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## ORIGINAL ARTICLE

# Some Physiological Responses of Mungbean to Salicylic Acid and Silicon under Salt Stress

**Kazem Ghassemi-Golezani[1*], Ramin Lotfi[1] and Nosratollah Najafi[2]**
[1] Department of Plant Eco-Physiology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
[2] Department of Soil Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
Corresponding author: golezani@gmail.com

**ABSTRACT**

*Salt stress is one of the most important abiotic stresses affecting plant growth and development.A greenhouse experiment was conducted to evaluate the effects of foliar application of salicylic acid (1mM) and silicon (2mM) on some physiological characteristics of mungbean under salt stress (control, 3, 6 and 9 dSm$^{-1}$). Exogenous foliar application of silicon and salicylic acid positively affected physiological performance of plants by decreasing $Na^+$ and increasing $K^+$ accumulation in roots and leaves. Salicylic acid stimulated shoot and root growth, but silicon only slightly increased shoot growth. The amount of $Na^+$ in both roots and leaves decreased with application of silicon and especially salicylic acid. Thus, $Na^+$ absorption may be limited by applications of silicon and salicylic acid. Foliar application of salicylic acid and silicon enhanced relative water content, stomatal conductance, chlorophyll content index, leaf area index and consequently seed yield under both saline and non-saline conditions. The effects of silicon on these traits were disappeared when it was applied together with salicylic acid. Therefore, foliar application of salicylic acid can improve physiological performance and seed yield of mung bean by diminishing salt stress injuries.*

***Keywords***: *Chlorophyll content, Leaf area, Salt stress, Seed yield, Stomatal conductance*

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## INTRODUCTION

Salinity reduces plant growth due to osmotic and ionic effects on soil solution. Short-term effects include reduction on growth by salt due to osmotic effects, which reduces cell expansion. Long-term effects include excessive salt absorption, which causes plants to suffer ionic stress, leading to premature leaf aging following a reduction in the available photosynthetic area to maintain growth [18]. The major constraints for plant growth and productivity are ion toxicity with excessive uptake of mainly $Na^+$ as well as nutrients imbalance caused by disturbed uptake of essential mineral nutrients such as $K^+$.Salinity can reduce the leaf photosynthetic activity by affecting stomatal and non-stomatal factors. Loss of turgor by osmotic effect can cause stomata closure which lowers the supply of $CO_2$ to leaves. Salt stress can also reduce photosynthetic activity by affecting the non stomatal attributes such as destruction of green pigments, lowering the leaf area or by decreasing the activity of photosynthetic enzymes in Calvin cycle [15].

Salicylic acid is classified as a phenolic compound that can regulate plant growth and also provides protection against biotic and abiotic stresses such as salinity [11]. The effect of salicylic acid on plant physiological processes varies and depends on species, developmental stage and environmental conditions [24].Salicylic acid application influences a wide variety of plant processes, including stomatal regulation, chlorophyll content, photosynthesis [26] and also seed yield [12].Intracellular salicylic acid concentration and signaling pathway(s) are associated with the functions in controlling cell growth, cell death and defence [3].

Exogenous application of some nutrients ameliorates the adverse effects of salt stress [6]. Silicon is a non-essential element for plant growth, however, various studies have demonstrated that silicon application significantly increases plant growth under normal and stress conditions such as salt stress [21]. It is most commonly found in soils in the form of silicic acid $[Si(OH)_4]$, and its absorption occurs directly from this form [4]. A number of possible mechanisms are proposed through which silicon may increase salinity tolerance in plants, e.g., improving water status, stimulation of antioxidant system and alleviation of specific ion effect [22] by reducing $Na^+$ uptake or by $H^+$-ATPase dependent enhancement in $K^+$ in shoots [14].

Some strategies are proposed for alleviation of salt stress such as developing salt-resistant cultivars, leaching excess soluble salts from upper to lower soil layers and reducing salt by harvesting salt-accumulating aerial plant parts which are very difficult and costly. A new scheme for amelioration salt stress is to overcome the irregularities in plant physiological mechanisms by some of the plant growth regulators and nutrients. Therefore, the objective of this research is to investigate the effects of foliar application of salicylic acid and silicon on physiological properties and seed yield of mung bean under salt stress.

## MATERIALS AND METHODS

A factorial experiment (using RCB design) with three replications was conducted in 2013 (Tabriz, Iran) to investigate the changes of physiological characteristics of mung bean under salt stress in response to exogenous foliar application of salicylic acid and silicon. In each plastic pot (25× 25 cm) containing 1.0 kg of perlite and coco peat (4:1 ratio) 15 seeds of mung bean were sown at a depth of 3cm and then tap water (0.8 $dSm^{-1}$) and NaCl solutions (3, 6 and 9 $dSm^{-1}$) were added to achieve 100% FC. All pots kept inside a glass greenhouse under natural light. Minimum and maximum temperatures of greenhouse were 25 and 30 °C, respectively. After emergence and establishment, plants were thinned to 10 plants per pot. During the growth period, the pots were weighed and the losses were made up with Hoagland solution (EC =1.3 $dSm^{-1}$and pH= 6.5-7). Perlites within the pots were washed every 20 days and non-saline and salinity treatments were re-applied in order to prevent further increase in electrical conductivity (EC), due to adding the Hoagland solution. Two levels of salicylic acid (SA; 0 and 1 mM) and silicon as silicic acid (Si; 0 and 2 mM) were sprayed at vegetative growth and flowering stages.

Leaves of two plants from each sample were dried at 60 $^{o}$C for 48 h. Then 1 g of leaves was powdered and burned in 560 $^{o}$C and the ashes digested in 10 ml of 1N HCL. The concentration of $Na^+$ and $K^+$ in the digested samples was determined, using a flame photometer (corning flame photometer, 410).

Shoots and roots of two plants were cut after flowering and dried in an oven at 75°C for 48 h and then separately weighed. Chlorophyll content index and stomatal conductance plant leaves in each pot were monitored with a chlorophyll meter (CCM-200, Opti- Science, USA) and AP4 leaf poro-meter, respectively.

At pod formation stage,two plants from each pot were harvested and leaf area index was measured by leaf area meter (LI-3100, LI-COR, Inc., Lincoln, NE, USA). Fresh weight (FW) of the leaves was recorded and then samples were subjected to rehydration for 2 h and turgor weight (TW) was determined. Subsequently, leaves were dried at 75°C for at least 48 h anddry weight (DW) was recorded. Relative water content (RWC) was determined as:

$$RWC\ (\%) = (FW - DW)/(TW - DW) \times 100$$

At maturity, plants of each pot were harvested and seed yield per plant was determined.

All the data were analyzed on the bases of experimental design, using SAS 9.1 software. The means of each trait were compered according to Duncan multiple range test at $p \leq 0.05$.

## RESULTS

### $K^+$ and $Na^+$ Accumulation in Leaves and Roots

Accumulation of $K^+$ in mung bean leaves and roots significantly ($P \leq 0.01$) increased, but $Na^+$ considerably decreased by exogenous application of Si and SA. Maximum $K^+$and minimum $Na^+$ in both leaf and root under both saline and non-saline conditions were recorded for SA treated plants, compared with other treatments. However, there was no significant difference between SA and SA+Si in minimum$Na^+$ and maximum $K^+$in leaf and root under severe salinity conditions (Table 1).

**Table 1.**Accumulation of $K^+$ and $Na^+$ in mung bean leaf and root under different salinity levels in response to application of salicylic acid (SA) and silicon (Si).

| **Treatments** | | | **Leaf** | | **Root** | |
|---|---|---|---|---|---|---|
| | | | $K^+$ | $Na^+$ | $K^+$ | $Na^+$ |
| $S_1$ | $SA_0$ | $Si_0$ | 21.06 d | 6.44 g | 46.27 b | 10.78 f |
| | | $Si_1$ | 21.84 c | 5.48 h | 46.68 a | 9.47 h |
| | $SA_1$ | $Si_0$ | 26.79 a | 5.20 h | 52.36 a | 8.44 j |
| | | $Si_1$ | 24.85 b | 5.32 h | 51.51 a | 9.23 hi |

| | | | | | | |
|---|---|---|---|---|---|---|
| **$S_2$** | $SA_0$ | $Si_0$ | 16.78 g | 12.03 d | 31.92 f | 13.25 d |
| | | $Si_1$ | 19 e | 9.41 f | 32.99 e | 12.21 e |
| | $SA_1$ | $Si_0$ | 19.71 e | 6.15 g | 45.05 c | 8.88 ij |
| | | $Si_1$ | 18.1 f | 6.34 g | 41.27 d | 9.22 hi |
| **$S_3$** | $SA_0$ | $Si_0$ | 11.22 k | 15.93 b | 21.93 i | 13.98 c |
| | | $Si_1$ | 14.32 i | 12.93 c | 26.27 h | 12.2 e |
| | $SA_1$ | $Si_0$ | 16.28 gh | 9.67 f | 31.68 f | 10.24 g |
| | | $Si_1$ | 15.82 h | 11.05 e | 27.59 g | 10.74 f |
| **$S_4$** | $SA_0$ | $Si_0$ | 9.23 l | 17.86 a | 16.69 l | 15.23 a |
| | | $Si_1$ | 9.77 l | 15.38 b | 18.86 k | 14.64 b |
| | $SA_1$ | $Si_0$ | 13.77 i | 13.45 c | 22.14 i | 13.31 d |
| | | $Si_1$ | 12.71 j | 15.73 b | 20.16 j | 13.21 d |

Different letters in each column for each factor indicate significant difference at $P \leq 0.01$.
$S_1$, $S_2$, $S_3$: and $S_4$: 0, 3, 6 and 9 $dSm^{-1}$ of NaCl.
$SA_0$ and $SA_1$: control and 1 mM of salicylic acid. $Si_0$ and $Si_1$: control and 2 mM of silicon.

### Plant Growth Parameters

Root and shoot dry weights of mungbean plants were significantly ($P \leq 0.01$) decreased by increasing salt stress. Both root and shoot dry weight under severe salt stress ($S_4$) was about 31% less than that of control (Figure 1). Exogenous application of SA significantly improved root dry weight(Figure 2), but Si had no significant effect on this trait ($P>0.05$).

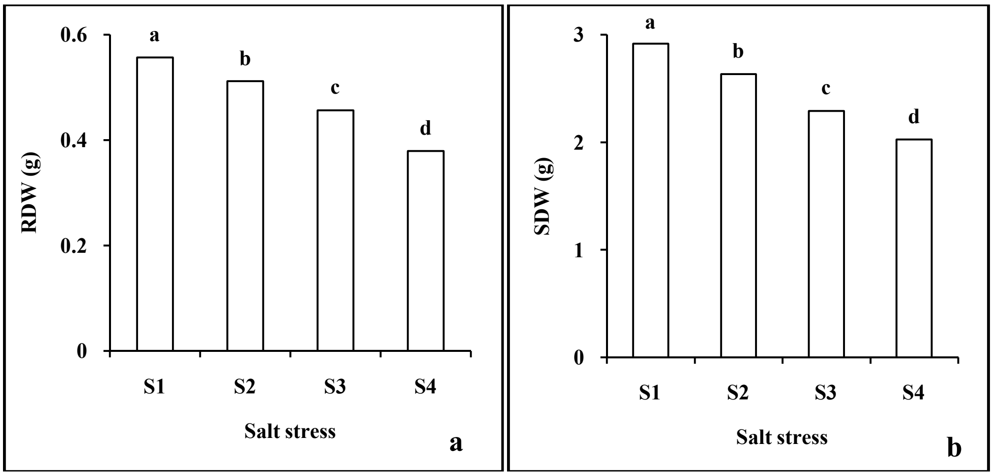

**Figure 1.** Root dry weight (RDW) (a) and shoot dry weight (SDW) (b) Of mung bean plants under different salinity levels.$S_1$, $S_2$, $S_3$: and $S_4$: 0, 3, 6 and 9 $dSm^{-1}$ of NaCl.

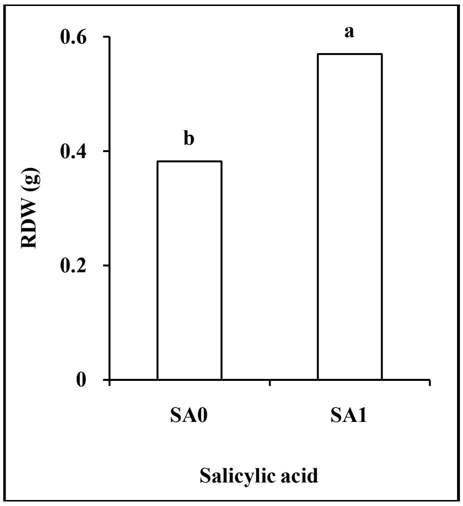

**Figure 2.** Root dry weight (RDW) of mung bean plants under application of salicylic. $SA_0$ and $SA_1$: control and 1 mM of salicylic acid.

## Physiological Parameters

The effect of SA application on CCI under non-saline condition was not significant. However, exogenous application of SA improved CCI of mung bean plants under different salinity conditions. CCI of non-treated plants decreased under different salinity levels, but application of SA prevented CCI to decline significantly in salinities up to 6 dSm$^{-1}$. Exogenous application of SA under saline and non-saline conditions significantly improved $g_s$. The highest $g_s$was recorded under non-saline condition with the application of SA and the lowest $g_s$was obtained from plants under severe salinity ($S_4$) without application of SA (Table 2).

**Table 2.** Means of the chlorophyll content index (CCI), stomatal conductance ($g_s$) and seed yield (SY) of mung bean plants under salt stress (S) with and without application of salicylic acid (SA)

| **Treatments** | | **CCI** | **$g_s$ (cm s$^{-1}$)** | **SY (g plant$^{-1}$)** |
|---|---|---|---|---|
| **$S_1$** | **$SA_0$** | 22.13 a | 0.6367 b | 2.192 c |
| | **$SA_1$** | 23.15 a | 0.9983 a | 2.991 a |
| **$S_2$** | **$SA_0$** | 19.26 b | 0.3650 c | 1.679 e |
| | **$SA_1$** | 22.05 a | 0.5733 b | 2.568 b |
| **$S_3$** | **$SA_0$** | 18.28 b | 0.1817 d | 1.473 f |
| | **$SA_1$** | 21.98 a | 0.3550 c | 2.183 c |
| **$S_4$** | **$SA_0$** | 16.12 c | 0.1417 d | 1.234 g |
| | **$SA_1$** | 18.83 b | 0.2967 c | 1.830 d |

Different letters in each column for each factor indicate significant difference at $P \leq 0.05$.
$S_1$, $S_2$, $S_3$: and $S_4$: 0, 3, 6 and 9 dSm$^{-1}$ of NaCl.
$SA_0$ and $SA_1$: control and 1 mM of salicylic acid.

Interaction of SA×Si indicated that SDW, CCI and $g_s$ were increased by application of both SA and Si. In general, the highest SDW, CCI and $g_s$ were recorded for plants with application of SA. However, shoot dry weight of plants with application of SA was statistically similar to those plants that sprayed with SA+Si (Table 3).

**Table 3.** Means of some physiological parameters and seed yield (SY) of mung bean in response to salicylic acid (SA) and silicon (Si).

| **Treatments** | | **CCI** | **$g_s$ (cm/s)** | **SDW (g)** | **SY (g/plant)** |
|---|---|---|---|---|---|
| **$SA_0$** | **$Si_0$** | 18.11 d | 0.2733 d | 2.033 c | 1.425 d |
| | **$Si_1$** | 19.78 c | 0.3892 c | 2.317 b | 1.864 c |
| **$SA_1$** | **$Si_0$** | 21.92 a | 0.6358 a | 2.770 a | 2.584 a |
| | **$Si_1$** | 21.08 b | 0.4758 b | 2.745 a | 2.202 b |

Different letters in each column for each factor indicate significant difference at $P \leq 0.05$.
$SA_0$ and $SA_1$: control and 1 mM of salicylic acid. $Si_0$ and $Si_1$: control and 2 mM of silicon.

Mung bean plants under non-saline condition with the application of SA+Si had the highest LAI. Under $S_2$,maximum LAI was recorded for plants that treated by SA and SA+Si. With increasing salinity levels ($S_3$ and $S_4$) plants with application of SA had more LAI, compared with other treatments. The lowest LAI under different saline conditions was obtained from control plants, which was statistically similar toSi treated plants (Figure 3a).

Maximum RWC under $S_1$, $S_2$ and $S_3$was recorded for plants with applications of SA and SA+Si. However, the highest RWC under severe salinity ($S_4$) was obtained from SA treated plant. Control plants had the lowest RWC under non-saline and different salinity conditions. However, differences between plants with application of Si and control was not significant under$S_2$ and $S_3$ (Figure 3b).

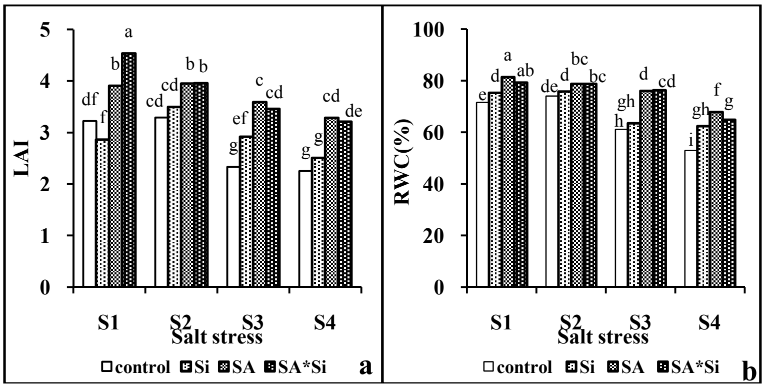

**Figure 3.** Means of leaf area index (LAI) (a) and relative water content (RWC) (b) of mung bean Plantsunder different salinity levels affected by salicylic acid and silicon.
$S_1$, $S_2$, $S_3$: and $S_4$: 0, 3, 6 and 9 $dSm^{-1}$ of NaCl.
$SA_0$ and $SA_1$: control and 1 mM of salicylic acid. $Si_0$ and $Si_1$: control and 2 mM of silicon.

## Seed Yield

Interactions of SS×SA and SA×Si on seed yield of mung bean were significant ($P \leq 0.05$). Seed yield of mung bean was enhanced by application of SA under both saline and non-saline conditions, but this enhancement was more under S1 and S2 treatments (Table 2). SY was also improved by individual application of Si. However, when Si applied with SA, seed yield was less than that of SA treated plants (Table 3).

## DISCUSSION

Exogenous foliar application of Si and SA positively affected physiology of mung bean by decreasing $Na^+$ and increasing $K^+$ accumulation in leaf and root under salt stress (Table 1). This may be occurred by preventing or decreasing enzyme inhibition [19] under salt stress. In mung bean plants SA alleviates salt stress by minimizing $Na^+$accumulation in leaves[20]. Similarly, Gunes*et al.* [7] showed that SA treatment inhibited $Na^+$ accumulation, but stimulated $K^+$ uptake by salt stressed maize plants.

Plant adaptations to salinity are of three distinct types: osmotic stress tolerance, $Na^+$ exclusion and the tolerance of tissue to accumulation of $Na^+$. SA positively stimulated shoot and root dry weights, but Si only slightly increased shoot dry weight (Figure 2, Table 3).On the other hand, the amount of $Na^+$ in both root and leaf decreased as a result of application of Si and especially SA (Table 1). Thus, $Na^+$ absorption may be decreased by applications of Si and SA. Improving root growth under salinity by SA treatment (Figure 2)indicate that accumulation of $Na^+$ in roots somehow helped to reduce the concentration of accumulated $Na^+$ in the shoot, similar to that reported for Arabidopsis [10].Under salinity stress, endogenous level of SA increased along with the increase in the activity of SA biosynthetic enzyme in rice seedling [23]. Arabidopsis seedling pre-treated with SA showed up-regulation of $H^+$-ATPase activity, thereby improving $K^+$ retention during salt stress.

Reduction in CCI under salinity (Table 2) may be associated with increasing the activity of the chlorophyllase, inducing the destruction of the chloroplast structure and the instability of pigment protein complexes [25]. The extent of these changes may be dependent on the biological processes and developmental stages of the plant and also on the levels of the salt stress.Similar results were reported for pinto bean [5]. The increase in chlorophyll content with application of SA (Table 2) was supported by the reports of Gunes *et al.*, (7) for maize and Yildirim *et al.*, (26) for cucumber.

High salt concentration in the soil solution is bound to create high osmotic pressure in the root zone and reduce availability of water to plants. Salinity causes stomatal closure (Table 2) and reduces transpiration rate [8].High $Na^+$ concentrations in turn increase the cytosolic $Ca^{2+}$ concentration and subsequently activate plasma membrane-localized anion channels [13], guard cell depolarization, potassium efflux, loss of guard cell turgor and volume and finally affectstomatal closure [9].Exogenous application of SA under saline and non-saline conditions significantly enhancedstomatal conductance ($g_s$) (Table 2). Root systems respond to salinity not only with a reduction in growth rate, but also with deduction in water and nutrient uptake. Reduction in water uptake decreased relative water content in leaves (Figure 3b), leading to closure of stomata and reducing stomatal conductance and photosynthesis rate [2]. This study showed that foliar application of SA induced an increase in RWC of the salt stressed plants as compared to the control plants (Figure 3b), which can be attributed to increasing root growth in treated plants by SA.

Decreasing leaf area index (LAI) due to salinity (Figure 3a) may be caused by shortening the length of the leaf elongating zone and decreasing the growth intensity in its central and distal portions. When plants are grown under saline conditions, as soon as the new cell starts its elongation process, the excess of salts modifies the metabolic activities of the cell wall causing the deposition of various materials which limit the cell wall elasticity. Cell walls become rigid and consequently the turgor pressure efficiency in cell enlargement is decreased [1]. Application of Si and SA in particular improved LAI of mung bean plants under both saline and non-saline conditions (Figure 3a). It seems that improvement of LAI with exogenous application of Si and SA was due to increasing turgor pressure in leaf cells (Figure 3b).

Reduction in seed yield under saline condition (Table 2) may be resulted from adverse effects on gametogenesis and fertilization, or loss in viability of pollen grains [16]. A decline in photosynthesis due to salinity stress could be caused by lower stomatal conductance, depression in carbon uptake and metabolism, inhibition of photochemical capacity, or a combination of all these factors [17]. The positive effects of Si and particularly SA on seed yield under salt stress could be attributed to an increase in chlorophyll content index, stomatal conductance (Table 2), leaf area index andrelative water content (Figure 3).

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