NISEB JOURNAL Vol. 13, No 1&2, July, 2013
Printed in Nigeria

1595-6938/2013

http://www.nisebjournal.org

Performance of Soybean (*Glycine max* L.) Variety in Salt-treated Soil Environment Following Salicylic Acid Mitigation

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ABSTRACT: The application of salicylic acid (SA) as mitigation for depression of crop performance in saline soil environments has gained good attention from scientists. In this study, the growth performance of *Glycine max* (L.) Merril (variety TGx 1448-2E) plants subjected to salinity treatments for four weeks before salicylic acid application as soil drench was investigated. Sodium chloride concentrations applied to soil were 0mM, 30mM, 50mM, 70mM, and 90mM. These were applied to the soil before and after planting up till four weeks after planting (4WAP) before SA mitigation for another 4 weeks. Plant parameters recorded were germination, plant height, number of leaves per plant, fresh and dry weights, number of fruits formed per plant and chlorophyll content of leaves. Soil physicochemical properties, pH and EC were determined also. The experiment was designed as a completely randomized one. The result indicated that percent germination of *G. max* seeds was affected by salt treatment generally. Nine weeks after planting (9WAP), plant heights were depressed in those plants grown in soils without SA as stress ameliorant. Values obtained for SA mitigated plants were higher than those of unmitigated plants. Differences in plant height recorded were significant (α=0.05). Data obtained for plant parameters in this study indicated the enhancement effects of SA mitigation of salt stress. pH records taken 8WAP indicated very negligible variation and as such the mechanism of amelioration by SA could not be ascribed to changes in soil condition even though application of SA was on the soil. Soil EC values recorded some significant differences (α=0.05). Future study should be able to explain the influence of SA application as soil drench on the partitioning of K, Na and Cl ions in roots and shoots. The study suggests that SA mitigation was by increased chlorophyll contents of plants.

Keywords: Amelioration, *Glycine max* L., mitigation, salinity, soil drench.

Introduction

Biotic and abiotic stresses exert reasonable limitations on yield potentials of field grown crops. Such stresses cause poor growth and development in crops. Salinity, as one of the challenges of irrigation farming, affects soil fertility and limit production. Soil salinity creates osmotic stress, decreasing water availability, uptake of various nutrient ions, changes in cellular ionic balance, dry matter production, fruit development, carbon assimilation and partitioning. An important consequence of salinity stress in plants is the excessive generation of ROS such as the superoxide anion (O_2^-), H_2O_2 and the hydroxyl radicals, particularly in chloroplast and mitochondria (Mittler, 2002). In order to survive under stress conditions, plants are equipped with oxygen radical detoxifying enzymes such as superoxide dismutase,
ascorbate peroxidase, catalase (and antioxidant molecules like ascorbic acid), α-tocopherol and reduced glutathione (Jaleel *et al*. 2007). Plants initially perceive environmental stresses and activate a range of defensive mechanisms (Sticher *et al*., 1997). These mechanisms may also be induced or enhanced by the application of some chemicals to the plants (Raskin, 1995; Rajasekaran and Blake, 1999, Janda *et al*., 1999). These chemicals include calcium chloride ($CaCl_2$) and salicylic acid (SA). Researchers apply these chemicals, $CaCl_2$ and salicylic acid, as foliar and seed treatments, to inducing stress tolerance. In this work, SA was applied as soil drench as mitigation to saline condition. The use of chemicals to mitigate stresses will encourage the maintenance of arable lands and engagement of marginal farmlands with very limited significance. It was important to establish that crop performance following this method of SA application would be significant.
Salicylic acid (o-hydroxybenzoic acid) belongs to a group of phenolic compounds widely distributed in plants and plays an important role in the regulation of plant growth and development (Raskin, 1992; Klessig and Malamy, 1994). Salicylic acid (SA) is an endogenous signal molecule with many physiological functions. Pretreatment of seeds with salicylic acid increases the antioxidant activities of plants, and plays a role in the plant response to adverse environmental conditions such as heavy metals, herbicides, low temperatures and salt stress (Metwally *et al*., 2003; Ananieva *et al*., 2002; Janda *et al*., 1999; Borsani *et al*., 2001). However, its exogenous application to plants generates diverse physiological effects, such as inhibition of dry mass accumulation (Schettel and Blake, 1983), and control of ion uptake and their transport (Harper and Blake, 1981); and accelerated leaf area and dry mass production in corn and soya bean whereas plant height and root length were unaffected (Khan *et al*., 2003). Salicylic acid (SA) has gained attention from researchers because of its ability to induce systemic acquired resistance (SAR) in plants. Salicylic acid (SA)produces a protective effect on plants under the action of stress factors of different abiotic nature (Levent Tuna *et al*., 2007). Authors have also reported positive effect of SA on photosynthesis and plant growth under stress (Gomez *et al*., 1993; Rajasekaran & Blake, 1999).

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Materials and Methods

The seeds of soybean (*Gycine max* L. Merril , variety TGx 1448-2E) were collected from a commercial farmer in Benin City. Test for viability was done using the flotation method. Prior to sowing into soil, the viable seeds were primed in distilled water for one hour. Composite top soil (0 - 15 cm depth) collected from a fallow farm was weighed into experimental pots. Each pot contained three kilogrammes of soil. A total of forty pots were prepared divided into two batches. One batch received salt treatments only and the second batch was given salt treatments for the first four weeks after sowing and thereafter salicylic acid was introduced as amelioration. These salt treatments and salicylic acid were applied as soil drench. Concentrations of salt solution prepared were 0, 30, 50, 70 and 90mM respectively. This was done by dissolving the calculated weight of NaCl salt of respective concentrations in one litre of deionised water. The concentration of salicylic acid applied was 10mM. The different salt treatments were applied to the experimental pots to soak the soil before planting, according to the experimental design. Ten seeds were sown per pot. After planting, application of the salt treatments continued every fourth day, for eight weeks for one batch. Similarly, the other batch received salt treatments every fourth day for four weeks and salicylic acid every fourth day for the next four weeks.

Physicochemical analysis of the composite soil was carried out using standard procedures. Ten grammes (10 g) soil samples were collected from the experimental pots and used for pH and electrical conductivity after eight weeks of growth. A field based and nondestructive procedure was used to estimate the chlorophyll content of leaves using the chlorophyll meter (CCM-200 plus). The chlorophyll meter works on the principle that chlorophyll molecules have distinct optical absorbance and these are exploited to estimate relative chlorophyll concentration in leaf tissues. Two wavelengths are used for absorbance determinations. One wavelength falls within the chlorophyll absorbance range while the other serves to compensate for mechanical differences such as tissue thickness. The meter measures absorbance at both wavelengths and calculates a chlorophyll concentration index (CCI) value that is proportional to the amount of chlorophyll in the leaf tissue. Plant growth parameters employed in this study included germination, plant height, number of leaves per plant, average number of fruits per plant, chlorophyll content, fresh and dry weights. Germination was recorded every day after sowing for two weeks. Plant height and number of leaves were measured every week for nine weeks. Biomass of plants (fresh and dry weights) was also determined after plant harvest. The study was carried out as completely randomized design. Statistical analyses of data were done using the GENSTAT version 8.1 for one- and two-factor analysis of variance, and the comparison of the means, using Student Newman-Keuls test.

Results

The results obtained in the study are shown in Tables 1- 5 and Figures 1-4. The physicochemical analysis of experimental soil before salinity treatment is shown in Table 1. Percent germination of seeds sown in salt-treated soil is shown in Table 2. Germination was not observed in all treatments within the first three days. Percent germination recorded was below 50% in all salt – treated soils. Seed storage technique may be responsible for this observation. Data obtained for plant height are shown in Table 3. There was a general increase in plant height as the growth period increased. The higher values for mean plant height were obtained in salicylic acid ameliorated soils after 9 weeks of growth. Significant differences (α=0.05) were noted between the treatments. Number of leaves counted is shown in Table 5. Data obtained were not significant between treatments. Table 4 shows the pH and EC values recorded for soil samples obtained after some weeks of plant growth. The pH values (between 7 and 8) indicated that the soils provided near neutral condition for plant growth. Electrical conductivity test showed that the soils did not have similar ionic conditions. Significant differences (α=0.05) were observed for the treated soils. Fresh and dry weights of plants are shown in Figures 1 and 2 respectively. Significant differences (α=0.05) between treated plants were observed for these data. Figure 3 shows the average number of fruits formed by plants. The highest mean value obtained was in plants grown in 30mM NaCl-treated soil. Figure 4 shows the chlorophyll content index recorded for plants. Generally, higher CCI values were observed for plants grown in soils ameliorated with salicylic acid. Differences in CCI values obtained between leaves of plants grown in treated soils were also significant (α=0.05).

Table 1: Physico-chemical analysis of soil obtained from the field before salinity treatment

pH	**Organic carbon (%)**	**Total Nitrogen (%)**	**Average Phosphorus (mg/Kg)**	**Calcium (mg/Kg)**	**Magnesium (mg/Kg)**	**Potassium (mg/Kg)**	**Sodium (mg/Kg)**	**Particle size analysis (%)**		
								sand	Silt	clay
7.5	0.82	0.06	4.11	4.86	0.64	0.21	0.10	88	2	10

Each value is an average of duplicate measurements

Table 2: Percent germination (%) of seeds of *Glycine max* L. sown in NaCl-treated soils

NaCl salt concentration (mM)	**Percent germination (days after planting)**				
	3	**6**	**9**	**12**	**14**
0	0.00[a]	18.75[a] ± 5.49	31.25[a] ± 5.81	33.75[a] ± 5.96	33.75[a] ± 5.96
30	6.50[a] ± 1.64	15.00[a] ± 1.89	21.25[a] ± 2.95	21.25[a] ± 2.95	21.25[a] ± 2.95
50	0.00[a]	12.50[a] ± 4.91	23.75[a] ± 6.80	27.50[a] ± 7.01	27.50[a] ± 7.01
70	0.00[a]	16.25[a]±3.75	21.25[a] ± 4.79	23.75[a] ± 4.20	25.00[a] ± 4.63
90	4.25[a] ± 1.25	15.00[a] ± 2.68	22.50[a] ± 3.13	22.50[a] ± 3.13	22.50[a] ± 3.13

Figures=mean ±S.E.; means in the same column bearing similar alphabet are not significantly different using Student Newman-Keuls test

Table 3: Plant height (cm) obtained for *Glycine max* plants grown in NaCl-treated and NaCl +salicylic acid treated soils

Salt applied	Concentration (mM)	Plant height (cm) at different weeks after treatment						
		Week 3	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 9**
NaCl	0	17.12^{a} ± 1.31	18.38^{a} ± 1.74	22.77^{a} ± 3.27	26.23^{a} ± 2.53	29.45^{a,b,c} ± 2.42	33.85^{a,b} ± 1.40	38.60^{a,b,c} ± 0.93
	30	18.15^{a} ± 1.65	18.93^{a} ± 1.95	20.62^{a} ± 1.00	23.50^{a} ± 0.91	25.75^{a,b,c} ± 0.39	34.23^{a,b} ± 4.38	38.60^{a,b,c} ± 2.02
	50	9.43^{a} ± 2.11	10.13^{a} ± 3.97	11.88^{a}± 4.45	12.90^{a} ± 4.96	14.27^{c} ± 9.51	15.38^{b} ± 5.83	16.45^{c} ± 7.71
	70	13.78^{a} ± 9.20	13.68^{a} ± 9.12	15.10^{a} ± 10.08	18.40^{a} ±12.29	22.57^{a,b,c} ± 15.15	27.23^{a,b} ± 8.22	29.98^{a,b,c} ± 20.00
	90	11.50^{a} ± 7.90	11.93^{a} ± 8.20	12.68^{a} ± 8.67	14.55^{a} ± 9.99	16.25^{b,c} ± 11.21	19.75^{b} ± 13.92	23.50^{b,c} ± 16.55
NaCl + Salicylic acid	0	14.30^{a} ± 2.59	16.00^{a} ± 2.04	17.35^{a} ± 2.80	29.15^{a} ± 0.50	37.33^{a,b} ± 0.67	45.62^{a} ± 1.78	53.52^{a} ± 1.04
	30	18.87^{a} ± 0.90	19.65^{a} ± 1.47	20.45^{a}± 1.39	29.95^{a} ± 0.66	39.28^{a} ± 0.68	48.45^{a} ± 0.42	55.55^{a} ± 0.79
	50	13.30^{a} ± 8.90	14.10^{a} ± 9.43	15.82^{a} ± 10.79	22.35^{a}± 14.93	29.20^{a,b,c} ± 19.48	35.60^{a,b}±23.75	40.60^{a,b,c} ± 27.08
	70	18.70^{a} ± 0.62	19.37^{a} ± 0.93	22.00^{a} ± 1.12	29.45^{a} ± 0.84	37.85^{a,b} ± 1.59	46.22^{a} ± 2.17	52.25^{a} ± 2.89
	90	15.60^{a} ± 3.28	16.45^{a} ± 3.50	18.15^{a} ± 3.86	27.12^{a} ± 2.15	33.10^{a,b,c} ± 2.43	38.95^{a,b} ± 3.50	47.55^{a,b} ± 2.34

Figures = mean ± S.D., means bearing the similar alphabets in the same column are not significantly different using Student- Newman- Keuls test

Table 4: pH and EC values obtained for soil samples taken after eight weeks of *Glycine max* plant growth

Salt applied	Concentration (mM)	pH	Electrical conductivity (μS)
NaCl	0	7.700^{a} ±0.100	64.64^{a} ±20.18
	30	7.767^{a} ±0.058	45.25^{a,b}±5.60
	50	7.767^{a} ±0.058	45.25^{a,b}±5.60
	70	7.733^{a} ±0.058	38.78^{b} ±0.00
	90	7.733^{a} ±0.058	45.25^{a,b}±5.60
NaCl + Salicylic acid	0	7.833^{a} ±0.115	51.71^{a,b}±5.59
	30	7.633^{a} ±0.153	42.01^{b}±5.60
	50	7.733^{a} ±0.153	48.48^{a,b} ±0.00
	70	7.800^{a} ±0.100	48.48^{a,b} ±9.70
	90	7.833^{a} ±0.058	38.98^{b} ±0.73

Figures=mean ± S.D.; means in the same column bearing similar alphabet are not significantly different using Student Newman-Keuls test

Table 5: Number of leaves per plant obtained for *Glycine max* plants grown in NaCl-treated and NaCl + salicylic acid treated soils

Salt applied	Concentration (mM)	Number of leaves per plant at different weeks						
		3	**4**	**5**	**6**	**7**	**8**	**9**
NaCl	0	8.00^{a} ± 0.00	15.25^{a}± 0.50	19.25^{a} ± 0.50	22.00^{a}± 4.00	26.00^{a} ± 2.71	27.00^{a} ± 2.71	28.00^{a} ± 3.22
	30	9.25^{a} ± 3.20	15.00^{a} ± 2.16	18.25^{a} ± 1.89	20.50^{a}± 2.65	24.25^{a} ± 3.77	27.75^{a} ± 6.80	28.75^{a} ± 7.21
	50	5.50^{a} ± 4.12	9.50^{a} ± 6.60	11.75^{a}± 8.06	14.25^{a}± 9.60	15.00^{a} ± 10.29	1 5.50^{a} ±10.85	16.50^{a} ± 9.53
	70	7.50^{a} ± 5.01	11.50^{a} ± 7.85	13.50^{a}± 9.43	15.00^{a}± 10.23	17.00^{a} ± 11.03	19.25^{a} ± 13.09	21.25^{a} ± 10.02
	90	7.25^{a} ± 4.92	10.50^{a} ± 7.00	13.00^{a}± 8.68	14.75^{a}± 9.84	16.75^{a} ± 11.47	18.50^{a} ± 13.18	20.50^{a} ± 9.23
NaCl + Salicylic acid	0	9.25^{a} ± 0.50	14.25^{a} ± 1.71	17.50^{a} ± 2.38	21.25^{a} ± 4.03	23.50^{a} ± 5.20	28.00^{a} ± 6.88	29.25^{a} ± 6.20
	30	10.25^{a} ± 0.96	14.25^{a} ± 0.96	17.00^{a} ± 1.83	20.00^{a} ± 3.56	23.00^{a} ± 5.48	25.50^{a} ± 2.38	26.25^{a} ± 5.36
	50	7.25^{a} ± 4.92	12.25^{a} ± 8.18	14.75^{a} ± 4.84	16.00^{a} ±10.80	18.00^{a} ± 12.19	19.75^{a} ± 13.38	21.75^{a} ± 11.41
	70	11.75^{a} ± 0.50	16.00^{a} ± 1.49	19.25^{a} ± 1.71	24.00^{a} ± 2.94	28.50^{a} ± 3.70	32.75^{a} ± 4.65	33.25^{a} ± 4.88
	90	10.25^{a} ± 0.96	13.50^{a} ± 2.65	17.25^{a} ± 2.22	21.00^{a} ± 4.32	24.25^{a} ± 4.99	28.75^{a} ± 5.25	30.25^{a} ± 5.22

Figures = mean ± S.D., means bearing the similar alphabets in the same column are not significantly different using Student- Newman- Keuls test

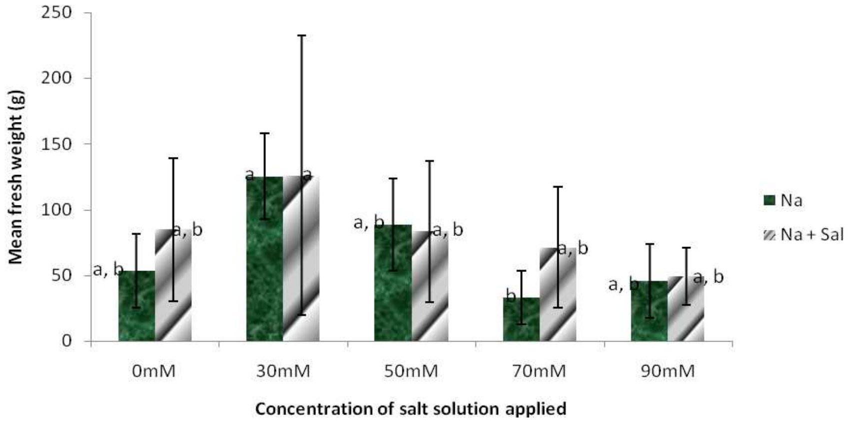

Figure 1: **Mean fresh weight of *Glycine max* plants obtained after eleven weeks of growth in salt-treated and salicylic acid ameliorated soils (error bars indicate standard deviation).**

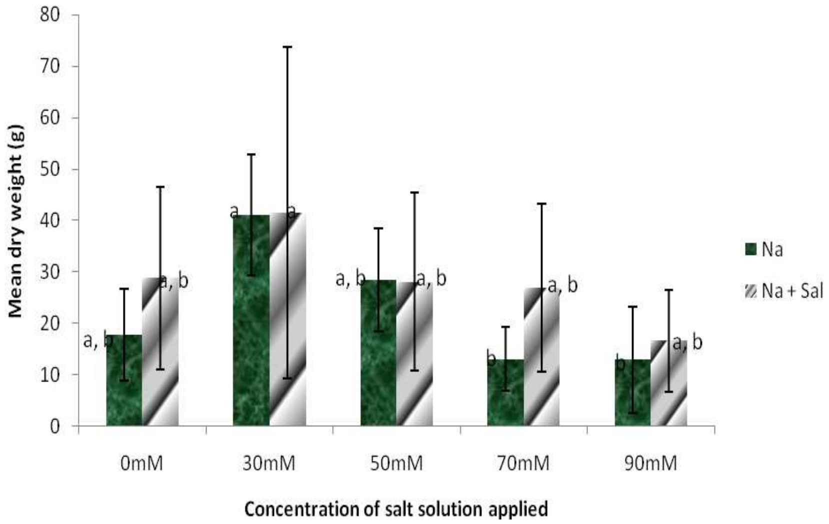

Figure 2: **Mean dry weight of *Glycine max* plants obtained after eleven weeks of growth in salt-treated and salicylic acid ameliorated soils (error bars indicate standard deviation).**

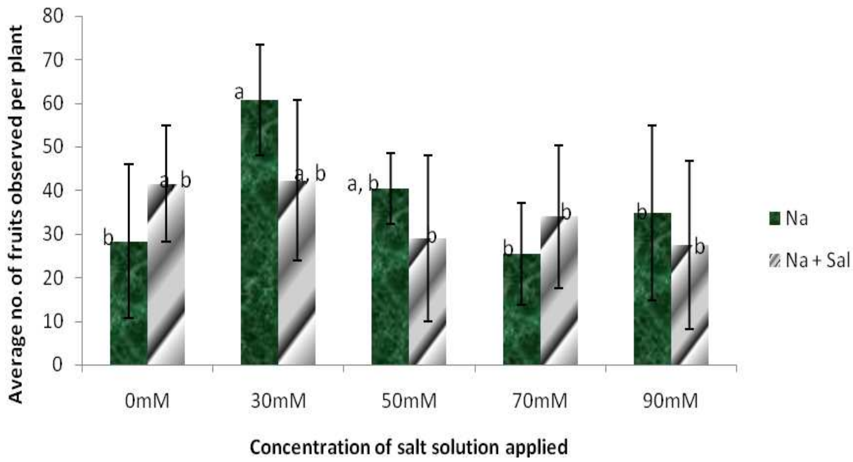

Figure 3: **Average number of fruits of *Glycine max* plants obtained after eleven weeks of growth in salt-treated and salicylic acid ameliorated soils (error bars indicate standard deviation).**

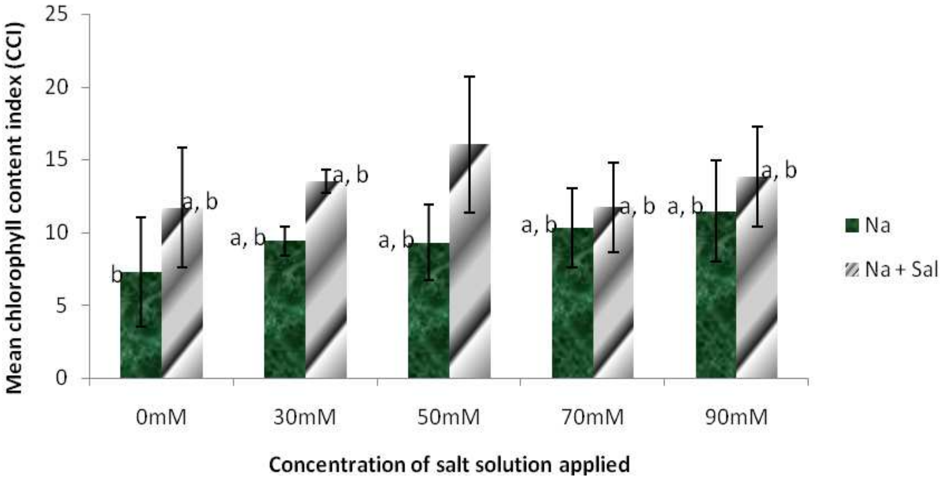

Figure 4: Mean chlorophyll content index (CCI) of *Glycine max* plants obtained after eleven weeks of growth in salt-treated and salicylic acid ameliorated soils (error bars indicate standard deviation).

Discussion

Developments of crop plants exposed to salt treatments have often been reported as inhibitory. To reverse such inhibition, the use of chemicals ($CaCl_2$ and SA) have been suggested and reported in many plants (Metwally *et al*., 2003; Ananieva *et al*., 2002; Janda *et al*., 1999; Borsani *et al*., 2001), though their applications are on the plants directly. In this study, SA was applied as soil drench, where it is expected to ameliorate the inhibitory effect created by the salt treatments. This amelioration was measured as good growth performance. It has been suggested that signaling compounds that are able to reduce the effect of stresses on plants and increase productivity are important to restoration of natural ecosystems as well as agricultural, horticultural and forestry production systems around the world (Sharareh *et al*., 2009). Foliar application of SA accelerated leaf area and dry mass production in corn and soybean (Khan *et al*., 2003). The SA reduced the negative effects of salinity stress on shoot and root dry weights in *Rosmarinus officinalis* (Sharareh *et al*., 2009). The SA induced protection of photosynthetic apparatus in plants under stress (Rajasekaran and Blake, 1999). The positive effect of SA on plants under stress was attributed to its ability to activate the synthesis of carotenoids, xanthophylls and the rate of de-epoxidation (Moharekar *et al*., 2003). In addition, SA has an enhancing effect on photosynthetic capacity by stimulating the activity of RUBISCO in plants under stress and induction of antioxidant defenses (Cornelia *et al*., 2010).

Kaydan *et al*. (2007) reported higher percentage emergence for wheat seeds pretreated with SA grown under saline condition as compared to non-treated seeds. Shakirova *et al*. (2003), El-Tayeb (2005) and Afzal *et al*. (2005) observed that SA pretreatment of seeds increases emergence percentage under salinity conditions. In this study, the germination of *Glycine max* seeds was below 50% in both salt treated and untreated soils. Though the highest mean percent germination of *G. max* was in the untreated soil, the difference in germination between salt treated and untreated was insignificant (Table 2). The SA was not applied to soil within the first 14 days after sowing. The germination result showed that salt treatment depressed seed emergence. The amelioration and enhancement effects of SA were observed in *G. max* plants after six weeks of growth. The SA application started after four weeks of plant growth in saline soil (Table 3). The mean values obtained for plant height nine weeks after planting (9WAP) for example, exhibited amelioration and enhancement effects. Under control soil condition, plants grown in SA-treated soil produced mean plant height approximately 140% of that for plants grown in soil not treated with SA. This is enhancement effect of SA. Under salt-treated soil condition, for example, 90mM concentration, mean plant heights (9 WAP) were 23.50cm and 47.55cm respectively for plants grown in SA-untreated and SA-treated soils. One can clearly observed the amelioration and enhancement effects of SA. Similarly, plants grown in other concentrations of salt treatment exhibited this amelioration and enhancement effects. For growth parameter like number of leaves per plant (Table 5), the enhancement and ameliorating effects of SA were indicated in plants grown on 70mM and 90mM salt-treated soils. The differences between plants grown in SA ameliorated soils and SA-untreated soils were however insignificant.

Khodary (2004) reported that SA increased fresh and dry weights of shoots and roots of salt stressed maize plants. Fresh and dry weights of plants were also improved following SA – application in soil at 70mM and 90mM salt concentrations in soil (Figure 1 and 2). Kaydan *et al*. (2007) stated that salinity decreased dry weights of shoots and roots while SA improved dry weights of seedlings. The number of fruits formed per plant was observed not to follow a particular pattern. The striking observation was that the application of SA to 0mM and 70mM salt treated soils, made plants grown in them to produce more fruits than in plants grown in SA untreated soil (Figure 3). Generally, the sizes of the pods formed by plants grown in SA-applied soils were larger than those of plants grown only in salt treated soil. The data are not included here. This study suggests that SA effects on fruits formed were on the sizes rather than on the number. The differences in the number of fruits formed per plant between plants grown in salt treated and SA ameliorated soils were generally not significant (α=0.05). Chlorophyll content index (CCI) measured, showed that plants grown in SA ameliorated soils consistently gave higher chlorophyll contents than those grown in SA untreated soils (Figure 4). The differences were significant (α=0.05). Kaydan *et al*. (2007) observed that under the influence of salinity, the photosynthetic pigments generally decreased in wheat seedlings. On the other hand, SA treatments increased pigment content of the plants under saline and non-saline conditions. El-Tayeb (2005) stated that in barley, chlorophyll a, b and carotenoids decreased significantly in NaCl-treated plants in comparison with control. Zhou *et al*. (1999) reported that photosynthetic pigments were increased in corn following SA application. Similarly, Khan *et al*. (2003) showed that SA increased photosynthetic rate in corn and soya bean. The implication of higher photosynthetic pigments (like chlorophyll) in plants grown in saline soil ameliorated with SA should be directly recorded as increased fresh and dry weights of the plants. In this study, this higher CCI values did not reflect directly as higher fresh and dry weights of plants grown in SA ameliorated soil. This study has shown that applying SA as soil drench mitigated the negative effects of salinity to plants. The study suggests that SA mitigation was by increased chlorophyll contents of plants. This has implication in reduction of oxidative damages (Raskin, 1992, 1995; Mittler, 2002; Levent Tuna *et al*., 2007).The soil condition measured 8 WAP (Table 4) indicated no significant changes in pH of soil and all soil samples were slightly alkaline. The range of pH was between 7.70 and 7.83 units for both SA ameliorated and SA untreated soils. Soil EC values recorded some significant differences (α=0.05). pH records taken 8 WAP indicated very negligible variations and as such the mechanism of amelioration by SA could not be ascribed to soil changes, even though the application of SA was on the soil. The study has shown that SA can ameliorate salinity stress (and even significantly stimulated growth 9 WAP) by applying it as soil drench and the suggested mechanism is by increase in chlorophyll content of plant leaves. Future study should be able to explain the influence of SA application as soil drench on the partitioning of K, Na and Cl ions in roots and shoots.

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