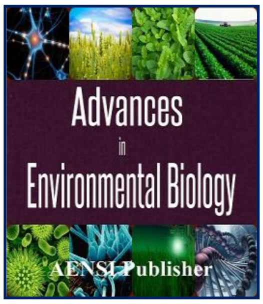
Advances in Environmental Biology, 9(5) April 2015, Pages: 229-235

AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/



Effects of Salinity Stress and Drought Due to Different Concentrations of Sodium Chloride and Polyethylene Glycol 6000 on Germination and Seedling Growth Characteristics of Pinto Bean (Phaseolus vulgaris L.)

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ARTICLE INFO

Article history:
Received 12 December 2014
Received in revised form 26 January 2015
Accepted 17 February 2015
Available online 8 March 2015

Keywords:
stress, pinto bean, drought, salinity, germination

ABSTRACT

Salinity and drought are the two main limiting factors for agricultural products in arid and semi-arid regions, especially Iran. Two separate factorial experiments based on completely randomized design with three replications were conducted in order to assess the effects of salinity and drought stresses on germination and growth of pinto beans (including local masses Khoy and Shahindeh), at Agricultural and Natural Resources Research Center of West Azarbaijan Province in 2013. Salinity and drought stress treatment included different levels of osmotic potential caused by different concentrations of sodium chloride and poly ethylene glycol 6000 in three levels of -4, -8, -12 bar and control treatment (distilled water), respectively. The results indicated that applying various levels of salinity and drought, there were significant decreasing effects on studied traits such as; germination percentage, germination rate, radicle length, plumule length, and seedling fresh weight. The mean comparison showed that in drought stress and in all parameters (except the radicle length to plumule length ratio) the decreasing effects were significant between control and -4 bar treatment. In salinity stress, except the germination percentage the decreasing effects were significant in other parameters from control to -4 bar treatment. Meanwhile, group comparison of inhibitory effects of salinity and drought stress indicated showed that the drought stress had a significant decreasing effect on rate and percentage of germination and radicle length traits compared to salinity stress.

To Cite This Article: Rasoul Khodaverdivand Keshtiban, Vahid Carvani, Mehdi Mojab., Effects of Salinity Stress and Drought Due to Different Concentrations of Sodium Chloride and Polyethylene Glycol 6000 on Germination and Seedling Growth Characteristics of Pinto Bean (Phaseolus vulgaris L.). ***Adv. Environ. Biol., 9(5),*** *229-235, 2015*

INTRODUCTION

Legumes are the second important nutrition in Iran [8]. One of the most important legumes is bean worldwide which has the first place in terms of cultivation levels between legumes. Various kinds of beans contain high amounts of protein so that its dry seeds contain 22% protein. Pinto bean is one of green beans subspecies with very similar properties to green beans [37]. The area under cultivation of legumes worldwide is about 70 million hectare and its production is approximately 60 million tons. In Iran, 697000 hectares, equivalent to 87/6% of arable lands, in 2007-8 cropping season, dedicated to the legumes products in which 110,248 hectares (225,720 tons of production) is dedicated to beans [5].

Salinity and drought stresses are important problems of agricultural production in many parts of the world, especially in arid and semi-arid regions [33,12]. The seed germination which is the first stage of and plant growth, may be encountered with stress [38]. Seed germination is usually the most critical factor determining the success or failure of plant establishment [4].

Salinity is one of the environmental stressors that leads to unfavorable conditions for seed germination and seedling establishment and about 20 to 30 percent of beans cultivated lands are affected by salinity [10]. Germination and seedling establishment are sensitive stages of plants growth to salinity stress [17]. Salinity mainly causes delay in germination and reduction in germination rate [23]. So, the success of plant species under saline conditions is that first, they should be able to maintain their lives and then, when the salinity level is decreased, germination is began [34]. Salinity affects the seeds germination and growth by reducing the water

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potential and toxicity of specific ions such as sodium and chloride ions and reducing nutrition requirements, such as calcium and potassium [24,39,25]. Bakhtyary and Hamedy [9] showed that in studying the effects of salinity stress on kidney beans, increasing salinity resulted to signifisant decreasing effect on rate and percentage of germination and also decreased the vegetative traits such as; radicle and plumule length, fresh weight of radicle and plumule, and plumule dry weight.

Drought stress may delay germination, reduce or completely prevent it [48]. Several reports suggested that the seeds that could have a good response to drought stress in this stage, would show better growth in the seedling stage [11,26].

The experiments in the field of stress are considered in laboratory conditions due to soil heterogeneity and lack of control on environmental factors such as drought stress in farm conditions. One of these methods that can be pointed is to determine the response of genotypes to solutions of osmoticom materials such as polyethylene glycol [14]. The different experiments in polyethylene glycol 6000 were mentioned to be successful in producing negative potential and drought stress on crops like lentil [20] and mung bean [16]. Prisco and *et al*. [50] reported that increasing drought stress would decrease the germination rate. Similar results were suggested by Bradford and *et al*. [13].

The studies showed that the germination stage is one of the critical stage of plant growth and the seeds that can suitably germinate in stress condition, have produced better vigorous seedlings and stronger root system in the next stages of growth [49]. So, germination and suitable seedling establishment are primarily considered as a determining factor in the yield amount [6]. Accordingly, this research aimed to study the effect of different osmotic potentials of salinity and drought stress on the attributes related to germination of pinto bean plants was and were performed to study and compare the sensitivity of the studied local masses in relation to the these stress tests.

MATERIALS AND METHODS

Two separate factorial experiments based on completely randomized design with three replications was carried out to study the effects of drought and salinity stress on two pinto bean local masses (including khoy and Shahindezh local masses) in laboratory of Natural Resources and Agricultural Research Centre in western Azerbaijan.

Experimental treatments included different levels of osmotic potential due to different concentrations of sodium chloride and poly ethylene glycol 6000 in four levels (0, -4, -8, -12 bar). The Van't Hoff's law (Equation 1) was used to produce different salinity potentials from sodium chloride and the Michel and Kaufman method (Equation 2) was utilized to apply drought stress from poly glycol 6000 [43].

$$\psi_s = miRT \qquad (1)$$

Where; ψ_s is the osmotic potential (bar), m is the solution molarity, i is ionization coefficient, R is gas constant (bar. l. 0.0832mol^{-1}.K^{-1}), and T is the temperature (K).

$$\psi_s = -(1.18 \times 10^{-2})C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T \qquad (2)$$

Where; ψ_s is the osmotic potential (bar), C is the amount of poly ethylene glycol (g/l), and T is the temperature (℃).

Every experimental unit included one 9 cm diameter petri dish containing sterile filter paper and 10 aseptic seeds were placed in the dish. Disinfecting the seeds, first, they were washed with distilled water, second, they were dipped in solution of 5% sodium hypochlorite for one minute then were leaching with distilled water, and third, seeds were placed in a solution of 2 per thousand benomyl fungicide for one minute and again were leaching with distilled water for three times. 10 ml of produced solutions with definite levels of salinity and drought was added to every petri dish then the dishes were closed by parafilms and transferred to the incubator with 25±1℃ temperature.

Daily monitoring of the samples were done and the number of seeds germinated with radicle length of 1-2 mm were counted and recorded at the same time of the day and to the end of the experiments on the eighth day. Counting was continued until the number of the germinated seeds was constant in each sample during three consecutive days [45].

The equation 3 was utilized in order to determine the rate of seed germination [2].

$$R_s = \sum_{i=1}^{m} \frac{S_i}{D_i} \qquad (3)$$

Where; R_s is the germination rate (number of seeds per day), S_i is the number of germinated seeds were counted on i-th day, and D_i is the number of days until i-th counting. The germination percentage was calculated by equation 4 [2].

$$GP = \frac{N_i}{N} \times 100 \qquad (4)$$

Where; GP is the germination percentage (%), N_i is the number of germinated seeds up to i-th day, and N is the total number of seeds.

Finally, in every treatment 3 random samples were chosen and radicle length, plumule length, radicle to plumule length ratio, radicle fresh weight, and seedling fresh weight were measured and calculated. Before the analysis of the data, the normalization of data was done in required cases (transformation equation: $\sin^{-1}\sqrt{x}$). The statistical analysis of data was done by using SPSS software and the mean comparison was based on LSR test in probability level of 5%. Group comparison of drought and salinity stresses was conducted by t test in probability level of 5%.

RESULTS AND DISCUSSION

1- The effects of drought stress:

The analysis of variance (ANOVA) of studied traits showed that applying various levels of drought stress had significant effects on all of the traits related to germination, so that the traits were quantitatively decreased by more negative osmotic potential (Table 1). These results were along with the studies of Shafarody and Zavareh [53] on pinto beans and Rahimy and Rahimy [51] on mung beans. Murillo and *et al*. [46] indicated that ions and organic solvents accumulated in cells under stressed conditions and the harmful effects of PEG on germination were due to the osmotic effect that created the concentration of these ions in solution and cells. The ANOVA also showed that there was no significant difference between the local masses that indicated the identical sensitivity in them in relation to drought stress.

The mean comparison showed that, more negative potential from control to -4 and -8 bar significantly decreased the germination percentage 61.45% and 100%, respectively, compared to control treatment (Table 2). It could be mentioned that in -8 and -12 bar treatments the amount of this trait (and also other studied traits) was equal to zero due to lack of germination. The rate of germination trend indicated that more negative water potential the germination rate decreased from 4.79 numbers per day in control treatment to 1.06 numbers per day in -4 bar treatment. Ghanbary Motlagh and *et al*. [22] studied the effects of drought stress on kidney bean local varieties (Drakhshan, Sayad, and Goli) and reported that more negative potential reduced the percentage and rate of germination, there was a significant difference between the control and -4 bar treatment. Kiany *et al*. [36] and De and Kar [16] suggested that the influence of drought stress on physiologic and metabolic processes during seed germination, leading to a reduction in the rate of germination.

Plumule and radicle length traits both had reducing trend in relation to different levels of drought stress so that with more negative potential the plumule and radicle length from control to -4 bar decreased significantly 66.40% and 48.71%, respectively, compare to control. Davoody [15] and Nezamy and *et al*. [47] reported in similar results that both radicle length and plumule length decreased with increasing osmotic potential during drought stress. Kafy and *et al*. (2005) assessed the drought stress effects in germination stage of different lentil genotypes and reported a reduction in radicle length and plumule length with decreasing water potential, meanwhile declared that the plumule length was more sensitive to drought stress and so that it could be used as an evaluation index of germination in lentil genotypes under drought stress. This was also reported by Alsherkavy. One of the causes of the reduction in plumule length in the stress condition was mentioned the decrease or no transfer of food from the cotyledon(s) to the fetus [54].

The radicle to plumule length ratio showed that in the potential level of -4 bar the ratio had more average than control although the difference between them was not significant. This result was in line with Turk and *et al*. [55] and Maasoumy and *et al*. [41].

The evaluation of fresh weight of radicle, plumule, and seedling showed that more negative potential declined the average of these traits, compared to control treatment so that there was a significant difference between -4 and -8 bar treatments. Kazeroony Monfared *et al*. [32] also reported in similar research in assessment of drought stress on germination of lentil and mung bean genotypes and suggested that drought stress significantly decreased the seedling fresh weight. Studies indicated that the reduction in water uptake by seeds under drought stress reduced the secretion of hormones and enzymes and thereby impaired the growth of seedlings (radicle and plumule) [29].

2- The effects of salinity stress:

The ANOVA showed that applying various levels of salinity significantly effected on the germination percentage, germination rate, plumule length, radicle length, plumule fresh weight, and seedling fresh weight while its effect was not significant on radicle to plumule length ratio and radicle fresh weight (Table 3). Ahmadian and *et al*. [1] reported the effect of salinity on all of the studied traits at germination stage was significant in the study of salinity resistance of 18 pinto bean genotypes in 5 levels of sodium chloride (0, 60, 120, and 180 mM). Ashraf and Rao [6], suggested that salinity effects on germination and seedling growth through osmotic potential and prevent water absorption or the toxic effects of chlorine and sodium ions.

The ANOVA results also showed that there was no significant difference between studied local masses in relation to measured traits except for the germination percentage, radicle to plumule length ratio, and radicle length. The interaction between salinity and genotypes was not significant except for the germination rate. Eradatmend and Mehrpanah [19] indicated that due to high sensitivity of bean plant to salinity, it can be used as a model plant in researches of salinity stress.

The results of mean comparison revealed that the germination percentage and germination rate declined with increasing levels of salinity from the control level to -12 bar treatments so that in seed germination percentage, this reduction between the control and -4 bar treatment was not significant but from -4 bar to -8 bar there was a significant difference (Table 4). Also survey of the germination rate revealed that more negative water potential decreased the germination rate from 4.79 (number per day) in the control treatment to 3.51, 2.32, and 1.24 (number per day) for the potential of -4, -8 and -12 bar, respectively that was significant between control and -4 and -8 bar but was not significant between -8 and -12 bar treatments. The rate of germination is one of seed vigor indexes which is affected by real salinity. Ghanbary Motlagh and *et al*. [22] also found the same results. The use of common salt levels reduced germination percentage and increased the mean of germination time [44]. Reduction in germination rate with increasing salinity can be attributed to the presence of excess cations and anions that due to their soluble in water reduces the water potential in addition to poisoning, thus due to the lack of plant ability to absorb water and low water absorption the activities within the seed conducted lightly and increased the time of radicle exit from seed [21].

Radicle and plumule length had the same decreasing trend in various levels of salinity so that, in relation to the plumule length from control to potential levels of -4, -8, and -12 bar, this trait showed a significant decrease of 41.02%, 63.33% and 71.02% respectively, compared to the control treatment, although this reduction was not significant between -8 and -12 bar treatments. Also, in relation to the radicle length from control to potential levels of -4, -8, and -12 bar, there were a significant decrease of 22.31%, 57.62% and 0.03% respectively, compared to the control treatment, however, this reduction was not significant between -8 and -12 bar treatments. Hasanbeygy and *et al*. [28], studied the effects of different levels of common salt solutions on germination of bean varieties (including Jules, Daneshkadeh, Khomeini and the J 14088) with four levels of sodium chloride (0, 1, 2, 3 dS/m) and reported that in the stress level of 3 dS/m the radicle and plumule length traits reduced 20% and 45% compared to control, meanwhile, the plumule length was affected more than radicle length. Hardgree and Emmerich [27] also found that chlorine ion had a negative effect on seedling growth. Makar and *et al*. [42] reported that under the salinity stress in both hydropriming and seedling establishment in addition to reducing water absorption, extra adsorbed ions due to the decrease in water potential between the outer and the inner environment of the seed, prevented the emergence of radicle.

Also the results out the plumule fresh weight and seedling fresh weight revealed that increase in salinity stress from control level to -12 bar treatment, the average of these traits were reduced. The most amount of plumule and seedling fresh weight were occurred in control treatment with 0.4118 and 0.5248 g, respectively and the least amount of them was observed in -12 bar treatment with 0.1023 and 0.1218 g, respectively. Jamil and *et al*. [30] believed that salinity stress also reduced cell division in addition to activate certain enzymes and ions and leaded to interference in effective growth activities and reduce the water in the tissues and ultimately causing reduction in radicle and plumule fresh weight with increasing salinity. Salehyfar and *et al*. [52] in the comparison of responses of 8 genotypes of beans to germination and growth under salinity stress found that increasing salinity stress from the control (zero) to 180 mM, the average of radicle and plumule fresh weight decreased and concluded that increasing salinity levels reduced the growth of the seedling components.

3- Comparison and evaluation of inhibitory effect of salinity and drought stresses:

An independent group comparison was carried out for germination percentage, germination rate, radicle length, and plumule length in order to compare and evaluate the effect of salinity and drought stresses (Table 5). The comparison showed that the average of the traits in salinity stress is less than the drought stress and the differences in the traits was significant except in the plumule length. Thus, the inhibitory effect of drought stress was more than salinity stress in studied traits and did not have the same effects. Khajeh Hosseiny and *et al*. [35] found that soybean germination in was affected less in sodium chloride solution than poly ethylene glycol 6000 and its cause was attributed to absorb water rapidly by seeds and receive necessary moisture for germination in solution of sodium chloride. Lynch and Lauchli [40] realized that PEG molecules absorbed more water because of their large size and prevented water absorption by seeds by reducing the osmotic potential. Al Ibrahim and *et al*. [3] reported the same results.

According to the results of these experiments, it could be concluded that the local masses were sensitive to drought stress, in germination stage, while resistance to moderate salinity. The comparison of the effects of these stresses on pinto beans also showed the negative effects of drought stress was greater than the salinity.

Yet, further testing should be done in greenhouse and field to evaluate the effects of stresses and comparison of local masses.

Table 1: The ANOVA of germination traits of pinto bean in drought stress.

	df	Germination percentage	Germination rate	Plumule length (cm)	Radicle length (cm)	Radicle to plumule ratio	Radicle fresh weight (g)	Plumule fresh weight (g)	Seedling fresh weight (g)
Drought	3	12626.27**	31.021**	20.469**	14.210**	2.146**	0.016**	0.233**	0.368**
genotype	1	230.454ns	0.388ns	0.940*	0.150ns	0.042ns	1.820ns	0.001ns	0.005ns
Drought* genotype	3	403.042ns	1.410*	0.487ns	0.055ns	0.031ns	0.002*	0.001ns	0.006ns
Error	16	121.141	0.173	0.173	0.149	0.035	000	0.005	0.007

ns: not significant, * and **: significant in probability levels of 5% and 1%, respectively.

Table 2: Mean comparison and effect of various levels of drought on germination and growth characteristics of pinto bean.

Drought levels (bar)	Germination percentage	Germination rate	Plumule length (cm)	Radicle length (cm)	Radicle to plumule ratio	Radicle fresh weight (g)	Plumule fresh weight (g)	Seedling fresh weight (g)
Control	97.17a	4.79a	3.90a	3.15a	0.88a	0.107a	0.418a	0.525a
-4	37.44b	1.06b	1.61b	1.85b	1.15a	0.499b	0.133b	0.189b
-8	000c	000c	000c	000c	000b	000c	000c	000c
-12	000c	000c	000c	000c	000b	000c	000c	000c

In each column, means with different letters are significantly different, according to ANOVA and the LSR test.

Table 3: The ANOVA of germination traits of pinto bean in salinity stress.

	df	Germination percentage	Germination rate	Plumule length (cm)	Radicle length (cm)	Radicle to plumule ratio	Radicle fresh weight (g)	Plumule fresh weight (g)	Seedling fresh weight (g)
salinity	3	4038.103**	12.828**	9.320**	5.068**	0.134ns	0.004ns	0.130**	0.201**
genotype	1	929.642*	0.44ns	0.447ns	0.675*	0.548*	0.008ns	0.020ns	0.031ns
salinity* genotype	3	411.925ns	1.356*	0.597ns	0.283ns	0.005ns	0.004ns	0.005ns	0.007ns
Error	16	124.910	0.388	0.201	0.124	0.089	0.004	0.007	0.011

ns: not significant, * and **: significant in probability levels of 5% and 1%, respectively.

Table 4: Mean comparison and effect of various levels of salinity on germination and growth characteristics of pinto bean.

salinity levels (bar)	Germination percentage	Germination rate	Plumule length (cm)	Radicle length (cm)	Radicle to plumule ratio	Radicle fresh weight (g)	Plumule fresh weight (g)	Seedling fresh weight (g)
Control	97.167a	4.79a	3.90a	3.115a	0.883a	1.107a	0.418a	0.525a
-4	86.22a	3.51b	2.30b	2.42b	1.12a	0.670a	0.242b	0.309b
-8	55.433b	2.23c	1.43c	1.32c	0.903a	0.046a	0.112b	0.158bc
-12	41.608b	1.48c	1.13c	1.245c	1.75a	0.068a	0.102b	0.122c

In each column, means with different letters are significantly different, according to ANOVA and the LSR test.

Table 5: Group mean comparison between drought and salinity stresses on decreasing germination percentage, germination rate, plumule length, and radicle length.

	Ave. germination percentage	Ave. germination rate	Ave. plumule length	Ave. radicle length
Drought	33.651	1.463	1.379	1.252
Salinity	70.109	3.001	2.192	2.034
Significant level between the two groups.	0.001	0.005	0.062	0.026

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