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Vegetative Growth and Ion Relations in Soybean with Potassium Sulphate Application under Saline Environment

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the effect of $K_2SO_4^{2-}$ application on growth of soybean cultivars besides chemical parameters under salt stress.
Study Design: Laid out the experiment in Complete Randomized Design in triplicates. Analyzed the data statistically by using the statistical software Statistix 8.1.
Place and Duration of Study: The study was conducted in Soil Salinity and Bio-saline Research green house and in the laboratory of Land Resources Research Institute at National Agricultural

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Research Centre, Islamabad (under PARC), Pakistan for three months.
Methodology: To study germination parameters under salt stress, NaCl was used @ 0, 100, 120 and 140 mmol L^{-1}. At seedling stage, interactive effect among salt stress (0 and 4.5 dSm^{-1}), potassium sulphate (KS) application @ 10 mmol L^{-1} and soybean (Cvs. NIBGE-301 and NIGBE-158) was recorded for growth and ion relations.
Results: Germination, biomass, bio vigor, mass vigor and sap vigor of the seedlings and ion relations were affected highly significantly ($p \leq 0.01$). Under interactive effect of KS and salt stress at vegetative stage, $Na^{+/}K^{+}$ in the plants declined with KS application. Sulphur and phosphorus concentration in the plants changed under the same conditions.
Conclusion: Induced salt stress revealed germination, seed bio vigor, seeding mass vigor, and seedling sap vigor seedling and other vegetative parameters of soybean cultivars. Potassium sulphate application revealed genotypes differential response to ion relations and growth under salt stress. NIBGE-301 was more tolerant to salt stress and more responsive to potassium sulphate application than NIGBE-158.

Keywords: Soybean; salinity; potassium sulphate; vegetative growth; ion relations.

1. INTRODUCTION

Soybean (*Glycine max* L.), a leguminous plant, is native to tropical and warm temperate regions of Asia. There are over 2,500 different types of varying size, shape and color. It was first introduced for cultivation in Europe in the 1700s. It has become one of the most important bean in the world, providing oil, protein as soybean meal besides animal feed and biodiesel. The United States, Brazil and Argentina are leading exports of soybean. China and Japan are the leading importers. Soybean plant has unique chemical composition [1]. It is a major source of dietary protein besides highly digestible oil [2]. As a non-conventional crop it is cultivated in Pakistan as autumn and spring crop preferably in loamy soil. In irrigated areas crop rotation consists of rice-soybean-rice and cotton-soybean-cotton. In rain fed areas, crop rotation consists of wheat - soybean-wheat.

Salinity causes growth and yield reductions in most plant species [3]. Saline soil impairs physiological functions through water stress, specific ion toxicity, ion imbalance stress, induced nutrient deficiency, or a combination of these factors [4]. Most of the salt stresses in nature are due to sodium salts, mostly NaCl [5]. Salinity induces deficiencies or imbalances of nutrient in plants and these effects vary among species and even among varieties of a crop [6]. Salinity presumes ion ratios (e.g., Na^{+}/Ca^{2-}, Na^{+}/K^{+}, Cl^{-}/NO^{3-}) in the soil solution. In these ratios, increasing numerator value causes improper nutrient availability and irregularly uptake and/or distribution of a nutrient within the plant. Growth of soybean is limited by soil salinity [7] and its germplasm may differ in resistance to this stress. As the seedling stage of soybean is more sensitive to salt stress [8]. Soybean exhibits considerable genotypic differences in salt tolerance [9]. Salinity impairs the normal uptake of K^{+} as well as other essential plant nutrients in soybean. The application of potassium sulphate fertilizer may be beneficial for growth of soybean. Its genotype response may be variable due to genetic makeup. Its exploration under salt stress is a continuous process due to genetic variability, macro and micro environment changes and nutrients availability. This study was conducted to evaluate the effect of $K_2SO_4^{2-}$ application on growth of soybean cultivars besides chemical parameters under salt stress.

2. MATERIALS AND METHODS

2.1 Seed Testing and Sterilization

Seeds of *Glycine max* (L.) Cvs. NIBGE-301 and NIGBE-158 having uniform size, color and weight were selected. For surface sterilization, seeds of soybean cultivars were treated with 1.0 percent (v/v) sodium hypochlorite [10] for minutes and then washed three times with tap water.

2.2 Seed Germination and Germination Parameters under Salt Stress

Ten seeds of each cultivar were placed in each of Petri dishes. The seeds were treated with NaCl solutions having concentrations @ 0, 100, 120 and 140 mmol L^{-1}. After two days, the seeds started germination and this process continued for next consecutive four days. On fourth day, plant heights were measured besides fresh mass. The samples were dried at 70^{0}C and dry mass was recorded. The samples were digested in an acid mixture of hydrochloric acid and nitric acid (1:2). The following parameters were calculated as under:

- Seed germination percentage = (Seeds germinated as per salt stress level)*100/(Total number of seeds germinated)
- Seed bio vigor (SBV) = Fresh mass /seedling length (dilution of FM µg / cm of length)
- Seeding mass vigor (SMV) = Dry mass / seedling length (Attenuation of FM µg / cm of length)
- Seedling sap (SS) = (Fresh mass - Dry mass)/ Fresh mass
- Seedling sap Vigor (SSV) = SS/ Seedling length (Reduction in sap of seedling µg / cm of length)

2.3 Seed Germination to Seedling Stage under Salt Stress, Potassium Sulphate Treatment

Seeds of two soybean cultivars (NIGBE-158, NIBGE-301) were washed with sodium hypochlorite (1:100 v/v %), sown in trays of washed sands. Plugged seedlings uniformly on seventh day, in pots containing aerated nutrient solutions [11]. Temperature was maintained up at 25± 2^0C with 16 hours photoperiod. The nutrient solution was replaced weekly. Light intensity of 450 µmol m^{-2} s^{-1} was maintained. The pH of the solution was adjusted to 6.0 either with HCl or $Ca(OH)_2$ and was monitored regularly. Potassium sulphate was applied @ 0 and 10 mmol L^{-1}. For salt stress applied NaCl to maintain ECe 4.5 dSm^{-1}. The treatments were applied in triplicates.

2.4 Plant Sampling, Physical and Chemical Data

The plants were sampled on 28th day after seedlings transplantation. After recording fresh mass (FM), the plants were rinsed with deionised water. Seedling length was measured. Dried plant samples at 65°C to constant mass. Recorded dry mass (DM) of each sample and ground to pass a 40-mesh Wiley Mill. Digested ground samples separately in 1:2 perchloric-nitric di-acid mixture [12]. Determined phosphorus in the plant digested material as given by [13]. Measured the concentration of Na and K ions by flame photometer. Sulphur in the digested material was found out as given by [14].

2.5 Statistical Data Analysis

Laid out the experiment in Complete Randomized Design in triplicates. Analyzed the data statistically by using the statistical software [15].

3. RESULTS

Salt stress @ 0, 100, 120 and 140 mmol L^{-1} of NaCl affected significant ($p \leq 0.01$) on seed germination and seedling growth response besides interactive effect among salt stress (0 and 4.5 dSm^{-1}), potassium sulphate (KS) application @ 10 mmol L^{-1} and soybean (Cvs. NIBGE-301 and NIGBE-158) for growth and ion relations.

3.1 Seed Germination and Seedling Growth Response to Salt Stress

3.1.1 Seed germination under salt stress

At germination level, both the varieties performed well. Under control and at 10 mmol L^{-1} NaCl application, in both, the germination was 100 percent. However, at 120 and 140 mmol L^{-1} NaCl application, the germination remained 80 percent (Fig. 1 A).

3.1.2 Bio mass of the seedlings

Fresh mass (FM) of both the varieties decreased 5, 9 and 14 percent on 100, 120 and 140 NaCl mmol L^{-1} application than the respective control. The trend was the same for dry mass (DM) in both the varieties. However, in NIBGE-301, DM decreased 13, 22 and 24 percent on 100, 120 and 140 NaCl mmol L^{-1} application than the control. In NIBGE-158 DM decreased 12 and 24 percent on 120 and 140 NaCl mmol L^{-1} application than the control (Table 1).

3.1.3 Seedling heights and seedling bio vigor

Seedling heights (SL) of both the varieties decreased 12, 32 and 44 percent on 100, 120 and 140 NaCl mmol L^{-1} application than the respective control. In NIBGE-301 dilution of seedling bio vigor (SBV) increased 9, 33 and 37 percent on 100, 120 and 140 NaCl mmol L^{-1} application than the control. In NIBGE-158 dilution of seedling bio vigor (SBV) increased 6, 15 and 59 percent on 100, 120 and 140 NaCl mmol L^{-1} application than the control (Table 2).

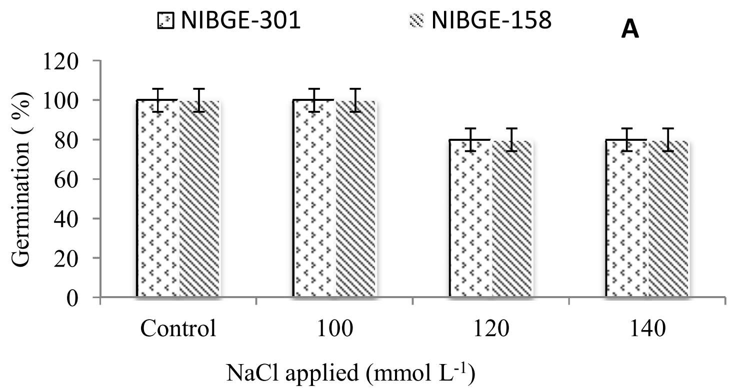

Fig. 1A. Screening of soybean cultivars under salt stress for germination

Table 1. Effect of salts tress on fresh and dry mass of soybean seedlings

NaCl (mmol L^{-1})	Fresh mass (g $seedling^{-1}$)			Dry mass (g $seedling^{-1}$)		
	(Cvs.)		Means	(Cvs.)		Means
	NIBGE-301	NIBGE-158		NIBGE-301	NIBGE-158	
Control	0.527 a	0.409 e	0.468 A	0.037 a	0.025 e	0.031 A
100	0.498 b	0.389 f	0.444 B	0.032 b	0.025 e	0.029 B
120	0.474 c	0.372 g	0.423 C	0.029 c	0.022 f	0.026 C
140	0.452 d	0.350 h	0.401 D	0.028 d	0.019 g	0.024 D
Means	0.488 A	0.380 B		0.032 A	0.022 B	

Means sharing similar letter(s) for a parameter do not differ significantly at $p < 0.01$

Table 2. Effect of salt stress on seedling length and seedling bio vigor in soybean

NaCl (mmol L^{-1})	Seedling length (cm)			Seedling bio vigor (SBV)		
	(Cvs.)		Means	(Cvs.)		Means
	NIBGE-301	NIBGE-158		NIBGE-301	NIBGE-158	
Control	17.6 a	16.0 b	16.8 A	29.3 e	24.1 h	26.7 D
100	15.5 c	14.0 d	14.7 B	32.0 d	25.6 g	28.8 C
120	12.2 e	10.9 f	11.6 C	38.9 b	27.8 f	33.4 B
140	10.3 g	9.1 h	9.7 D	43.7 a	38.3 c	41.1 A
Means	13.9 A	12.5 B		36.0 A	28.9 B	

Means sharing similar letter(s) for a parameter do not differ significantly at $p < 0.01$

3.1.4 Seedling mass vigor (SMV) and seedling sap vigor (SSV)

In NIBGE-301 attenuation of SMV increased 15 and 29 percent on 120 and 140 NaCl mmol L^{-1} application than the control. In NIBGE-158 SMV increased 8, 25 and 27 percent on 100, 120 and 140 NaCl mmol L^{-1} application than the control. In NIBGE-301 reduction of SSV in NIBGE-301 increased 14, 46 and 73 percent on 100, 120 and 140 NaCl mmol L^{-1} application than the control. In NIBGE-158 SSV increased 14, 47 and 77 percent on 100, 120 and 140 NaCl mmol L^{-1} application than the control. In NIBGE-158 SSV was 12 % higher than that of NIBGE-301(Fig. 1 B, C).

3.2 Interactive Effect of Potassium Sulphate and Salt Stress at Vegetative Stage

Fresh mass (FM) of the plants improved with potassium sulphate (KS) application under salt non-stress and stress conditions. However, the varietals response was variable. In NBGE 301, FM decreased 9 percent under salt stress than that of its control (Non-stress) with KS application. In NBGE 158, FM decreased 11 percent under salt stress than that of its control (Non-stress) with KS application (Table 3). Dry mass (DM) of the plants improved with KS application under salt non-stress and stress conditions. In NBGE 301, DM decreased 10 percent under salt stress than that of its control (Non-stress) on KS application. In NBGE 158, FM decreased 15 percent under salt stress than that of its control (Non-stress) on KS application (Table 4).

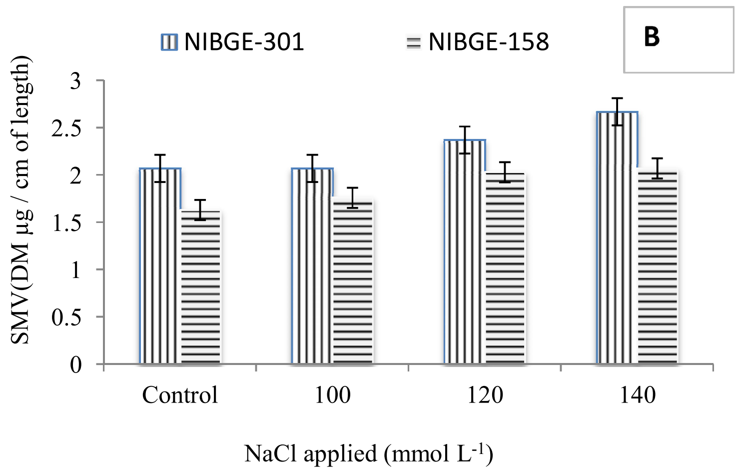

Fig. 1B. Effect of salt stress on seedling mass vigor (SMV)

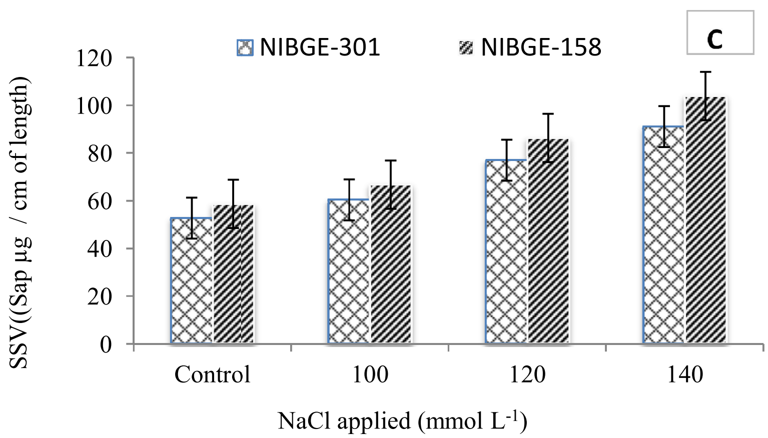

Fig. 1C. Effect of salt stress on seedling sap vigor (SSV)

Table 3. Interactive effect of salt stress and potassium sulphate application on fresh mass (g plant^{-1}) in soybean

Soybean (Cvs.)	Salt stress level (dSm^{-1})						Mean of means
	0			4.5			
	Potassium sulphate (mmol L^{-1})						
	Control	10	Means	Control	10	Means	
NIBGE-301	1.27 bc	1.42 a	1.35 A	1.25 c	1.29 b	1.27 B	1.31 A
NIBGE-158	1.21 d	1.28 b	1.25 C	1.07 f	1.11 e	1.09 D	1.17 B
Means	1.24 B	1.35 A		1.18 C	1.18 C		
Mean of means	1.30 A			1.18 B			

Means sharing similar letter(s) for a parameter do not differ significantly at p < 0.01

Table 4. Interactive effect of salt stress and potassium sulphate application on dry mass (mg plant^{-1}) in soybean

Soybean (Cvs.)	Salt stress level (dSm^{-1})						Mean of means
	0			4.5			
	Potassium sulphate (mmol L^{-1})						
	Control	10	Means	Control	10	Means	
NIBGE-301	56.8 d	63.6 a	60.2 A	50.1 f	57.2 c	53.7 C	57.0 A
NIBGE-158	51.2 e	57.9 b	54.5 B	44.4 h	49.5 g	47.0 D	50.8 B
Means	54.0 B	60.8 A		47.2 D	53.4 C		
Mean of means	57.4 A			50.4 B			

Means sharing similar letter(s) for a parameter do not differ significantly at $p < 0.01$

Table 5. Interactive effect of salt stress and potassium sulphate application on Na^+/K^+ ($X10^{-4}$) ratio in soybean

Soybean (Cvs.)	Salt stress level (dSm^{-1})						Mean of means
	0			4.5			
	Potassium sulphate (mmol L^{-1})						
	Control	10	Means	Control	10	Means	
NIBGE-301	8.1 c	3.2 g	5.7 C	6.2 d	2.7 h	4.5 D	5.1 B
NIBGE-158	10.8 a	4.1 e	7.5 A	8.4 b	3.9 f	6.2 B	6.9 A
Means	9.5 A	3.7 C		7.3 B	3.3 D		
Mean of means	6.6 A			5.4 B			

Means sharing similar letter(s) for a parameter do not differ significantly at $p < 0.01$

Table 6. Interactive effect of salt stress and potassium sulphate application on sulphur (%) in soybean

Soybean (Cvs.)	Salt stress level (dSm^{-1})						Mean of means
	0			4.5			
	Potassium sulphate (mmol L^{-1})						
	Control	10	Means	Control	10	Means	
NIBGE-301	0.69 e	1.51 a	1.10 A	0.58 f	1.30 b	0.94 B	1.02 A
NIBGE-158	0.48 g	1.11 c	0.80 C	0.38 h	0.88 d	0.63 D	0.72 B
Means	0.59 C	1.31 A		0.48 D	1.09 B		
Mean of means	0.95 A			0.79 B			

Means sharing similar letter(s) for a parameter do not differ significantly at $p < 0.01$

Table 7. Interactive effect of salt stress and potassium sulphate application on phosphorus (%) in soybean

Soybean (Cvs.)	Salt stress level (dSm^{-1})						Mean of means
	0			4.5			
	Potassium sulphate (mmol L^{-1})						
	Control	10	Means	Control	10	Means	
NIBGE-301	0.37 c	0.42 bc	0.40 C	0.43 bc	0.43 bc	0.43BC	0.42 B
NIBGE-158	0.55 ab	0.54 ab	0.55 A	0.44 abc	0.57a	0.51AB	0.53 A
Means	0.49^{ns}	0.46		0.44	0.50		
Mean of means		0.48^{ns}			0.48		

Means sharing similar letter(s) for a parameter do not differ significantly at $p < 0.01$
$^{ns:}$ Non-significant

3.3 Salt Stress and Status of Sulphur and Phosphorus

Sodium potassium ratio (Na^{+}/K^{+}) of the plants declined on KS application under salt non-stress and stress conditions. In NBGE 301, Na^{+}/K^{+} decreased 16 percent under salt stress than that of its control (Non-stress) on KS application. In NBGE 158, Na^{+}/K^{+} decreased 4 percent under salt stress than that of its control (Non-stress) on KS application (Table 5). Sulphur concentration in the plants changed on KS application under salt non-stress and stress conditions. In NBGE 301, sulphur decreased 14 percent under salt stress than that of its control (Non-stress) on KS application. In NBGE 158, sulphur decreased 20 percent under salt stress than that of its control (Non-stress) on KS application (Table 6). Phosphorus concentration in the plants also changed on KS application under salt non-stress and stress conditions. In NBGE 301, phosphorus increased 2 percent under salt stress than that of its control (Non-stress) on KS application. In NBGE 158, phosphorus increased 6 percent under salt stress than that of its control (Non-stress) on KS application (Table 7).

4. DISCUSSIONS

4.1 Seed Germination Parameters

Seed germination under salt stress is a crucial stage for crop growth and development. Damages created during seed germination due to salt stress cannot be rectified usually at the later stages of growth. If management process is not carried out on seeds before germination, the seeds cannot show their natural potential for germination under salt stress. In this way the seed germination level under saline conditions is revealed. Fresh and dry mass of the seedlings under salt stress indicates the genetic potential of the embryo in the seed. As per micro environment, fresh mass of a plant indicates the moisture having water and dissolved chemicals in it for binding the tissues [16]. In both the varieties, fresh mass declined with increasing salinity in the growth medium. This decline was higher in NIBGE-158 than that of NIBGE-301. In a plant the resultant metabolic activities are in the form of dry mass [17]. Dry mass in NIBGE-301 was higher than that of NIBGE-158 under salt stress. The difference in dry mass indicates variable response of the varieties for salt stress. Like bio mass, plant length also decreased under increasing salt stress. Seedling bio vigor (SBV) is a relation between fresh mass (FM) of a plant to its length (PL). With favorable conditions, a plant has normal growth potential, geotropism of roots and photo tropism of shoot remain in a good coordination. Fresh mass and seedling length are in normal proportion. In some conditions, FM and PL may not be in harmonic relations. FM may change per unit PL. The higher the SBV, the lower harmonic relation. In this study SBV of both the varieties declined with salt stress, however SBV of NIBGE-301 remained lower than that of NIBGE-158. Seedling mass vigor (SMV) is affinity between dry mass (DM) of a plant to its length (PL). Under non stress conditions, a plant has normal growth where dry mass and seedling length are in normal proportion. In salt stress conditions, DM may change per unit of PL. The higher the SMV, the lower harmonic relation. In this study SMV of both the varieties declined with salt stress, however SMV of NIBGE-301 remained lower than that of NIBGE-158. Seedling sap Vigor (SSV) is a comparison between seedling sap (SS) of a plant to its length (PL). In salt stress conditions, SSV may change per unit PL. The higher the SSV, the lower harmonic relation. In this study SSV of both the varieties declined with salt stress, however SSV of NIBGE-301 remained lower than in NIBGE-158.

4.2 Interactive Effect of Potassium Sulphate and Salt Stress at Vegetative Stage

Synergistic or antagonistic linkage between specific ions out side a plant exists in micro environments of roots. Growth of plants is dependent on positive association of ions within the system [18]. When potassium sulphate is induced through root system of a crop plant, then both ions i.e. potassium and sulphate reduce adverse salinity effects on water and tissue growth. Under non-stress condition, the application of KS improved fresh and dry mass of the plants. Under salt stress, these parameters also improved significantly, although the magnitude effect of KS was a little bit lesser under salt stress than non-stress. Synergism between potassium and sulphate ions support to overcome salt stress. Potassium and sulphate ions are in synergistic relations with in and out side of a plant under normal conditions or salt stress. In addition potassium ion is well known for accelerating enzymatic metabolism [19]. Potassium ion has antagonistic relation with sodium ion [16]. Plants intake sulphur as sulphate ion through their root system [20].

Sulphur as nutrient is synergistic to potassium ion and sulphur has gained importance as the fourth important macronutrient for crop plant known as tetrad [18].

5. CONCLUSIONS

Induced salt stress revealed germination, seed bio vigor, seeding mass vigor, and seedling sap vigor seedling and other vegetative parameters of soybean cultivars. In addition, potassium sulphate application exposed genotypes differential response to ion relations and growth under salt stress. NIBGE-301 was more tolerant to salt stress and responsive to potassium sulphate application than NIGBE-158.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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