Journal of Scientific Research and Development 2 (5): 118-121, 2015
Available online at www.jsrad.org
ISSN 1115-7569

Effect of salt stress on Seedlings growth and ions homeostasis of soybean (*Glysin max)* Cultivars

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Abstract: This experiment was carried out in Islamic Azad university (Shoushtar Branch) for investigate effect of salt stress on seedling growth of soybean cultivars. The soybean cultivars (Hill, Klark and Williams) obtained from Iran seed and plant institute, Karaj, Iran. The experimental design was 2 factorial, arranged in completely randomized design with 4 replication. First factor was soybean cultivars and second factor was salt stress levels (0, 4, 8, 12 and 16ds/m NaCl solution). These salinity treatments named S0, S1, S2, S3 and S4. Results indicated salt stress led to decrease germination, seedling fresh weight, seedling growth and seedling K^+ percentage in dry matter but increase Mean germination time and seedling Na^+ percentage. Results showed cultivar Hill K^+/Na^+ ratio was higher compared Williams and Klark. Results indicated under salt stress seedling growth of cultivar Hill was better compared other soybean cultivars.

Key words: Soybean; Salt stress; K^+/Na^+

1. Introduction

Soil and water salinity is recognized as the most important problem involved in crop plant establishment and growth. Transpiration and evaporation from the soil surface, low quality of irrigation water and lack of proper drainage are major causes of area land salinity leading to crop loss. World salty area land is thought to be 950 million hectar. Thus, screening of salt tolerant crop cultivars is of crucial importance (Okcu et al., 2005).

Soybean is a strategic crop plant grown to obtain edible oil and forage. High sensitivity to soil and water salinity is one of the biggest problems with soybean crop. Results have indicated that salinity affects growth and development of plants through osmotic and ionic stresses. Because of accumulated salts in soil under salt stress condition plant wilts apparently while soil salts such as Na^+ and Cl^- disrupt normal growth and development of plant (Khajeh-Hosseini et al., 2003; Farhoudi et al., 2007; Letly ,1993; Makki and Asif, 1987). Chen et al. (1996) reported salt stress led to decreased seedling growth of soybean cultivars. They also reported a positive correlation between Na^+ and Cl^- content of soybean seedlings and susceptibility level to salinity. Roger et al. (1994) suggested salt stress decrease germination percentage of white clover. Cultivars accumulating Na^+ and Cl^- in roots inhibited transportation of these ions to air organs were more resistant to salt stress circumstances, salinity led to decreased dry weight of shoot and root. Farhoudi et al. (2007) found salt tolerance of canola cultivars have a direct relationship with Na^+/K^+ ratio so that the ratio increased with the increase of salinity level but less increase is observed in tolerant cultivars, They concluded that Na^+/K^+ ratio can be a measure of salt stress tolerance.

The aim of this study was to evaluate the effects of salt stress on germination, seedling growth and ions homeostasis of three soybean cultivars.

2. Materials and Methods

The research was carried out to evolution effect of salt on three soybean (Glysin max) cultivars (Williams, Hill and Clark) growth at germination stage in Islamic Azad University, Shoushtar Branch, in 2010. Salt stress treatments were applied using NaCl solutions with EC values of 4, 8, 12 and 16ds/m. These solutions were called S1, S2, S3 and S4, respectively. Distilled water used for control (S0).

Required amount of each solid salt for preparing one liter salt solution was calculated through the following formula (Al-Ansari, 2002):

$$TDS\ (mg/lit) = EC \times 640$$

Where: TDS= total soluble solid salt amount (mg/lit)

EC= given electro conductivity value (ds/m)

Then EC value of each solution was read by means of EC meter and reached the desirable EC with addition of solid salt or distilled water.

Treatments were assessed in a factorial experiment based on a completely randomized design at four replications. 25 similar sized soybean seeds for each cultivar were grown in a Petri dish. Each Petri dish was considered as a replication.

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Seeds were incubated under 16hr light, 8hr dark, 70% humidity and 24ºC ±1. Seed count was carried out 24hr after applying treatments and continued for 10 days. Assessed traits were Germination percentage (GP), Mean Germination Time (MGT), root and shoot length, seedling fresh weight, Na^+ and K^+ contents of the seedling shoot and root. For the determination of Na+ and K+ in the seedling, 1mg dry mater was placed in test tubes containing 20 ml distilled deionized water, and heated in a boiling water bath for 1 h. The tubes were then autoclaved at 120°C for 20 min and cooled. The Na^+ and K^+ content in 15 time's diluted extract were determined by atomic absorption spectrophotometry (Al-Ansari, 2002).

Mean germination time (MGT) was calculated using Schilin et al. (2003) method:

$$MGT = \frac{\sum f_i x_i}{N}$$

fi: Day number during germination period

ni: Number of germinated seeds per day

N: Sum of germinated seeds

Experimental data were statistically analyzed using MSTATC software and mean values were compared with Duncan statistical test.

3. Results and discussions

Data variance analysis indicated GP and MGT, root and shoot length and Na^+ and K^+ contents were affected by salt stress, cultivar and salinity-cultivar interaction at 0.05 statistical level.

3.1. Germination percentage (GP) and Mean Germination Time (MGT)

Results showed the most germination percentage obtain in control treatment in all cultivars. Increase salt stress level, decrease soybean germination percentage (Table 1). Under highest salinity level, Hill cultivar had more germination percentage (45%) compared other soybean cultivars. Results showed increased salinity level decreased germination percentage of three soybean cultivars but lowest decrease was observed in cultivar Hill compared other soybean cultivars. Results showed salt stress increase MGT of soybean cultivars. Under S4 salinity level, Hill cultivar show lowest MGT (Table 1). Khajeh-Hosseini et al. (2003) suggested salt stress decrease the germination rate and germination percentage of soybean. They recognized negative osmotic and ionic effects of Na^+ on seedling growth and development as the cause of this observation. Results indicate with the increase of salinity level, cultivar Hill germinates more quickly and seeds germinate within shorter period of time in comparison to cultivars Williams and Clark. Chippa and Rana (1995) reported irrigation water with EC 14ds/m decreased the seed germination level of soybean significantly.

3.2. Seedling fresh weight and seedling growth

Salt stress decreased seedling fresh weight of soybean cultivars but seedling fresh weight of cultivar Hill was significantly higher than Williams and Clark seedling fresh weight. Similar results have also been obtained with soybean. Above researches have emphasized Na^+/Cl^- toxicity and its effects on seedling growth as the main cause of weight loss. Increasing salinity level caused significant decrease in soybean seedling biomass. Enhanced transpiration is thought to be the major cause of such weight loss under the salt stress (Bajji et al., 2002). Ashraf and Mecneilly (2004) suggested harmful ion like Na^+ increased under salinity stress and decrease growth of canola.

Results showed root and shoot length significantly decreased with the increase of salinity level so root and shoot were not growth in cultivars Williams and Clark highest salinity level (Table1). The longest root and shoot were observed in S0 and S1 treatments in three soybean cultivars. Compared with S0 treatment, S2 treatment significantly decreased root and shoot length in cultivars Williams and Clark but only decreased root length and did not significantly affect shoot length in cultivar Hill. Increase of salinity level to S3 and S4 treatments significantly decreased root and shoot length in all three cultivars. Root and shoot length of cultivar Hill were significantly higher than those of Williams and Clark in S3 treatment. But no significant differences in shoot length were observed among all three cultivars in S4 treatment, however, root length of cultivar Hill were significantly higher than those of Williams and Clark in S4 treatment. Researchers reported that accumulation of ions in plant growth environment causes osmotic and Pseudo-drought stress leading to decrease of water absorption by plant tissues. Decrease of tissue water content results in reduction of cellular growth and development. Therefore, restriction of water absorption and its consequences for cellular growth and development is one of the most important causes of decreased growth of stem and root. Root cells have a much less turger threshold pressure than that of stem cells thus root growth is more than stem growth under salt and drought stresses. Therefore, root is significantly less affected by salt stress in comparison to stem (Abd-Ala et al., 1998; Ashraf, 2001; Morant et al., 2004).

3.3. Na+ and K+ content of Root and Shoot

Results showed salt stress increase soybean seedling Na^+ content but decrease K^+ content. Na^+ content of seedling tissues of cultivar Hill was significantly lowest than those of two other cultivars in all treatments except at S1 level. Shoot Na^+ content of cultivar Hill was significantly less than Na^+ content of cultivar Clark but it was not significantly different with that of cultivar Williams in some applied treatments (Table 2).

According to the results, it can be concluded that cultivar Hill is salt stress tolerant due to its less Na^+ absorption and more Na^+ accumulation in root compared with two other studied cultivars. Additionally, more K^+ absorption gave cultivar Hill the advantage of well responding to salt stress during germination and seedling establishment stages in comparison to cultivar Clark. Although Na^+ contents measured for both cultivars, Hill and Williams were similar, cultivar Hill may have the advantage of salt stress tolerance due to its less Na^+ absorption compared with cultivar Williams (Table 2). K^+ / Na^+ ratio of cultivar Hill was more than cultivar Williams (except at S0 and S1 levels) and the latter was more than cultivar Clark which in turn plays a great role in making cultivar Williams a salt stress tolerant one (Fig. 1).

Table 1: Effect of salt stress on seedling growth of soybean cultivars[1]

Cul*	Salt stress	GP (%)	MGT (day)	Root length (mm)	Shoot length (mm)	Seedling fresh weight (gr)
W	S0	95 a	2.6 a	110 a	98 a	1.17 a
	S1	96 a	2.6 a	111 a	98 a	1.18 a
	S2	92 b	2.9 c	75 b	54 c	0.73 c
	S3	65 b	3.8 d	30 d	20 e	0.57 d
	S4	22 e	4.9 f	19 e	9 f	0.35 f
C	S0	96 a	2.6 a	108 a	97 a	1.19 a
	S1	96 a	2.4 a	109 a	96 a	1.17 a
	S2	93 a	2.4 d	79 b	57 c	0.72 c
	S3	68 c	3.5 c	31 d	19 e	0.51 d
	S4	28 e	4.3 e	18 e	8 f	0.31 f
H	S0	96 a	2.5 a	116 a	95 a	1.13 a
	S1	96 a	2.4 a	112 a	97 a	1.14 a
	S2	92 a	2.9 b	108 a	69 b	0.89 b
	S3	79 b	3.2 b	41 c	29 d	0.71 c
	S4	45 d	3.7 b	18 e	14 e	0.46 e

1: Means followed by the same letter(s) are not significantly different at P = 0.01 according to Duncan test
*: W;Williams, C;Clark, H;Hill

Table 2: Effect of salt stress on ion percentage of soybean tissue[1]

Cul*	Salt stress	Seedling K (%)	Seedling Na (%)	Shoot K^+ (%)	Root K^+ (%)	shoot Na^+ (%)	Root Na^+ (%)
W	S0	10.8 a	3.0 a	6.4 a	4.5 a	0.7 a	2.3 a
	S1	9.8 a	3.7 a	5.9 a	3.9 b	1.2 a	2.5 a
	S2	7.4 b	5.6 c	4.2 b	3.2 c	1.9 b	3.7 b
	S3	5.1 c	8.3 e	3.7 c	1.4 d	3.1 d	5.2 d
	S4	2.9 g	9.8 d	2.5 e	0.4 f	3.7 e	6.1 f
C	S0	9.8 a	3.5 a	6.1 a	3.7 b	0.9 a	2.6 a
	S1	8.4 b	4.1 a	5.3 a	3.1 c	1.1 a	3.0 a
	S2	5.2 c	6.3 d	3.6 c	1.6 d	2.1 b	4.2 c
	S3	4.1 e	9.4 f	3.2 d	0.9 e	3.4 d	5.7 d
	S4	2.4 h	10.8 d	1.9 f	0.5 f	3.7 e	6.9 f
H	S0	10.4 a	3.2 a	6.1 a	4.3 a	0.8 a	2.4 a
	S1	9.6 a	3.9 a	5.7 a	3.9 d	1.0 a	2.9 a
	S2	7.5 b	4.8 b	4.4 b	3.1 c	1.2 a	3.6 b
	S3	5.7 d	6.9 d	3.9 c	1.8 d	2.2 c	4.7 e
	S4	3.4 f	8.3 e	2.3 e	1.1 e	3.2 d	5.1 e

1: Means followed by the same letter(s) are not significantly different at P = 0.01 according to Duncan test
*: W;Williams, C;Clark, H;Hill

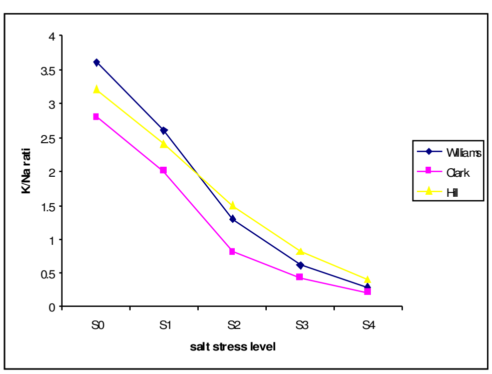

Fig. 1: Efeect of salt stress on K/Na ratio of soybean cultivars

Having studied soybean, wheat, maize and cotton, Chen et al. (1996) suggested Na^+ concentration increased with the increase in salinity level in all of these plants. Researchers suggested K^+ concentration observed in salt stress tolerant soybean plants were more than susceptible cultivars led to decreased Na^+ toxicity. Morant et al. (2004) found K^+ / Na^+ ratio was higher in salt tolerance of Triticale cultivars. Increased Na^+ content led to decrease in seed germination level and seedling fresh weight in such plants (Munns, 2002).

Salinity stress companied by increasing soil ions concentration and charging the ions balance cause to decreasing the germinating and plant growth rate. The results of this investigation showed that salinity decreased the germinating percentage, seedling fresh weight, shoot and root length of soybean cultivars. Overall by increasing the salinity level, potassium content of plant tissues decreased, however sodium content of plant tissue increased. These results have conformity with the Chippa and Rana results (1995). There is a relationship between potassium decreasing and sodium increasing in seedling tissue with sensivety to salinity. Sodium affected the cell membrane permeability, deconstructed the cell membrane and destroyed the selectivity property (Munns, 2002). Other investigations demonstrated that resistant plants to salinity not only have the lower sodium – potassium ratio in compare of sensitive plants to salinity, but also deposited higher sodium in root tissue and therefore it inhibits to sodium transmit to shoot tissue, and also inhibits of their damage. The results of this study showed cultivar Hill has higher tolerance to salinity in compare of cultivars Clark and Williams. At the other side it's related to absorption and distribution of ions in shoots and root tissue in cultivar Hill. In spite of cultivar Hill resistance to salinity, it can't be recommended to cultivar Hill species in salt ness situation absolutely, and therefore we propose to investigate the different growth and development steps and performance of these spices in salinity situation and finally with considering all aspects, in can be introduce the best cultivar(s).

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