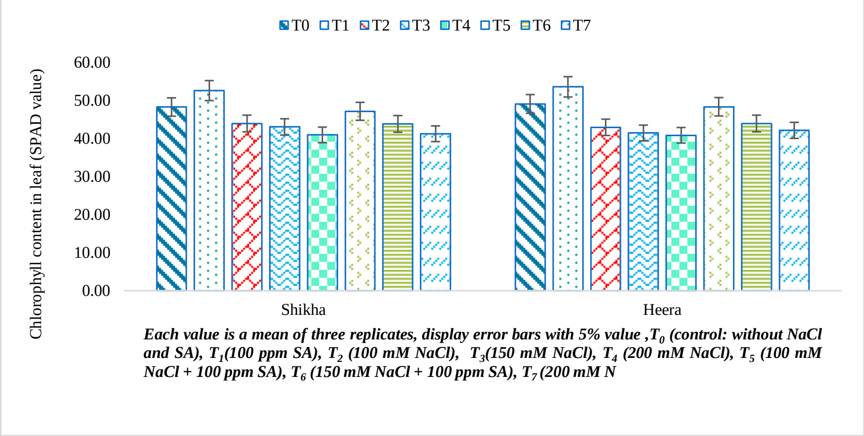
Treatment	Plant height (cm)		Leaf area index (LAI)	
	Shikha	**Heera**	**Shikha**	**Heera**
T_0 (Control: 0.0 mM NaCl & SA)	59.90	63.03	3.10	3.28
T_1 (100 ppm SA)	63.23	65.13	3.33	3.47
T_2 (100 mM NaCl)	50.87	52.73	2.98	3.05
T_3 (150 mM NaCl)	38.93	40.03	2.73	2.77
T_4 (200 mM NaCl)	31.57	33.80	2.40	2.48
T_5 (100 mM NaCl + 100 ppm SA)	59.17	61.97	3.52	3.34
T_6 (150 mM NaCl + 100 ppm SA)	40.30	42.93	2.89	2.84
T_7 (200 mM NaCl + 100 ppm SA)	25.70	28.27	2.15	1.88
	SEm ±	CD 5%	SEm ±	CD 5%
Genotypes (G)	0.70	2.02	0.30	0.88
Treatments (T)	0.35	1.01	0.15	0.44
GXT	0.25	0.71	0.11	0.31

Table 2: Influence of foliar spray of Salicylic acid (SA) on mungbean plant on total dry weight plant^{-1} (g) and relative water content (%) under induced salt stress

Treatment	Total dry weight plant^{-1} (g)		Relative water content (%)	
	Shikha	**Heera**	**Shikha**	**Heera**
T_0 (Control: 0.0 mM NaCl & SA)	31.93	32.64	73.13	73.30
T_1 (100 ppm SA)	32.67	32.75	75.12	75.66
T_2 (100 mM NaCl)	30.63	30.04	68.62	64.94
T_3 (150 mM NaCl)	28.91	29.44	63.22	58.54
T_4 (200 mM NaCl)	28.21	28.48	51.95	50.31

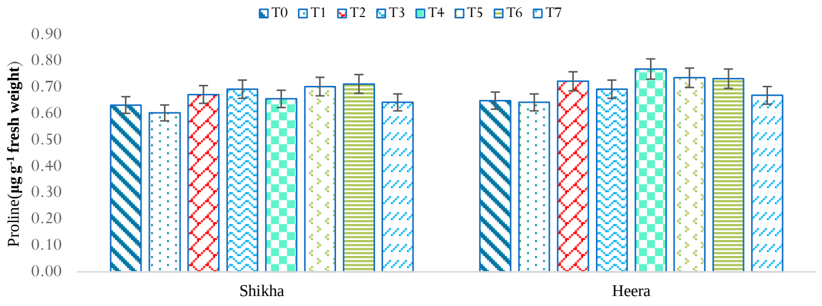
T_5 (100 mM NaCl + 100 ppm SA)	31.70	31.27	75.63	72.05
T_6 (150 mM NaCl + 100 ppm SA)	29.01	32.42	70.45	69.75
T_7 (200 mM NaCl + 100 ppm SA)	26.27	26.74	54.15	52.28
	SEm ±	CD 5%	SEm ±	CD 5%
Genotypes (G)	1.16	3.34	1.45	4.17
Treatments (T)	0.58	1.67	0.72	2.09
GXT	0.41	1.18	0.51	1.48

Fig 1: Influence of foliar spray of Salicylic acid (SA) on total chlorophyll (SPAD) in mungbean leaf under induced salt stress



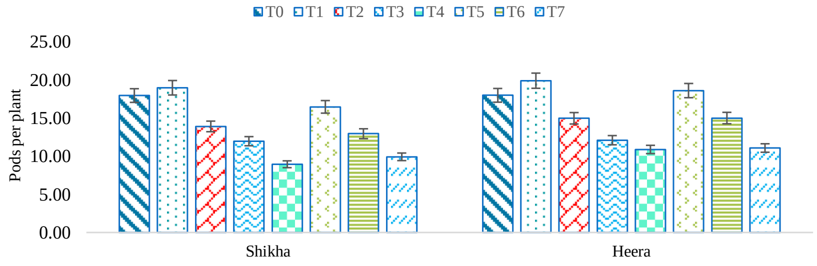
Each value is a mean of three replicates, display error bars with 5% value ,T_0 (control: without NaCl and SA), T_1(100 ppm SA), T_2 (100 mM NaCl), T_3(150 mM NaCl), T_4 (200 mM NaCl), T_5 (100 mM NaCl + 100 ppm SA), T_6 (150 mM NaCl + 100 ppm SA), T_7 (200 mM N

Fig 2: Influence of foliar spray of Salicylic acid (SA) on proline content (µg g^{-1} fresh weight) in mungbean leaf under induced salt stress



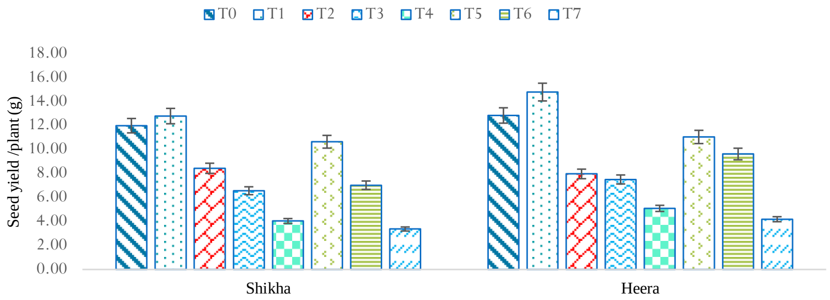
Each value is a mean of three replicates, display error bars with 5% value ,T_0 (control: without NaCl and SA), T_1(100 ppm SA), T_2 (100 mM NaCl), T_3(150 mM NaCl), T_4 (200 mM NaCl), T_5 (100 mM NaCl + 100 ppm SA), T_6 (150 mM NaCl + 100 ppm SA), T_7 (200 mM N

Fig 3: Influence of foliar spray of Salicylic acid (SA) on pods plant^{-1} on mungbean plant under induced salt stress



Each value is a mean of three replicates, display error bars with 5% value ,T_0 (control: without NaCl and SA), T_1(100 ppm SA), T_2 (100 mM NaCl), T_3(150 mM NaCl), T_4 (200 mM NaCl), T_5 (100 mM NaCl + 100 ppm SA), T_6 (150 mM NaCl + 100 ppm SA), T_7 (200 mM N

Fig 4: Influence of foliar spray of Salicylic acid (SA) on seed yield plant^{-1} (g) on mungbean plant under induced salt stress



Each value is a mean of three replicates, display error bars with 5% value ,T_0 (control: without NaCl and SA), T_1(100 ppm SA), T_2 (100 mM NaCl), T_3(150 mM NaCl), T_4 (200 mM NaCl), T_5 (100 mM NaCl + 100 ppm SA), T_6 (150 mM NaCl + 100 ppm SA), T_7 (200 mM N

Conclusion

The present study provides valuable findings for the management of salt stress in mungbean crop by using Salicylic acid effectively. However, the exogenous supplementation of SA significantly restored these impairments and played a protective role in mitigating the adverse effects of salt stress on mungbean genotypes. This comprehensive analysis contributes to a deeper understanding of the complex interactions between SA and salt stress responses in mungbean tested genotypes and concluded the way of mode of application of Salicylic acid to enhanced the salt tolerance capacity and combating the economic losses.

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