

Journal homepage: http://www.journalijar.com INTERNATIONAL JOURNAL

 OF ADVANCED RESEARCH

RESEARCH ARTICLE

AMELIORATIVE EFFECTS OF SALICYLIC ACID ON SOME PHYSIOLOGICAL AND BIOCHEMICAL ATTRIBUTES IN MASHBEAN (*Vigna mungo* **L. Hepper) UNDER NaCl STRESS**

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Manuscript Info Abstract

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Manuscript History:

Received: 11 January 2014 Final Accepted: 25 February 2014 Published Online: March 2014

Key words: Salt stress, mashbean, salicylic acid , growth, water status, biochemical attributes, yield ******Corresponding Author*

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The study was conducted in an open air facility equipped with a rainout shelter to determine the effects of salicylic acid on growth, biochemical attributes and yield in mashbean genotypes varying in salt sensitivity. Plants were grown under saline and non-saline conditions in 27 cm diameter plastic pots. The saline treatment ω 3.0 and 4.5 ds m⁻¹ NaCl was applied in split dose: at the time of sowing and 15 days after sowing. Salicylic acid @ 0.5 and 1.0 mM concentrations was sprayed at 25 DAS and observations were recorded at pod formation (65 DAS) stage. Increasing salt concentration adversely affected the plant height and leaf area at all the stages of development. A decline in leaf relative water content and water potential of leaves was observed under the influence of salt at both the concentrations in all the four genotypes. Salinity also decreased membrane permeability. A reduction in chlorophyll content, carotenoid content and Hill reaction activity in leaves was recorded under salt stress. Salt stress increased the levels of proline and different metabolites viz. total soluble sugars, total soluble proteins and free amino acids in the leaves. However, the tolerant genotypes registered greater increase as compared to sensitive ones. SA application conferred protection to the plants by improving plant height, leaf area, leaf relative water content and water potential in all the genotypes. The membrane permeability was restored and photosynthetic efficiency of plants was also enhanced. The level of various biochemical constituents in salt stressed plants was enhanced and it helped the plants to overcome the adverse affects of salinity. Significant reduction in seed yield and its attributes were recorded under both levels of salinity. SA application protected plants against salinity induced decline in yield components. The recovery was more pronounced at 3.0 ds m^{-1} salinity as compared to 4.5 ds m⁻¹ salinity level particularly with 0.5mM SA.

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Introduction

Salt stress is one of the major abiotic stresses in arid and semi arid regions of the world and hampers the agricultural output by lowering the yield of various crops (Kapoor and Srivastava, 2010). Accumulation of soluble salts in the soil leads to osmotic stress, biochemical imbalance, specific ion toxicity and ionic imbalance in plants. In order to overcome the adverse effects of unfavourable saline conditions, plants develop various strategies which include accumulation of compatible solutes, like glycinebetaine, proline and soluble carbohydrates (Munns, 2003).

Black gram or mashbean (*Vigna mungo* L. Hepper) is the third most important pulse crop in India (Hussain et al., 2011) and occupies a unique position in Indian agriculture. Its cultivation in India is about 3.25 million hectares with an annual production of 1.45 million tonnes (Arulbalachandran et al., 2010). Although India is the main producer of black gram but its production is limited due to various biotic and abiotic stresses (Varalaxmi et al.,

2007). Salicylic acid (SA) is as an important signaling molecule for modulating plant responses to environmental stresses (Breusegem et al., 2001). It can induce abiotic stress tolerance by improving plant growth, stimulating the synthesis of protein and retarding or enhancing the accumulation of proline content. Convincing data have been obtained concerning the salicylic acid induced alleviation of salinity stress in bean, tomato, pea, wheat, maize and rice (Arfan et al., 2007, Gunes et al., 2007 and Senaratna et al., 2007). However, the effects of salicylic acid on growth, physiological and biochemical changes and yield parameters of mashbean under salt stress have not been established. Therefore, the present investigation was designed to assess the ameliorative effects of salicylic acid on salt stress in resistant and sensitive genotypes of mashbean.

Material and Methods

The present investigation was carried out with mashbean genotypes grown under salt stress. Based on our previous studies (Kaur, 2009), seeds of four mashbean genotypes differing in salinity tolerance i.e. KUG 363 and KUG 310 (salt sensitive); KUG 253 and KUG 529 (salt tolerant) were procured from the Department of Plant Breeding and Genetics, PAU, Ludhiana.

Experiment layout

Plants were grown under NaCl stress and non-stress conditions in 27 cm diameter plastic pots containing 8 kg of soil taken from experimental field following recommended package of practices for mashbean. Normal soil (EC 0.085 ds m-1 , pH 7.6) from the field was non-saline and requisite amount of NaCl solutions were added to create different levels of stress. The experiments were carried out in an open-air facility equipped with a rainout shelter.

Treatments

The salt treatment ω 3.0 and 4.5 ds m⁻¹ NaCl was applied in split dose: 50% at the time of sowing and 50% at 15 days after sowing (DAS) in a sufficient volume to wet the soil to field capacity. The pots with control treatments had drainage holes whereas the pots with other treatments were without drainage holes. This helped in maintaining the salinity levels at different stages. Non-saline controls were irrigated with tap water (EC 0.266 ds m-¹). In all the treatments, six seeds were sown in each pot and later thinned to 3 plants per pot at 20 DAS. Salicylic acid @ 0.5 and 1.0 mM concentrations was sprayed at 25 DAS. The experiment was conducted with twelve replications; nine for physiological and biochemical estimations and three for seed yield.

Measurements of plant growth and yield:

Five plants in each replication were uprooted randomly in order to record their heights (cm) and leaf area at pod formation stage (65 DAS). For yield contributing parameters**,** number of pods of three replications of each treatment was counted at harvest and average was recorded. Twenty pods from nine plants of each genotype were selected randomly at the time of harvest and number of seeds in each pod was counted and average was recorded.100-seeds were counted from nine randomly selected harvested plants of each genotype and their weight was taken. Weight was expressed in grams. All the seeds from each genotype were collected and weighed.

Relative water content:

For relative water content (RWC) estimation, ten leaf discs from each treatment were weighed immediately to obtain their fresh weight. Then the discs were submerged in distilled water in beakers till saturation. After 6 h the discs were removed from beakers. Surface water of the discs was blotted off without putting any pressure and then they were weighed to obtain saturated weight. After drying the discs at 70°C for 72 h their dry weights were determined. From these data RWC was calculated as follows and expressed as percentage (Weatherley, 1950) $F = 1$ $F = 0$

$$
RWC = \frac{Fresh \text{ weight} - Dry \text{ weight}}{Saturated \text{ weight} - Dry \text{ weight}} \times 100
$$

Leaf water potential:

Leaf water potential was measured with psychrometer (Wescor, USA).

Membrane injury index:

For assaying the percent leakiness, leaf was excised from the main stem of the randomly selected plants with the help of blade and washed with distilled water to remove adhering exogenous electrolytes and immersed in the test tubes containing 15 ml of distilled water for 24 h at $24\pm1^{\circ}$ C. The conductivity of the water was determined using conductivity meter. The samples were then placed in boiling water bath (100°C) for 20 minutes, cooled to 24 ± 1 ^oC and conductivity was determined again. The membrane permeability expressed as membrane injury index was calculated as the ratio of conductivity before boiling to conductivity after 24 h of boiling (Premachandra et al., 1990).

Membrane injury index =
$$
\frac{CAB - CBB}{CBB} \times 100
$$

CBB= Conductivity before boiling, CAB= Conductivity after boiling

Estimation of biochemical parameters:

Total Chlorophyll content (Hiscox and Israeltam, 1979), Carotenoid content (Kirk and Allen, 1965), Hill reaction activity (Cherry, 1973) **,** total soluble sugars (Dubois et al., 1956), total soluble proteins (Lowry et al., 1951), free amino acids (Lee and Takahashi, 1966) and proline content (Bates et al., 1973) were estimated in leaves. For comparing the effects of treatments on various parameters, data were subjected to one -way ANOVA using CPCS 1 software package.

Results and Discussion

Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate as well as changes in plant metabolic processes (Munns, 2002). In our studies, salt stress (3.0 & 4.5 ds m⁻¹ NaCl) adversely affected the plant height (Table 1) and leaf area (Fig.1) in both sensitive as well as tolerant genotypes. Significant reduction in plant height in relation to salt stress has also been observed in many legumes such as soybean (Essa and Al-Ani, 2001) and common bean (Gama et al., 2007). Application of SA (0.5 &1.0 mM) stimulated growth in all the four genotypes under salt stress (at both 3.0 $\&$ 4.5 ds m⁻¹ NaCl) conditions. The relative water content (RWC) of leaves also showed a decline under the influence of salt at both the concentrations in all the four genotypes (Table 2). Increasing salinity also decreased the water potential in all the genotypes and this decrease was more pronounced $(43\%$ at 65 DAS under 4.5 ds m⁻¹ NaCl stress) in sensitive genotypes (Table 2). However, treating the plants with SA caused an increase in water potential and RWC to some extent. The lower concentration of SA (0.5 mM) was more effective in 3.0 ds m⁻¹ NaCl stressed plants and tolerant genotypes responded more than sensitive ones. The reduction of plant growth under saline conditions may either be due to osmotic reduction in water availability which resulted in increasing stomatal resistance (Gunes et al., 1996) or due to excessive Na⁺ and Cl accumulation in the plant tissues (Cusido et al. 1987, Gunes et al. 1996 and Yousif and Al-Saadawi, 1997). Yildirim et al., (2008) also noticed that plant height decreased significantly with the increasing NaCl concentration $(6.0 \text{ and } 12.0 \text{ ds m}^{-1})$ and all SA treatments (0.25-1.0 mM) increased the plant height compared to non-treated plants both in absence and presence of salinity in cucumber.

Salinity acts to inhibit plant access to soil water by decreasing the osmotic potential of soil solution. As the soil dries, the soil solution becomes increasingly concentrated, thus lowering the soil water potential, thereby limiting the plants access to soil water. This reduced availability of water to plant lowers its water potential. Decrease in water potential of broad bean plants under saline conditions has also been reported by Katerji et al., (1992). SA treatments induced an increase in leaf relative water content of the stressed plants compared to the nontreated plants.

Salinity affected the membrane permeability (Fig.2) and both sensitive and tolerant genotypes reported a sharp increase in membrane injury under the influence of salt. This increase was in a dose dependent manner. Treatment of salt stressed plants with SA helped them to recover from the salinity induced increase in membrane injury there by restoring the membrane permeability (Fig.2). Application of 0.5mM SA to 3.0 ds m⁻¹ NaCl stressed plants caused about 17% decrease in electrolyte leakage and helped them to withstand stress conditions. Salinity induced increase in membrane injury and ameliorating effect of SA on NaCl grown plants have been reported in tomato (Stevens et al., 2006), maize (Gunes et al., 2007) and cucumber (Khan et al., 2010).

Salt treatment reduced chlorophyll content, carotenoid content and Hill reaction activity (Fig. 3) in leaves of all the four genotypes. Salicylic acid improved the chlorophyll content, carotenoid content and Hill reaction activity in leaves of salt stressed plants. The genotypes KUG 253 and KUG 529 recorded 19% and 21% increase in Hill reaction activity, respectively, over salt stressed plants with 0.5 mM SA under 3.0 ds m⁻¹ NaCl.

The reduction in Hill activity can be correlated with decrease in chlorophyll content and other pigments. Photosynthetic capacity increased upon treatment with SA in salt stressed maize plants (Khodary, 2004). Improvement in photosynthetic performance of *Triticum aestivum* and *Hordeum vulgare* plants under stress conditions following SA application has also been reported by Deef, 2007. The enhancement of Hill activity with leaf maturation may be due to increased synthesis of chlorophyll (Maity and Bera, 2009).

Various biochemical constituents in leaves of control, salt stressed and SA treated plants were analysed (Table 3 and 4). Under salt stress, tolerant genotypes accumulated these biochemical constituents more than sensitive ones. 0.5 mM SA application caused an increase in total soluble sugars, proteins and free amino acids and it ranged between 17 to 25, 21 to 29 and 21 to 28 per cent in salt sensitive and salt tolerant genotypes, respectively, growing under 4.5 ds m-1 NaCl stress.

Accumulation of sugar in plants is enhanced in response to a variety of environmental stresses (Gill et al., 2001). An increase in free amino acids has also been worked out in various crops under saline conditions (Hamid et al., 2010). Increase in amino acid content may be due to degradation of intracellular proteins to meet the requirements for biosynthesis of new proteins and a few other molecules needed to support growth (Ashraf and Naqvi, 1996). Moreover amino acid must be accumulated to high levels to create an osmotic potential gradient to facilitate inward movement of water (Khan et al., 2000). Plants produce a variety of proteins under biotic and abiotic stresses. SA is known to induce the production of these proteins which contribute to enhanced plant resistance to salinization (Kang and Saltveit, 2002).

Our results are in agreement with those obtained by Ahmed and Arain (1999) in wheat, Kumar et al. (1999) in soybean and Akhtar (2004) in grasses, who also reported an increase in protein content in leaves of salt stressed plants. The present investigations revealed that the level of total soluble sugars, proteins and amino acids increased further in all the genotypes under salt stress, when SA (0.5 and 1.0 mM) was applied as foliar spray, the effect was similar at both levels of salt stress. Increased accumulation of sugars (reducing and non reducing), starch and soluble proteins following SA application has also been observed by Maity and Bera, 2009, in leaves of green gram. Pooja and Sharma (2010) also reported an increased accumulation of carbohydrates, total soluble proteins and free amino acids in salt stressed mungbean plants following SA applications. Similar results were reported by Hamid et al., (2010) in wheat.

In the present investigation, salt stress induced proline accumulation was more in salt tolerant (KUG 253 and KUG 529) than sensitive genotypes (Fig.4). SA treatments also increased the proline content in all the genotypes but maximum increase was shown in genotype KUG 529 (27% with 4.5 ds m-1 NaCl and 0.5 mM SA) followed by KUG 253 (25% with 4.5 ds m⁻¹ NaCl and 0.5 mM SA). However, the sensitive genotypes (KUG 363) and KUG 310) showed less increase (20% with 4.5 ds m^{-1} NaCl and 0.5 mM SA) in proline content. Similar results were recorded by Tasgin et al., (2006) in wheat where the proline accumulation increased by salicylic acid treatment, under oxidative stresses. The more tolerant plants stored more proline (Desnigh and Kanagaraj, 2007). Hussein et al., (2007) also reported an increase in proline concentration when salicylic acid was used as foliar application in maize plants under salt stress. Proline content also increased by SA treatment under saline conditions in cucumber plants (Quing-Moo et al., 2007).

The yield attributes viz; number of pods per plant, number of seeds per pod and 100 seed weight decreased significantly at both levels of salt stress (Table 5). Seed yield decreased upto 41% (average basis) with increasing level of salt $(4.5 \text{ ds m}^{-1} \text{ NaCl})$ as compared to control plants. The foliar application of SA $(0.5 \text{ and } 1.0 \text{ mM})$ increased the yield attributes in salinity stressed plants. Lower concentration of SA (0.5 mM) was more effective under salt stress than higher concentration. Maximum increase in seed yield was observed in KUG 529 (14%) followed by KUG 253(11%), KUG 310 (6%) and KUG 363(5%) at 3.0 ds m^{-1} NaCl and 0.5 mM SA in comparison with plants under 3.0 ds m⁻¹ NaCl only. The reduction at higher salinity level in most of the yield attributing characters may be because of adverse affect on growth. With increase in SA concentration there was corresponding increase in yield due to improved water status at both levels of salinity. Salinity induced reduction in yield has also been reported by Ghai et al., (2010) in mashbean and an improvement in yield following SA application under salt stress in mungbean by Pooja and Sharma, (2010).

Table 1: Effect of NaCl and salicylic acid treatments on plant height (cm) in mashbean genotypes at pod formation stage

Table2: Effect of NaCl and salicylic acid treatments on relative water content (%) and water potential (Mpa) in mashbean genotypes at 65 DAS

Table 3: Effect of NaCl and salicylic acid treatments on total soluble sugars and free amino acids in mashbean genotypes at 65 DAS

Table 4 : Effect of NaCl and salicylic acid treatments on total soluble proteins in mashbean genotypes at 65 DAS

Table 5: Effect of NaCl and salicylic acid treatments on yield and its attributes in mashbean genotypes

Fig.1 Effect of NaCl and salicylic acid treatments on leaf area plant-1 in mashbean genotypes at 65 DAS

Fig.2 Effect of NaCl and salicylic acid treatments on membrane permeability index in mashbean genotypes at 65 DAS

Fig.3 Effect of NaCl and salicylic acid treatments on chlorophyll, carotenoid content and Hill activity (Ax= Absorbance at 420nm) in mashbean genotypes at 65 DAS

Fig.4 Effect of NaCl and salicylic acid treatments on proline content in mashbean genotypes at 65 DAS

Conclusion

In the present investigation, the tolerant genotypes (KUG 253 and KUG 529) performed better even under salt stress and also responded more to SA treatments than sensitive genotypes in terms of improved membrane stability index, photosynthetic efficiency , accumulation of osmoprotectants (sugars and proline) and enhanced accumulation of various biochemical constituents in leaves which might be responsible for promoting crop yield in these genotypes. Thus, salicylic acid can be used to enhance plant resistance to salt stress.

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