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INFLUENCE OF EXOGENOUS APPLICATION OF SALICYLIC ACID ON SALT-STRESSED MUNGBEAN (*VIGNA RADIATA*): GROWTH AND NITROGEN METABOLISM

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Abstract

To investigate the effect of salicylic acid (SA) on growth and nitrogen metabolism in mungbean grown under saline conditions, an experiment was conducted in wire house in plastic pots containing soil + sand at NIAB Faisalabd. Mungbean (*Vigna radiata* L.) varieties, two salt tolerant (NM-98 and NM-92) and two salt sensitive (NM-54 and NM-13-1), identified in laboratory experiments, were grown under four salinity levels, i.e., 1.2, 4, 8 and 12 dS m^{-1}. Salicylic acid @ 0, 100, 200 and 300 mg L^{-1} was applied as foliar spray at vegetative and flowering stages. Results indicated that salinity reduced the growth by decreasing plant height and fresh biomass of all the cultivars, however, the salt tolerant cultivars performed better than sensitive ones. Foliar application of SA @ 100 mg L^{-1} significantly improved all the growth parameters in all the cultivars under saline conditions. The SA levels of 200 and 300 mg L^{-1} did not show appreciable performance regarding growth attributes under normal and saline conditions. Similarly biochemical attributes like nitrate reductase activity (NRA), nitrite reductase activity (NiRA), soluble proteins and N reduced while total free amino acids increased due to salinity and increase / decrease was progressively enhanced by the increase in salt concentration in the growth medium. Application of SA @ 100 mg L^{-1} was helpful in reducing the adverse effects of salinity on all the above mentioned parameters while other levels of SA did not perform better under all salinity treatments.

Introduction

Salinity is a major agricultural constraint which causes heavy losses to crop productivity. It is a worldwide problem and prevails in many parts of the world as a major threat (Ashraf *et al*., 2002). In Pakistan, out of 16.2 Mha of irrigated land, more than 6.7 Mha are salt-affected up to different degrees of salinity (Anon., 2009-2010). Saline soils contain sufficient salts in the root zone to impair the growth of crop plants and sodium chloride is the most predominant among the salts (Ashraf *et al*., 2005). The effects of salinity on plant development and productivity are of multifarious nature, i.e., they may be due to reduced cell expansion and leaf area (Gorham, 1995; Ashraf, 1994; Ashraf *et al*., 2002; 2012) or may be due to reduced supply of photosynthates or hormones to the growing tissues (Munns, 1993) possibly due to reduced activity of ribulose 1-5-biphosphate carboxylase (El-Shihaby *et al.*, 2002). The injurious effects of salt stress on plant activities are associated with decreased water supply in the soil due to osmotic effects (Cramer *et al*., 1990) thereby causing physiological drought, nutritional deficiencies, direct toxicity, or combination of all these factors (Marschner, 1995; Ashraf *et al*., 2002).

Different approaches like breeding, genetic engineering, use of soil amendments and growing of halophytes are used to cope with salinity problem. Several physiological mechanisms counteract to cope with the adverse effects of salinity (Parida & Das, 2005; Ashraf *et al*., 2008; Kausar *et al*., 2012), these mechanism could be accelerated by the application of chemicals, plant growth regulators to the plants. Plants successful in maintaining their nitrogen metabolism have shown considerable tolerance/resistance to salinity (Khan *et al*., 2009) and produce high yield (Bänziger *et al*., 2006).

Mungbean is a pulse crop of special importance and provides an inexpensive source of vegetable dietary protein. It is popular for its nutritive value and digestibility, containing higher protein contents (28%), fat (1.3%), carbohydrates (60.4%) and reasonable amount of, vitamins and essential micronutrients (Anwar *et al*., 2007). It takes less time to mature, can be cultivated during spring and winter, and fits well in existing cropping pattern of the country. Being leguminous in nature, it has the ability to fix nitrogen from the atmosphere by the roots having nodules through microbial symbiosis and thus helps in maintenance and improvement of soil fertility. In Pakistan, mungbean is cultivated on an area of 257.7 thousand hectares with total grain production of 537 kg ha^{-1} (Anon., 2009-2010). The production of mungbean in third world countries is very low (Anon., 2009-2010), which is mainly due to its cultivation on marginal lands mostly salt-affected. Its cultivation on such soils could only be made profitable by applying chemicals or plant growth regulators exogenously.

Salicylic acid (SA) is a plant origin phenolic compound that has shown its role as a growth enhancer (Arberg, 1981). It commonly occurs naturally in white willow (*Salix alba*) and was extracted from the herb meadowsweet (*Filipendula ulmaria*), formerly named as *Spiraea ulmaria* by the German scientists in 1839. It is called a useful "natural product" because it can be isolated from nature (Mohrig *et al*., 1998). Salicylic acid was used historically as an analgesic (pain reliever) but now a days it is becoming popular among plant physiologists as it also act as an important growth regulator especially under salinity stress. However, the information regarding nitrogen mechanism under the action of salicylic acid in enhancing salt tolerance and plant growth especially mungbean is not enough for making any concrete recommendation to the end-users. The present study, therefore, was planned to study effect of salicylic acid on the growth and nitrogen metabolism in mungbean.

Materials and Methods

Exogenous application of salicylic acid was used to improve the nitrogen metabolism and growth of mungbean grown under saline conditions. The study was conducted in plastic pots containing 8 kg clay-loam soil (pH 7.8 and EC_e 1.2 dS m^{-1}) at Plant Stress Physiology Laboratory, Salinity and Environmental Division, Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan.

Two salt tolerant (NM-98 and NM-92) and two salt sensitive (NM-54 and NM-13-1) mungbean cultivars identified in green house experiments (Akhtar, 2011), were grown under four salinity levels including control (1.2 , 4, 8 and 12 dS m^{-1}). Salinity levels were developed with analytical reageant grade NaCl. Salicylic acid @ 0, 100, 200 and 300 mg L^{-1} was applied as foliar spray at vegetative and flowering stages. The data for growth, nitrate reductase (NRA) and nitrite reductase activities (NiRA), total soluble protein(TSP), total free amino acids (TFAA), total nitrogen (TN), plant height and above ground biomass were recorded 10 days after foliar spray of SA at flowering stage. NRA was determined according to Sym (1984); NiRA as described by Ramarao *et al*., (1983), TSP (Lowry *et al*., 1951), TFAA (Hamilton & Vanslyke, 1973) and TN was estimated by micro–Kjeldhal method (Bremner, 1965). Data so collected were analyzed statistically using analysis of variance technique and the STATISTICA Computer Program was used for this purpose. The Least Significant Difference test at 5% probability level was used to assess the differences among significant means (Steel *et al.*, 1997).

Results

Plant growth: Plant height decreased with the increase in salinity level and the maximum reduction in plant height was recorded at the highest salinity level (12 dS m^{-1}). The foliar spray of salicylic acid (SA) significantly improved the plant height under saline conditions (Fig. 1A; Table 1). SA level of 100 mg L^{-1} was the most effective one in enhancing plant height under saline conditions by ameliorating the adverse effect of salinity. Mungbean cultivars, NM-92 and NM-98 were successful in attaining higher plant height than NM-54 and NM-13-1. Foliar application of SA improved height of all mungbean cultivars grown under saline conditions. The highest salinity level adversely affected the plant height and application of SA @ 100 mg L^{-1} mitigated the adverse effect of salinity.

Dry matter yield was significantly reduced by salinity in all mungbean cultivars (Fig. 1B; Table 1), the most severe effect was noted at the highest level (12 dS m^{-1}) of salinity. The foliar spray of SA had produced appreciable results by increasing dry matter yield under saline conditions and the effect was more pronounced in plants which were sprayed with 100 mg L^{-1} concentration of SA. The foliar application of other concentrations of SA i.e., 200 and 300 mg L^{-1} failed to produce economical dry matter yield under normal or saline conditions. Salt tolerant cultivars (NM-92 and NM-98) produced higher dry matter yield than non-tolerant (NM-54 and NM-13-1) cultivars.

From Fig. 1B, it is very clear that the highest salinity level adversely affected the production of dry matter yield and exogenous application of SA @ 100 mg L^{-1} was successful in alleviating the adverse effect of salinity.

Nitrogen metabolism: Salinity significantly reduced the nitrate reductase activity (NRA) and the highest reduction in NRA was recorded under the highest salinity level (12 dS m^{-1}). Response of application of different level of SA was significantly different under various levels of salinity (Table 1). SA level of 100 mg L^{-1} was the most effective in improving the NRA in all the mungbean (Fig. 2A). At the highest level of salinity, application of SA failed to enhance the NRA upto the desired level, however, it enhanced NRA upto 8 dS m^{-1} salinity. SA level 100 mg L^{-1} was the most effective in maintaining the highest NRA in all the mungbean varieties under all salinity levels (Fig. 2A). All the mungbean varieties showed maximum reduction in NAR at the highest level of salinity (12 dS m^{-1}). However, tolerant mungbean varieties (NM-98 and NM-92) maintained higher NRA than non-tolerant ones (NM-54 and NM-13-1).

Nitrite reductase activity (NiRA) decreased with the increase in salinity and it was the minimum at the highest salinity level (12 dS m^{-1}) while the highest NiRA was recorded in those plants growing under normal conditions (Fig. 2B). Foliar application of SA significantly enhanced the NiRA and its activity was the highest in plants sprayed with 100 mg L^{-1} SA, while plants sprayed with 200 and 300 mg L^{-1} SA did not show any appreciable enhancement in NiRA (Fig. 2B; Table 1). All the mungbean cultivars showed reduction in NiRA with increase in salinity however, NM-98 and NM-13-1 maintained the highest and the lowest NiRA, respectively under all levels of salinity (Fig. 2B).

Table 1. Analysis of variance table (mean squares) for different attributes recorded to study effect of exogenous application of salicylic acid on growth and nitrogen metabolism in salt stressed mungbean varieties.

Source of variation	df	Mean squares						
		Total nitrogen	Plant height	Dry biomass yield	Nitrate reductase activity	Nitrite reductase activity	Total soluble protein	Total free amio acid
Variety (V)	3	7.591**	76.262**	9.783**	0.064**	8.648**	2.565**	1.679**
SA	3	23.104**	56.590**	4.620**	2.184**	1.952**	1.092**	1.631**
V x SA	9	2.25NS	1.724NS	0.461NS	0.025NS	0.014NS	0.053NS	0.051NS
NaCl level	3	1040.908**	1262.720**	215.065**	18.304**	21.160**	9.105**	26.860**
V x NaCl	9	3.292*	9.286**	1.443**	0.268**	0.358**	0.157**	0.335**
Sa x NaCl	9	3.817**	5.615**	0.403NS	0.602**	0.664**	0.239**	0.124**
V x SA x NaCl	27	1.322NS	2.024NS	0.202NS	0.041NS	0.079NS	0.035NS	0.030NS
Error	128	1.445	2.297	0.332	0.032	0.065	0.053	0.029

NS = Non-significant (p>0.05); * = Significant (p<0.05); ** = Highly significant (p<0.01)

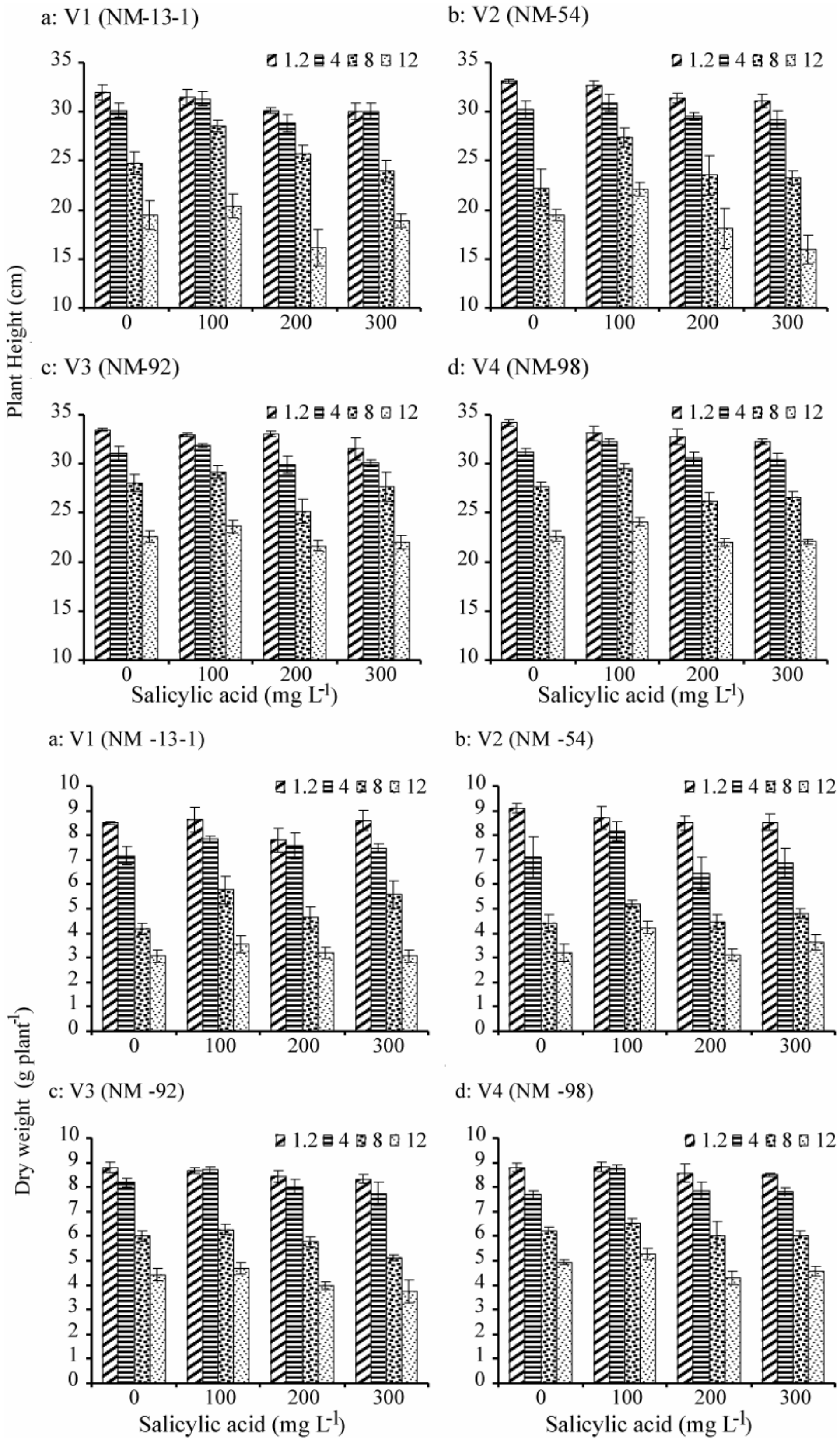
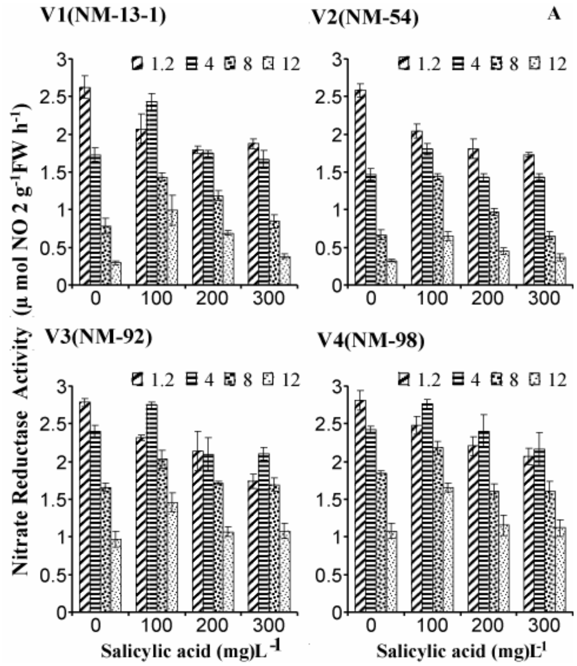
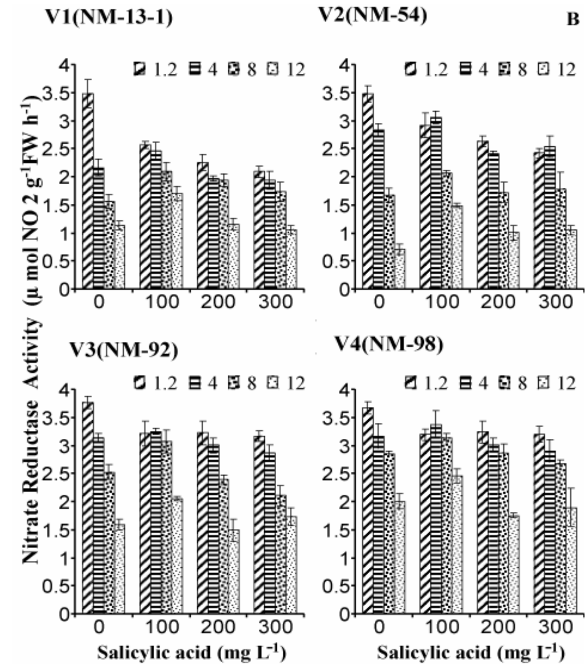


Fig. 1. Plant height (A) and dry biomass yield (B) of salt stressed and non-stressed plants of four mungbean cultivars after application of SA as a foliar spray at the vegetative and flowering stages.





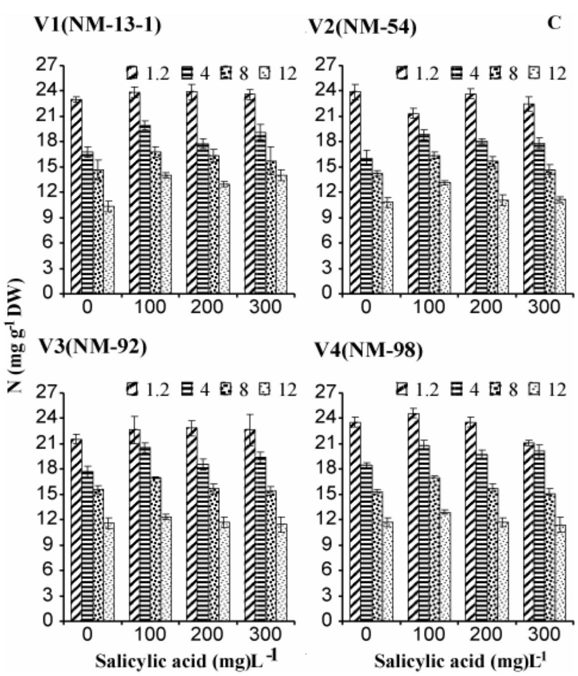


Fig. 2. itrate reductase (NRA), nitrite reductase activities (NiRA) and nitrogen contents in salt stressed and non-stressed plants of four mungbean cultivars as influenced by foliar spray of SA at vegetative and flowering stages.

Shoot total nitrogen contents (TN) were also reduced with the increase in salt concentration in the root zone and was the minimum at the highest salinity level (12 dS m^{-1}) and the highest TN content was found in plants growing under normal condition (Fig. 2C; Table 1). Foliar application of SA significantly influenced leaf TN concentration. Mungbean cultivars NM-98 exhibited the highest value for TN contents under all salinity levels which was closely followed by NM-13-1. While other two formed group with low TN under all salinity levels and statistically maintained similar values for TN contents. At the highest salinity level, TN uptake was minimum in all mungbean cultivars, however, application of SA @ 100 mg L^{-1} enhanced this uptake which was more pronounced in salt tolerant cultivar i.e., NM-98 (Fig. 2C).

Total soluble proteins (TSP) decreased as the salinity level increased and maximum reduction in TSP was observed at the highest salinity level (12 dS m^{-1}). Maximum TSP were recorded in plants grown under normal condition followed by 4, 8 and 12 dS m^{-1} (Fig. 3A; Table 1). The foliar application of SA significantly increased the TSP and it was maximum under 100 mg L^{-1}. SA treated plants followed by 0, 200 and 300 SA levels. Different mungbean varieties performed differently, regarding TSP and NM-98 (salt tolerant) maintained the highest TSP while it was the minimum in NM-13-1 (salt sensitive). The highest salinity level was detrimental and application of SA @ 100 mgL^{-1} was effective in alleviating the adverse effect of salinity. Salt tolerant varieties NM-98 and NM-92 performed better than the non-tolerant ones.

Total free amino acids (TFAA) accumulation increased with the increase in salinity and was the maximum under the highest salinity level (12 dS m^{-1}) in all mungbean varieties (Table 1). However, applications of SA significantly affected the total free amino acids and plants sprayed with 100 mg L^{-1} SA showed the highest accumulation followed by 0, 200 and 300 mg L^{-1} SA. The cultivar NM-98 was successful in maintaining the highest TFAA under all salinity levels while it was the minimum in NM-13-1. Plants grown under saline conditions and sprayed with SA showed higher TFAA than those grown under salinity and not sprayed with SA (Fig. 3B). The highest accumulation of TFAA was at the highest salinity level and application of SA under saline conditions increased the total free amino acid in all the mungbean varieties.

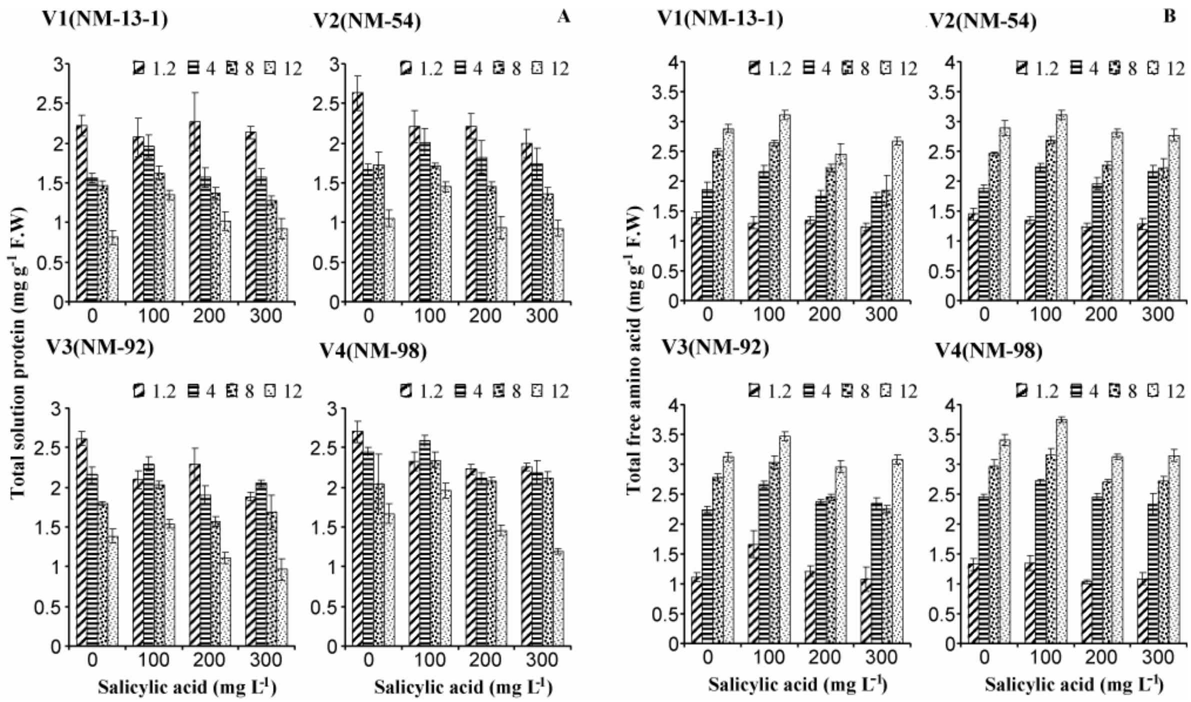


Fig. 3. Total soluble protein (A) total free amino acids (B) in salt-stressed and non-stressed plants of four mungbean cultivars after application of SA as a foliar spray at the vegetative and flowering stages.

Discussion

Results of the present study clearly indicated that salinity significantly reduced plant growth (plant height and dry biomass) of all the mungbean cultivars which decreased with the increase in salt concentration in the root zone (Fig. 1A,B). However, foliar application of SA @ 100 mg L^{-1} improved the plant growth in salt stressed plants of all the tested mungbean cultivars. The effect of 200 and 300 mg L^{-1} SA was not considerable under salinity stress conditions; similar findings are reported by Hamid *et al*., (2008) in wheat while in studies of Noreen & Ashraf (2008), SA application of 200 mg L^{-1} was quite effective in enhancing the growth of sunflower under salinity stress. Similarly Hussain *et al*., (2008) under water stress conditions reported that SA @ 100 mg L^{-1} increased the plant growth and biomass of sunflower. From the present study it is also very clear that salt tolerant mungbean cultivars (NM-98 and NM-92) responded more to the application of SA under saline conditions than salt sensitive (NM-54 and NM-13-1) cultivars. Many reports are in agreement with the present findings (Ghoulam *et al.*, 2001; Stevens *et al*., 2006; Bilal *et al*., 2011).

Nitrate and nitrate reductase activities (NRA and NiRA) significantly reduced by the salinity in all the mungbean cultivars, however, foliar application of SA was effective in improving the activity of both of these up to 8 dS m^{-1}, beyond that it failed to bring the activity up to desired level (Fig. 2 A, B) and only SA level 100 mgL^{-1} was successful in enhancing their activity. Decrease in NRA and NiRA due to salinity or other stress was reported by many workers (Khan *et al*., 1990; Ashraf *et al*., 1995). The process of nitrate reduction is well known for its sensitivity to environmental stresses (Iqbal *et al*., 2006). Nitrate reductase enzyme plays a key role in nitrogen metabolism and NRA is associated with protein synthesis and plant growth. Reduction in NRA and NiRA affected the plant growth and metabolic activities regarding nitrogen metabolism (Debouba *et al*., 2007) as recorded in the present study, the cultivars NM-98 and NM-92 maintained higher NRA and NiRA were superior in growth and dry matter yield than salt sensitive NM-54 and NM-13-1. Similarly Khan *et al*. (1990) observed that under saline conditions, NRA is reduced more in sensitive sorghum varieties whereas, Islam *et al*., (2007) did not find any genotypic differences for NRA, however, a consistent decrease was recorded in rice. NRA is an inducible enzyme and its concentration depends on the availability of substrate so improvement in NRA or NiRA in foliar applied SA was due to the availability of substrates. Reports indicated that application of SA increased the availability of nutrients by enhancing turgor of the plants under stress (Jakab *et al*., 2007), which is very true in present case where application of SA improved the total nitrogen (TN) uptake (Fig. 2C) uptake under saline conditions. Similar findings were also recorded by Noreen and Ashraf (2008). Application of SA increases the availability of substrate for NRA and NO_2 generated after NO_3 reduction is a substrate for NiRA so consequently it increases with the application of SA. The findings of present study confirmed the above statement. So, the application of SA is beneficial to get improvement in nitrogen uptake and nitrogen metabolism, which improves the protein/enzyme/growth hormone synthesis, thus enhances the metabolic activity resulting increase in growth and plant productivity. Results of present study showed positive relationship with growth yield and NRA and NiRA which also confirmed role of SA in improvement of growth and yield of mungbean (Fig. 1A,B).

In this research, total soluble protein (TSP) level decreased with salinity stress and application of SA as foliar spray enhanced it (Fig. 3A). Ashraf *et al*., (2002) also recorded reduction in TSP due to salinity in cotton. The reduction in soluble protein in the present study may be due to the reduction of NRA (Ashraf *et al*., 2005). However, there are different views regarding increasing or decreasing proteins in plants under saline conditions. Soluble proteins increased in leaves and stem of tomato growing under saline conditions (Amini & Ehsanpour, 2005). Study on maize conducted under saline conditions indicated that application of SA increases protein and amino acids (El-Tayeb, 2005). The increase in amino acids in plant organs under saline conditions is due to the breakdown of protein and as a result, reduction in protein and growth has been observed (Hussain *et al*., 2007). The reduction in protein may be due to its break down into amino acids (Fig. 3B) and as a result of decrease in the synthesis due to reduction of NRA, NiRA and substrate. The results showed that N uptake reduced in all the mungbean cultivars under saline conditions (Fig. 2C) which results in reduction in substrate concentration. The decrease in soluble protein may be due to reduction in the synthesis of protein (Kong-Ngern *et al*., 2005). It is also observed that under stress conditions some low molecular weight compounds such as amino acids, polyamine, polyols, proline, glycinebetain and organic acids are produced consequently leaf osmotic potential reduced (Dantas *et al*., 2005). The results of present study confirmed these findings. Reports indicate that under stress conditions, plants produced some stress proteins and some of them are taken by phytohormones such as SA (Hussain *et al*., 2007) resulting decrease in protein. The enhancement in NRA by SA application may be the action of SA on NO_3 inhibitors (Ahmad *et al*., 2003). Zahra *et al*., (2010) reported that salt stress enhances the protein contents in root but application of SA reduced their concentration and the accumulated proteins under salt stress environments acts as osmo-regulators (Ahmad *et al*., 2003). On the other hand, El-Bassiouny & Bakheta (2005) reported that salinity stress reduced the concentration of special proteins in the leaves and roots of barley. They also observed increase in amino acids in wheat leaves under saline conditions, which is very similar to present findings. Literature also indicated that change of protein into other organic compounds helps the plant to adjust itself with external environments (Kong-Ngern *et al*., 2005). Reduction in protein content under salinity stress may be related to nitrogen uptake and its consumption. It is very clear from the present study that nitrogen metabolism is disturbed due to the imposition of salinity, which may disturb its consumption in synthesis of different organic compound such protein, enzymes and phyto-hormones etc. (Meloni *et al*., 2004).

Conclusion

The results of present study suggest that SA application @ 100 mgL^{-1} under saline conditions is effective in improving the growth and plant productivity of mungbean through improving the nitrogen metabolism by enhancing NRA, NiRA, nitrogen uptake, protein and total amino acids.

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