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THE EVALUATION OF SEED GERMINATION ON 12 SOYBEAN CULTIVARS UNDER DIFFERENT SALINITY STRESS LEVELS

ABSTRACT

In order to study effects of saline water on seed germination of various soybean cultivars, an experiment was conducted using completely randomized design with factorial method and three replications in 2012.

The factors were salinity stress levels (A) (0, 30, 60 and 90) mM NaCl, soybean cultivars (B) (Including 12 seed cultivars of II and III maturity groups). Five traits including seed germination, seed vigor, dry weight of radical and, plumule and biologic yield were studied. The results of analysis of variance showed significant differences between cultivars on studied traits at 1 % level of significance. The highest biologic yield belonged to Hy-1 and Steel under 90 mM. It was belong to M9, Clark and Union under 60 mM and belonged to Hy-1, Halcor and Union under 30 mM.

The results of comparison means using Duncan's method showed L17 and Steel not only had the high seed germination and vigor but also they had dry weight of radicles and biologic yield than others. It was identified although Union, Elgine and Williams were in second grade on seed germination and vigor but they had considerable growth and dry weight. Based on display scatter plot with two STI and GMP indices, L17 and Elgine were placed in A region of plot under 30 mM NaCl, while they were in B region under 60 and 90 mM salinity stress. The cultivars such as Union and Hy-1 were in C region of mentioned scatter plot.

Key words: environmental stress, saline water, seed characters, soybean

Abbreviation: STI (Stress Tolerance Index), GMP (Geometric Mean Production)

INTRODUCTION:

The soybean (US) or soya bean (UK, AU) (*Glycine max* L.) is a species of legume that growing in East Asia. This plant is classified as an oilseed (FAO). Stress is

non-ordinarily phenomenon in physiological process that it is occur of combination of biologic and environmental effects (Levitt, 1980). Salinity is one of factors which damages to cell plants and inhibits of growing them. Its damage is due to different type of oxygen activation radicals that toxic and activator for their cells (Jiang and Zha, 2001). It is one of factors so that decrease legumes yields in arid and semi arid zone. It is estimated about 340 million hectares of farm lands were faced with salinity (Arzani, 2001). Based on the United Nations Environment Programs, It was estimated that approximately 20 % of agricultural lands and 50 % of croplands were settled in salinity regions in the world (Flowers and Yeo, 1995). Soil salinity is a major constraint to food production because it limits crop yield and restricts use of land previously uncultivated (Shuji Yokoi et al, 2002). The most ions of saline soils are Na^+ and Cl^- that affects on cell membrane via interaction competition and selectable ion penetration and disorder on absorption macro and micro elements (Francisco *et al.*, 2002; Grattan and Grieve, 1992). The extensive genetic diversity for salt tolerance was studied on plant taxa and distributed on other numerous genera (Flowers *et al.*, 1986; Greenway and Munns, 1980). The plant response to salinity are consists of many processes such as cellular hyper osmolarity and ion disequilibrium that must be function in coordination to salinity effects. Plant responses are either avoiding or tolerating to salt stress. The plants are either dormant during the salt episode or they must be adjust their cells to tolerate the salinity effects (Bohnert *et al.*, 1995). Salinity stress effect on soybean via toxicity of Cl^- than Na^+ . The susceptibility of seedling soybeans in *Glycin max* L. is more than *Glycin soja* and salinity decreased chlorophyll, photo synthesis and assimilation in soybean (Luo *et al.*, 2005). It was reported that root hairs were wrinkled under increasing salt. The elasticity of root hairs was decreased under 1% NaCl density and they were wrinkled under 1.5 % of salinity condition (Zahran and Sprent, 1986). Dry mass of root and stem of soybean was decreased under 80 mili/mol NaCl and it was lass in tolerant cultivars than others (Velagaleti *et al.*, 1990). Based on reports dry mass of whole bush was decreased under salinity (Ikeda, 1994; Wang and Shannon, 1999; Basra and Basra, 1997). Salinity is known for its depressing effects on germination percentage, length of shoot and root, fresh and dry weight of shoot and root. Its effects are either completely inhibits germination at higher levels or induces a state of dormancy at lower levels (Khan and Ungar, 1997). In a research, the effects of salinity on six soybean cultivars on germination stage under. 0, 3, 6, 9, 12 and 15 $\text{dS} \times \text{m}^{-1}$ NaCl were examined. The results showed that soybean cultivars significantly varied in averages of final germination percentage, mean germination time, germination index, vigor index and energy of germination. Results showed that germination percentage, germination index, and seedling vigor index were decreased under salinity levels from 0 to 15 $\text{dS} \times \text{m}^{-1}$ significantly (Kandil *et al.*, 2015). Khajeh-Hosseini *et al.* (2002) reported that salinity had negative effects on germination characters. Germination decreased under Nacl concentration of 330 mM Nacl (81% germination) while above at 420 mM NaCl, it is only 40% and at 500 mM NaCl there was no germination. Based on this study, soybean seeds were

more tolerance to salinity during germination than in the seedling phase. Ahmadvand *et al.* (2012) reported that increasing salinity levels from 0, 4, to 8 dS $\times$ m⁻¹ lead to decreasing final germination percentage. Seed vigor includes all of the characters that can cause to rapid and uniform seedling emergence under wide range of environmental condition after seed planting (Copeland and McDonald, 1985). Based on reports, Seed size is one of the important yield components which has effect role on cultivar adaptation to different environmental condition. It is effective on seed vigor (Morrison and Xue, 2007). Tobe *et al.* (1999) reported the derived toxicity of saline water is a cause for decreasing radicle in various plant species. Ten sesame cultivars were examined under (0, 5.3, 8.5, 12.05, 14.65 and 18.45 dS $\times$ m⁻¹) of salinity and it was resulted germination and seedling growth were strongly inhibited under 12.05 dS $\times$ m⁻¹. (Bahrami and Razmjoo, 2012).

The identification of the best cultivars about germination and the other seed characters and demonstration tolerant them under salinity stress levels and so identification the threshold of salinity tolerance in soybean in seedling stage were the objectives of this research.

MATERIALS AND METHODS

Experimental design and treatments

This experiment was conducted using completely randomized design with factorial method in seed technology laboratory of Islamic Azad University (Kermanshah branch) in 2012. A factor was included four salinity stress as (0, 30, 60 and 90) mM NaCl and B factor included 12 seed cultivars of soybeans that belonged to II and III maturity group.

The study method

At the first, the seeds were disinfected with sodium hypochlorite 25% (containing 5 % activated chloride), then they were treated in potassium nitrate soluble (2% in density) for raising dormancy. The bed cultures were filter paper that disinfected in sodium hypochlorite 25% (containing 5% activated chloride) under two stages washing with distilled water. They were placed in the Petri dishes with ten centimeter diameter that were disinfected at one hour under 120° centigrade. The disinfected seeds were washed and cultivated into petri dishes. Each replication included 25 seeds.

The petri dishes were cited into germinator under 25°C (day) and 15°C (night) under treatment with 16/8 hours light/ darkness regime (ISTA Rules, 2013).

The cultivated seeds were irrigated with mentioned different saline water versus distilled water as (normal condition) each three days.

Evaluation of quantitative traits:

The first counting seedling was the third day and final counting was at tenth day than cultivation time. For evaluation seedling growth and their radicles, the petris were irrigated for one week furthermore (17 days totally). After that, dry weight of radicles and (Plumules) were measured using digital balanced with 0.0001 gram accuracy. Total of them in each replication was recorded and their means was considered as biologic yield for each cultivar.

Data analysis:

The data were normalized and converted for analysis. The analysis of variance was conducted using completely randomized design with factorial method moreover the comparison means was conducted using Duncan's method at 1% level.

The percent of variation of traits was used for evaluation decrease and increasing of them. It was calculated with below formula. Two STI (Stress Tolerance Index) and GMP (Geometric Mean Production) indices were used for estimation amounts of tolerance of studied cultivars, (Fernandez,1992).

$$STI = \left(\frac{Y_p}{\bar{Y}_p} \right) \times \left(\frac{Y_s}{\bar{Y}_s} \right) \times \left(\frac{\bar{Y}_s}{\bar{Y}_p} \right) = \frac{(Y_p) \times (Y_s)}{\bar{Y}_p^2}$$

$$GMP = \sqrt{Y_s \times Y_p}$$

where:

Y_p = yield of each cultivar under non-stress condition

Y_s = yield of each cultivar under stress condition

$\bar{Y}_p$ = Mean of total cultivars under normal condition

$\bar{Y}_s$ = Mean of total cultivars under stress condition

In this research, the software's such as SPSS (version 16), MSTAT-C (version 2.10) were used for analysis of recorded data and drawing scatter plots and Micro-soft office (version 2007) (Excel environment) was used for drawing graphs.

RESULTS

Analysis of variance:

Based on analysis of variance of data, there were significant differences at 1% level between studied cultivars on five evaluated traits of them. In A factor (stress) the variation was significant only in dry weight of radicle at 5% level. It

was reported that root hairs were wrinkled under increasing salt. The elasticity of root hairs was decreased under 1% NaCl density and they were wrinkled under 1.5 % of salinity condition (Zahran and Sprent, 1986). The lowest of coefficient of variation belonged to seed germination (29.51 %) while the highest belonged to dry weight of plumule (76.85 %) (as shown in Table 1)

Table 1

The results of analysis of variance on studied traits of 12 soybean cultivars under different salinity effects

Source of variation	Degree of freedom	First counting (Seed vigor)	Second counting (Seed germination)	Radicle dry weight	Plumule dry weight	Biological yield
Means of squares						
(Stress) A	3	161.496	282.852	0.004*	0.005	0.001
(Cultivar) B	11	1939.877**	1251.424**	0.005**	0.013**	0.006**
AB	33	96.263	129.236	0.001	0.001	0.001
Error	96	243.453	265.836	0.00129	0.0034	0.00193
Total	143					
Coefficient of variation [%]	-	31.72	29.51	26.19	76.85	26.25
Max		75.66	76.50	31.68	19.04	42.43
Min		26.66	38.16	11.44	1.02	16.9

The comparison of means:

The results of comparison mean using Duncan's method at 1% level showed Baj-maj and Bonus with 75.67 and 63.17% had the highest seed vigor and LD9, L17, M9, Steel, Union and Williams had the amounts between 44.67 to 26.67% as the cultivars with the lowest of it. Seed vigor includes all of the characters that can cause to rapid and uniform seedling emergence under wide range of environmental condition after seed planting (Copeland and McDonald, 1985).

The cultivars including L17 and Steel with 75.63 and 63.17% had the highest seed germination while the cultivars such as Baj-maj, Hy-1, Union, Elgine, M9 and Bonus with 44.67 to 26.67% had the lowest in this trait (As shown in Table 2 and Fig. 1). The cultivars including Union, Elgine, Steel, Hy-1, L17 and LD9 had the highest dry weight of plumule between 138.3 to 69.54 mili gram. The others such as Steel, Hy-1, L17, LD9, Bonus, Baj-maj, Williams, M9, Halcor and Clark had the low amounts so that Clark had 1.02 mg as the lowest. Based on comparison mean at 1% level, LD9, Williams, Halcor, Clark, Baj-maj and Elgine had the highest amount between 31.6 to 18.7 mg in dry weight radical. The others including Clark, Baj-maj, Elgine, Union, LD9, Hy-1, Steel and Bonus had low amounts and M9 had the lowest as 11.4 mg (as shown in Table 2).

Table 2
The comparison means between 12 soybean cultivars on five traits under four salinity stress levels Percent of variation:

	Seed vigor ¹	Seed germination ¹	Radicle dry weight ¹			Plumule dry weight ¹			Biologic yield ¹			Radicle dry weight ²		
			B Factor (Soybean cultivars)									A Factor (Salinity stress)		
	Mean	Group	Mean	Group	Mean	Mean	Group	Mean	Mean	Group	Mean	Mean	Group	Group
b1(Baj-Maj)	44.67	bcd	56.00	bcd	4.40	bc	19.00	abcd	25.90	abc	(0 mM)a1	17.40	b	
b2(Bonus)	67.26	d	17.38	d	1.0	c	14.10	cd	19.30	c	30 mM)a2	17.90	b	
b3(Clark)	67.55	bc	17.59	abc	1.00	abc	21.60	abcd	23.70	abc	60 mM)a3	23.10	a	
b4(Elgine)	67.41	cd	17.45	bcd	1.60	c	18.70	abcd	34.20	abc	90 mM) a4	16.90	b	
b5(Halcor)	33.49	bc	50.56	bcd	4.90	abc	24.60	abc	30.20	abc				
b6(Hy-1)	33.43	cd	83.54	bcd	4.00	bc	15.10	cd	27.80	abc				
b7(LD9)	0.57	bc	33.60	abc	10.40	abc	15.10	cd	20.70	bc				
b8(L17)	67.75	a	50.76	a	19.00	a	31.60	a	42.40	a				
b9(M9)	33.38	cd	50.42	cd	1.60	c	11.40	d	16.90	c				
b10(Steel)	17.63	ab	83.63	ab	14.40	ab	14.60	cd	27.80	abc				
b11(Union)	17.43	cd	67.51	bcd	3.70	bc	16.90	bcd	38.00	ab				
b12(Williams)	67.51	bc	33.58	abc	7.00	abc	27.80	ab	33.40	abc				

¹ at $\alpha=0.01$; ² at $\alpha=0.05$

Table 3
The comparison of percent of variation of biologic yield on studied soybean cultivars under different salinity stress levels

Cultivar	Biological yield [mg]					Percent of variation of trait					30 mM			60 mM			90 mM		
	0 mM	30 mM	60 mM	90mM	30 mM	60 mM	90 mM	GMP	STI	GMP	STI	GMP	STI	GMP	STI	GMP	STI	GMP	STI
Baj-maj	23.83	32.76	32.73	22.00	-37/48	10/00	78/32	27.94	0.918	27.93	0.917	22.89	0.616						
Bonus	17.40	20.23	22.20	19.70	-16/28	-9/72	26/11	18.76	0.414	19.65	0.454	18.51	0.403						
Clark	27.53	19.40	30.23	19.30	2953/	-55/84	16/36	23.11	0.628	28.85	0.979	23.05	0.625						
Elgine	46.86	30.10	38.70	23.60	77/35	-28/57	01/39	37.55	1.659	42.58	2.133	33.25	1.300						
Halcor	21.96	52.96	26.10	26.70	-141/12	72/50	-2/29	34.11	1.368	23.94	0.674	24.21	0.689						
Hy-1	7.90	50.30	24.10	73.56	-536/70	52/08	-205/25	19.93	0.467	13.79	0.223	24.10	0.683						
LD9	22.23	19.06	22.10	20.76	14/24	-15/90	6/03	20.58	0.498	22.16	0.577	21.48	0.543						
L17	69.96	33.80	37.56	33.40	51/69	-111/142	11/09	48.63	2.781	51.26	3.091	48.34	2.748						
M9	37.50	5.20	18.10	16.73	86/13	-248/07	55/7	13.96	0.229	26.05	0.798	25.04	0.738						
Steel	21.13	24.56	27.33	40.60	-16/24	-11/25	-48/53	22.78	0.610	24.03	0.679	29.29	1.009						
Union	22.53	43.56	63.43	42.96	-93/34	-45/59	32/26	31.33	1.154	37.80	1.681	31.11	1.138						
Williams	31.03	33.1	35.03	36.73	-6/66	-5/84	-4/85	32.04	1.208	32.97	1.278	33.76	1.340

Fig. 1. The comparison of seed germination of 12 soybean cultivars under different salinity stresses

The cultivars including L17, Union, Elgine, Williams, Halcor, Steel, Hy-1, Baj-maj and Clark with the amounts between 42.4 to 23.7 mg in biologic yield had the highest means. On the other hand, Elgine, Williams, Halcor, Steel, Hy-1, Baj-maj, Clark, LD9 and Bonus had low amounts and M9 had the lowest mean (as shown in Table 2).

Fig. 2. The comparison of biological yield of 12 soybean cultivars under different salinity stresses

Based on percent of variation of biologic yield, Hy-1 and Union with -536.70 and -93.34% had the lowest amounts under 30 mM salinity. M9 and

Clark had the lowest amounts -248.07 and -55.84% under 60 mM salinity. The cultivars including Hy-1 and Steel with -205.25 and -48.53% in 90 mM salinity condition (as shown in Table 3 and Fig. 2,3). In a research was reported that salinity decreased chlorophyll, photo synthesis and assimilation in soybean (Luo *et al.*, 2005).

Fig. 3. The comparison of biological yield variation percent of 12 soybean cultivars under different salinity stress

Table 4
The comparison of percent of variation of studied traits under different salinity stress levels

Parameters	The mean of traits				Percent of variation		
	0 mM	30 mM	60 mM	90 mM	30 mM	60 mM	90 mM
Seed vigor	48.668	47.278	52.222	48.611	2.856086	-7.30254	0.11712
Seed germination	55.889	52.833	58.944	53.333	5.467981	-5.46619	4.573351
Dry weight of radicle	17.424	17.956	23.104	16.900	-3.05326	-32.5987	3.007346
Dry weight of plumule	7.569	7.225	3.969	5.041	4.544854	47.56243	33.39939
Biologic yield	28.561	28.224	29.584	25.281	1.179931	-3.58181	11.48419

These results showed that the most of traits under 90 mM were decreased while they had increasing under 60 mM salinity. Dry weight of radical was increased under 30 and 60 mM salinity as -3.053 and -32.59 % respectively. Seed vigor had only increasing under 60 mM as -7.30%. Dry weight of plumule was the only trait decreasing under 60 and 90 mM as 47.56 and 33.39 respectively (as shown in Table 4).

Environmental indices:

In evaluation of the cultivars using the indices such as GMP and STI, two cultivars, including L17 and Elgine had the highest amounts under whole of salinity conditions and Williams had the response similar to Elgine only in 90 mM condition (as shown in Table 3).

Scatter plot:

Based on display scatter plot with GMP and STI, L17 was the only cultivar with high Y_p and Y_s under 30 mM salinity and was cited in A region. After that elgine was cited in second grade, it was between A and B region. Although Williams, Halcor, Union and Baj-maj had high Y_s but they had median amounts in Y_p and were cited in C region of scatter plot (as shown in Fig. 4).

Fig. 4. Display12 soybean cultivars citation based on biologic yield and GMP and STI indices in scatter plot under 30 mM salinity

Fig. 5. Display12 soybean cultivars citation based on biologic yield and GMP and STI indices in scatter plot under 60 mM salinity

The cultivars including L17 and Elgine had the high Y_p but less than median in Y_s under 60 mM salinity and were cited in B region, while Union had the

high Y_s but less than median in Y_p so it was cited in C region (as shown in Fig. 5).

Fig. 6. Display 12 soybean cultivars citation based on biologic yield and GMP and STI indices in scatter plot under 30 mM salinity

L17 had the highest Y_p but less than median in Y_s under 90 mM salinity and was cited in B region. Although Hy-1 had the high amount in Y_s but it had low amount in Y_p so it was cited in C region (As shown in Fig. 6).

DISCUSSION AND CONCLUSIONS:

High genetic variation between studied cultivars on their traits can be a cause for high coefficient variation of evaluated traits.

Considering there was not significant different in A factor (stress), between studied cultivars on the traits such as seed vigor and germination and so dry weight of plumule and biologic yield, It is concluded that these cultivars had almost equal responses to different salinity levels and it's means they had the yield as normal condition.

Considering the percent of variation of traits, it seems soybean seeds had a good adaptability with 60 mM salinity because the most of studied traits had increasing under 60 mM saline water. It's may be salinity had positive effects on radicle growth and some seed character such as vigor and germination but negative effects on plumule growth.

Based on the results, it was concluded that considerable tolerance between studied cultivars in seed germination can be consider in growing stage in further. It can be promising for identification tolerant cultivars to environmental stresses such as salinity and its may some of studied cultivars be suitable for introduction to cultivation. In a research was reported that some seedling characters such as dry weight of root and stem of soybean was decreased under 80 mili/mol NaCl and it was less in tolerant cultivars than others (Velagaleti *et al.*, 1990). The results showed that germination percentage, germination index, and seedling vigor index were decreased under salinity levels from 0 to 15 $\text{dS} \times \text{m}^{-1}$ significantly (Kandil *et*

al., 2015). On the other hand, it is considerable that basing on the studies, soybean seeds were more tolerant to salinity during germination than in the seedling phase (Khajeh-Hosseini *et al.*, 2002). Other researches reported that salinity stress strongly inhibited seed germination under 12.05 dSm-1 (Bahrami and Razmjoo, 2012)

Although based on comparison of means, It was identified L17 and Steel not only had the high seed germination and vigor but also they had dry weight of radicles and biologic yield than others. However, L17 and Elgine were cited in A region (with high potential and stability if yield) of scatter plot based on tolerance indices such as STI and GMP.

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