



Amelioration of Salinity Stress in Mung Bean by Combined Application of PGPR and Silicon

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Abstract – Salt stress is one of the main environmental threats to crop productivity that hamper plant growth due to the osmotic stress and nutritional imbalances. This study investigated the effect of plant growth promoting rhizobacteria (PGPR) inoculation and foliar application of silicon (Si) on the growth and physiology of mung bean under saline irrigation conditions in a field trial. Mung bean seeds were inoculated with 2 PGPR strains SM16 and SM6 separately, and grown under 3 different saline irrigation water (3.12, 5.46 and 7.81 dS m⁻¹) using drip irrigation system. After three week of growth, foliar application of silicon, with 3 levels [0, 1 and 2 kg ha⁻¹] was carried out using potassium silicate. Preliminary, results indicated that PGPR inoculation and foliar application of Si both alone and in combination caused significant improvement in the growth and physiological attributes of mung bean under all salinity levels compared with control. Especially, the effect of inoculation with SM16, at Si 2 kg ha⁻¹, was more pronounced in improving the chlorophyll contents and transpiration rate up to 61.52 % and 74.16 %, respectively, over uninoculated control under salinity level 7.81 dS m⁻¹. Similarly, SM16 and SM6 inoculation, at silicon 1 and 2 kg ha⁻¹, made significant improvement in the fresh and dry weight of mung bean over control under saline conditions. The results suggested that combined application of PGPR and silicon could be effectively used to improve the mung bean productivity from salt-affected lands.

Keywords – Mung Bean, Saline Irrigation, Rhizobacteria Inoculation, Silicon Foliar Spray.

I. INTRODUCTION

Salinity is the most limiting environmental factor to successful crop production [1]. Globally about 800 m ha of land are salt-affected which is 6 % of total land area of world [2]. High salt concentration reduces the osmotic potential of soil water, which results in declined water uptake by plants and ultimately decreases transpiration. Moreover, greater uptake and accumulation of Na⁺ and Cl⁻ ions by plants under salinity causes nutritional imbalance [3, 4]. In addition, plants face oxidative stress under saline conditions due to increased synthesis of reactive oxygen species [5] and finally crop productivity decreased [6-8].

Silicon (Si) has been considered as beneficial element in plant growth and development especially under stress environment [9]. It is the second most abundant element in the soil and its concentration in plant tissues range from 1

to 10 % [9]. Improvement of salt tolerance by application of Si has been reported in many important agricultural crops including rice [10, 11], barley [12] Sorghum [13], wheat [14], maize [15], sugarcane [16], soybean [17], tomato [18], and canola [19]. The previous researches suggested that Si can enhance the salt tolerance of plants through decreasing Na uptake and accumulation in rice [20, 10], improving the water balance in tomato [21], minimizing the oxidative damage in cucumber [22] and increasing the plasma membrane H⁺-ATPase activity in barley [23]. However, above mentioned effects of Si may vary from crop to crop which strongly suggest that Si facilitated mechanisms for enhancing salt tolerance in plants are complex and need detailed investigation.

Use of bacterial inoculants for amelioration of salt stress in crop plants is also an emerging era in research. Plant growth promoting rhizobacteria (PGPR) are the beneficial free living soil-borne bacteria which can colonize with plant roots and are able to improve the plant growth through multiple mechanisms of action [24, 25]. Plant growth promoting rhizobacteria have been reported to improve the plant growth via biological fixation of atmospheric nitrogen, biosynthesis of phytohormone, nutrient solubilisation and increasing the host plant resistance to biotic and stress factors [26, 27]. PGPR are well adapted to worst environmental conditions and may safeguard the plants from the adverse effects of salt stress, thus increasing crop productivity from arid and semi-arid areas [28, 29]. Numerous PGPR are reported alleviate salt stress in important agricultural crops such as wheat, rice, mung bean, alfalfa, soybean and pea [28, 30-34].

Mung bean is an important pulse crop grown worldwide. Its importance is not only due to its nutritional value in human diet but also play vital role in improving soil fertility through biological nitrogen fixation in the nodules [35]. However, under agroclimatic condition of Saudi Arabia the performance of mung bean is poor due to occurrence of severe aridity and salinity in the Kingdom. Several individual studies have been conducted on Si and PGPR for enhancing salt tolerance in plants but up to date no report is available on their combined use for alleviation of salinity stress in crop plants. Therefore, on the base of above discussion, the present study aimed to investigate the combined use of Si and PGPR for enhancing salt tolerance in mungbean.

II. MATERIALS AND METHODS

Preparation of Inocula

The inoculum for the pre-isolated strains SM6 and SM16 were prepared in conical flasks using Luria Bertani (LB) broth media. LB broth media was prepared by using standard composition. Each flask containing broth was inoculated with the isolate SM6 and SM16, separately, and kept in shaking incubator (100 rpm) for 72 h at 28 ± 1 °C.

Field Trial

The effect of PGPR inoculation and foliar application of Si was evaluated under natural field condition using saline irrigation during the field experiment conducted at Agricultural Research Station, located at Hada Al-Sham, King Abdulaziz University, Jeddah, Saudi Arabia.

For inoculation of seed, inocula were prepared (as described earlier) for both SM6 and SM16 strain separately and mixed in sterilized peat. Mung bean seed dressing was done by mixing seeds in peat with 10 % sugar solution. For uninoculated control, seeds were treated with peat containing sterilized broth and 10 % sugar solution. Fourteen day after sowing, saline irrigation was started with three different saline water having EC value 3.12, 5.46 and 7.81 dS m⁻¹ through drip irrigation system for 10 minutes per day in the morning hours. Foliar application of Si was carried out at 0, 1 and 2 kg ha⁻¹ using potassium silicate after one week of saline irrigation treatment. The experiment was laid out according to split-split plot design keeping saline irrigation as main plot factor, silicon as sub-plot factor and PGPR strains as sub-sub plot factor with plot size 3 × 2 m and four replication. The plants were fertilized with NPK fertilizer (18:18:5) at 600 kg ha⁻¹ added in three equal doses.

Plant Growth and Yield Trait

At crop maturity stage, data on growth and yield attributes like fresh and dry weight production were recorded following standard protocols. For data collection, 1 m² area was randomly selected from each plot.

Gaseous Exchange Measurement

Gaseous exchange parameters like stomatal conductance (g_s) and transpiration rate (E) were recorded with poro meter during the morning hours. Fresh, fully developed leaf of both stressed and non-stressed plants was selected for gaseous exchange measurement.

Chlorophyll Measurement

The fully expanded leaves were used for chlorophyll measurement by following the protocol as described by Arnon [38]. Briefly, the chlorophyll was extracted with 80 % acetone from frozen leaf samples (0.5 g) in a shaker till the complete bleaching of leaf samples. The extract was centrifuged at 13000 rpm for 10 min and the supernatant was used for absorbance measurement at 645 and 663 nm using a spectrophotometer.

Statistical Analysis

The data were analyzed using analysis of variance (ANOVA) [42] and means comparison were carried out using LSD test. The software used for statistical analysis of data was Statistix 8.1 (Analytical Software, USA) by applying a split-split plot design.

III. RESULT AND DISCUSSION

Salinity stress significantly reduced the growth and physiological attributes of mung bean as shown in Table (1). Fresh biomass decreased significantly up to 3.99 t ha⁻¹ under saline irrigation at 7.81 dS m⁻¹ followed by 10.09 and 21.11 t ha⁻¹ at salinity level 5.46 and 7.81 dS m⁻¹, respectively. Likewise, mean values of dry weight follow the same pattern and maximum dry weight (9.70 t ha⁻¹) was recorded under saline irrigation at 3.12 dS m⁻¹ and minimum dry weight (1.23 t ha⁻¹) was observed at salinity level 7.81 dS m⁻¹. Foliar application of silicon significantly ($P \leq 0.05$) improved the fresh and dry weight of mug bean (Table 1). Significant improvement was recorded in fresh and dry weight up to 12.94 and 5.62 t ha⁻¹, respectively, by application of Si at 2 kg ha⁻¹. Rhizobacteria inoculation made significant ($P \leq 0.05$) improvement in growth attributes of mung bean. The maximum improvement in fresh and dry weight was observed under the rhizobacterial strain SM16 up to 13.88 and 6.25 t ha⁻¹, respectively. Analysis of variance (ANOVA) indicated that three way interaction is statistically significant.

Improvement in plant growth and development under saline conditions by silicon application has been widely reported in literature [43, 44]. In our study, foliar application of Si increased the fresh and dry weight of mung bean under saline irrigation condition which might be correlated to Si facilitated mechanisms in crop plants. These mechanisms may include reduced uptake and accumulation of Na⁺ and Cl⁻ [44, 45], improved nutrient uptake and regulation of osmolytes [17, 19, 46], modification in gaseous exchange parameters [8], minimizing the oxidative damage [11] and activation of genetic manifestation [6]. Our results are in line with Lee et al. [17] where they observed improvement in growth parameters of soybean under saline stress by Si application. Comparable results of Si on plant growth have been observed under saline condition in other crops such as tomato [47], cucumber [48], *Phaseolus vulgaris* L. [49] and faba bean [50]. In the present study it has been observed that fresh and dry weight increased due to rhizobacterial inoculation under salt stress. This increase in mung bean growth might be correlated to bacterial facilitated mechanisms in plants under stress conditions. These mechanisms may include inhibition of Na⁺ uptake by exopolysaccharide activity [51, 52], lowering the stress accelerated synthesis of ethylene via ACC-daminase enzyme activity [34, 53] improving the water balance and minimizing the oxidative damage [33] and modification in gaseous exchange [28]. Kang et al. [33] reported that shoot length and plant fresh weight of salt stressed soybean increased with rhizobacteria inoculation. Likewise Ahmad et al. [54] and Martínez et al. [32] observed similar effects of bacterial inoculation on mung bean and alfalfa, respectively, under salt stress conditions.

Data presented in Table (1) indicated that salinity significantly ($P \leq 0.05$) reduced the gaseous exchange and total chlorophyll contents. The percent reduction observed in stomatal conductance at salinity level 5.46 and 7.81 dS m⁻¹ was 26.47 and 47.32 %, respectively, compared with

stomatal conductance recorded under saline irrigation at 3.12 dS m⁻¹. Likewise, significant reduction in transpiration rate (64.78 %) and total chlorophyll (16.55

%) was registered at salinity level 7.81 dS m⁻¹ compared with salinity level at 3.12 dS m⁻¹. Application of Si

Table 1. Growth and physiological parameters of mung bean influenced by foliar application of Si and rhizobacterial inoculation under saline irrigation conditions in a field trial

	Fresh biomass (t ha ⁻¹)	Dry weight (t ha ⁻¹)	<i>g_s</i> (mmol m ⁻² S ⁻¹)	<i>E</i> (mmol m ⁻² S ⁻¹)	Total Chlorophyll (mg kg ⁻¹)
Salinity (dS m⁻¹)					
3.12	21.11 a	9.70 a	0.374 a	3.72 a	969.40 a
5.46	10.09 b	4.12 b	0.266 b	2.52 b	907.07 b
7.81	3.99 c	1.23 c	0.189 c	1.31 c	808.91 c
LSD	1.06	0.27	0.034	0.129	3.22
Silicon (kg ha⁻¹)					
0	10.39 c	4.40 c	0.224 c	1.87 c	727.6 c
1	11.86 b	5.1 b	0.291 b	2.16 b	937.6 b
2	12.94 a	5.62 a	0.332 a	3.51 a	1050.6a
LSD	0.59	0.31	0.036	0.047	2.19
PGPR Strain					
Uninoculated	9.55 c	4.05 c	0.180 c	1.51 c	752.6 c
SM6	11.76 b	4.76 b	0.272 b	2.42 b	894.0 b
SM16	13.88 a	6.25 a	0.381 a	3.61 a	1038.8 a
LSD	0.58	0.35	0.032	0.053	1.9
Significance					
Salinity (S)	**	**	**	**	**
Silicon (Si)	**	**	**	**	**
PGPR Strain (P)	**	**	**	**	**
S × Si	*	*	**	**	**
S × P	**	**	**	**	**
Si × P	*	*	**	**	**
S × Si × P	*	*	**	**	**

Means followed by different letters are significantly different according to LSD ($p < 0.05$); * Significant at $p < 0.05$; ** Significant at $p < 0.01$; g_s = Stomatal conductance and E =Transpiration rate

Markedly improved the physiological attributes of mung bean. Application of silicon at 2 kg ha⁻¹ made significant improvement in stomatal conductance, transpiration rate and total chlorophyll up to 48.21, 87.7 and 40.21 %, respectively, compared with control where silicon was not applied. Similarly, bacterial inoculation has significant effects on physiology of mung bean. Inoculation with rhizobacterial strain SM16 markedly improved the stomatal conductance, transpiration rate and total chlorophyll up to 118.89, 139.07 and 38.03%, respectively over uninoculated control. Analysis of variance (ANOVA) indicated that three way interaction of salinity, silicon and rhizobacteria is statistically significant for improving physiology of mung bean.

Si-facilitated improvement in gaseous exchange and chlorophyll contents under saline conditions has been observed in many crops plants including wheat, rice, tomato and canola [14, 19, 45, 55]. The findings of this

study are in line with Shi et al. [45] and Haghghi and Pessarakli [55] as they reported that silicon application improved the assimilation rate, substomatal conductance, water use efficiency and chlorophyll contents in salt stressed rice and tomato seedling, respectively. In current study, bacterial inoculation markedly improved the stomatal conductance, transpiration rate and chlorophyll contents under saline stress. These findings are also reinforced by the previous studies in which enhanced stomatal conductance, transpiration rate and chlorophyll contents due to PGPR inoculation has been reported [28, 30, 33, 54].

IV. CONCLUSION

It can be concluded from the results of the field experiment that the synergistic use of PGPR and silicon can enhance mung bean productivity in saline soils, thus

contribute to a better agricultural exploitation of low productive areas.

V. ACKNOWLEDGEMENT

This article was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah. The authors, therefore, acknowledge with thanks DSR technical and financial support.

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