P-ISSN: 2305-6622; E-ISSN: 2306-3599

International Journal of

Agriculture and Biosciences

www.ijagbio.com; editor@ijagbio.com

Research Article

Physiological Effects of Light, Salinity and pH on the Process of Seed Germination and Seedling Growth of Three Varieties of Red Beans

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Article History: Received: July 16, 2016 Revised: September 12, 2016 Accepted: October 18, 2016

ABSTRACT

To evaluate the effects of ecological factors on germination of three species of red beans factorial experiment was carried out in a completely randomized block design with three replications. Treatments included: light at two levels of darkness in the first 48 hours of germination and treatment period of 14 hours of light and 10 hours of darkness; salinity levels in three levels distilled water (control), 6 grams per liter of salt, 10 grams per liter of salt and pH treatments at three levels: 6.5,7.5 (control), 8.2 and varieties of three species of red beans (Goli, Akhtar and KS31169).The results for analysis of variance showed that the quadratic interaction was significant in the possibility level of one percent for all traits. The results for comparison of means showed that overall germination rate was reduced by applying the third level of salinity. But it was observed that germination rates were reduced if the first level of light was used with a combination of other factors compared to when second level of light was used with a combination of other factors. Generally, the treatment combinations that had maximum and minimum length of root and shoot had the highest seedling length. For seedling dry weight, the treatment combinations 3221 (1.41) and 2121 (1.41) showed the highest and treatment combinations 1232 (0.40) and 3132 (0.40) showed the lowest results. Results of correlation of characteristics showed that germination percentage did not have a high and meaningful correlation with any of the traits.

Key words: Germination, Red Beans, pH, Light, Darkness, Salinity

INTRODUCTION

Beans with the scientific name *Phasaeolous vulgaris* is an annual plant of the legume family and is one of the important sources of protein in many countries, especially developing countries, because economically it is cheaper than animal protein (Graham and Ranalli 1997). Germination is a complex physiological process which is influenced by genetic and environmental factors (Foley and Fennimore 1998; Meyer and Pendleton, R. L. 2000). Germination and seedling establishment is one of the most critical stages in the life cycle of plants and understanding the ecological requirements at this stage can help to control growth or stimulate the growth of a plant (Windauer *et al*, 2007). Many factors are involved that benefit or detriment conditions for favorable germination and growth of plants. Among these factors can be environmental stresses that after humans are the most important factors to effect product deployment in the areas of climate and soil. (Shalhevet, 1993). In environmental stresses, early Stage of plant growthis regarded as the most sensitive growth stage (Amir *et al*., 1390).

Germination like other vital activities is effected by environmental factors and non-environmental factors such as water and temperature. Although moisture, oxygen and temperature are necessary for germination of all seeds, some particular species need light to germinate. The effect of light on germination of seeds has long been known (Akram Qaderi *et al*., 1387). pH also affects the germination of seeds of different plants, some species prefer acidic conditions and some prefer neutral or alkaline conditions for germination, some species do not respond to pH changes (Pierce *et al*. 1999; Susko *et al*, 1999). Salinity is one of the most important problems in arid and semi-arid regions of the world. Salinity in Iran has always had adverse effect on the economic performance of agricultural products (Afzal 2005; Belaqziz *et al*., 2009). Reduction in the percentage of germination under salt stress is due to osmotic effects of

Cite This Article as: Anbohi FB, B Delkhosh and G Noormohammadi, 2016. Physiological effects of light, salinity and pH on the process of seed germination and seedling growth of three varieties of red beans. Inter J Agri Biosci, 5(6): 382-387. www.ijagbio.com

sodium chloride and salt toxicity on embryo or seed endosperm cells. The germination rate is more sensitive than germination percentage, fast germination will improve initial deployment of the plant (Ashraf and Foolad, 2005). Pretreatment of seeds with salt solutions of different osmotic potential (osmopriming or halopriming) is also an easy method with low cost and low risk. This is a common strategy to increase the percentage of germination, speed and uniformity of germination, emergence and improvement of product quality and quantity under adverse environmental conditions, which can increase resistance to salt stress in plants (Cayuela *et al*., 1996; Yadollahi, 2008). Halopriming with NaCl, KCl and CaCl salts has been successfully effective in mitigating the effects of drought and salinity stresses on germination and growth of plants such as peas, lentils, wheat and pepper (Ebadi and Kamel, 2009; Saglam *et al*., 2010; Yagmur and Kaydan, 2008; Patrik and Greg Cobb, 1991). By understanding the ecological conditions that would have the least damage on crops in the germination stage, the environment or area suitable for the cultivation of plants can be determined. Study of ecological factors affecting seed germination and plant growth and paving the way for further studies will be thegoal of this research.

MATERIALS AND METHODS

To evaluate the effects of ecological factors on germination of three species of red beans, a series of experiments were carried out in Zakariya Razi laboratory, Laboratory complexes of Agriculture lab, Science and Research Branch of Islamic Azad University of Tehran, during the year 1394. Factorial experiment was carried out in a completely randomized block design with three replications. Treatments included: light at two levels of darkness in the first 48 hours of germination and treatment period of 14 hours of light and 10 hours of darkness; salinity levels in three levels (distilled water (control), 6 grams per liter of salt, 10 grams per liter of salt and pH treatments at three levels: 6.5,7.5 (control), 8.2 and varieties of three species of red beans (Goli, Akhtar and KS31169).The third factor (pH treatments) was carried out by adding the required amounts of 1N NaOH (to make 1N NaOH, 10 g of solid NaOH was dissolved in 100 ml of distilled water) and 1N hydrochloric acid (to make 1N HCl, 100 ml of distilled water was mixed with 1.8 cc hydrochloric acid) to distilled water. pH was determined by pH meter.

The experiment was consisted of 18 ecological treatments (light on two levels, each with three levels of salinity and pH), 3 treatments of (bean) seeds and 3 repetitions with a total of 162 experimental units. Each unit consisted of a petri dish with diameter of 9 cm, containing 10 seeds and type of ecological treatment used. After placing two layers of number one Whitman filter paper, seeds under experiment were placed in each Petri dish and 6 ml of water was added. All tests for seed germination in Petri dishes were carried out in 10 days in front of light and temperature of 25°C. The traits such as seed germination, root length, shoot length, seedling length, root fresh weight, shoot fresh weight, seedling fresh weight, root dry weight, shoot dry weight, and seedling dry weight were measured in stressed conditions. After testing, data variance analysis was done using SPSS software and means comparison was performed with Duncan's multiple range test with probability level of 5%. For drawing diagrams and tables Excel and Word software were used.

RESULTS AND DISCUSSION

The results of variance analysis of traits (Table 1) showed that the varieties studied (Factor A) and salinity factors (C) and pH (D) of all traits had statistically significant differences in the probability level of one percent, but the effect of light factor (B) was significant only for the root, shoot and seedling length; for other traits no significant differences were observed in terms of this factor. Quadratic interactions were significant for all traits in probability level of one percent. In factorial experiments, when the interaction of all factors (quadratic interactions) are significant, mean of factors should be reviewed and compared and other interactions and original effects of the factors should be overlooked.

Rate and percentage of germination: the results showed the highest germination percentage for treatment combinations of 1111, 3123, 1212, 2211, 1222, and 3112 with 85 percent, and the lowest germination percentage for treatment combinations of 2123 and 3223 with 15% germination. Overall, the rate of germination was reduced by applying the third level of salinity. It was observed that germination rates were reduced if the first level of light

Table 1: Results for variance analysis of traits in the process of germination and seedling growth of three varieties of red beans

References	Degrees of freedom	Average Of Squares									
		Germination percentage	Root length	Shoot length	Seedling length	Root fresh weight	Shoot fresh weight	Seedling fresh weight	Root dry weight	Shoot dry weight	Seedling dry weight
Variety (A)	2	5037.5**	0.87**	2.25**	5.01**	0.02**	0.18**	0.10**	0.01**	0.18**	0.11**
Light (B)	1	12.5ns	0.55**	1.07**	0.09**	0.0003ns	0.0005ns	0.0002ns	0.002ns	0.0005ns	0.0003ns
Salinity (C)	2	262.5**	5.23**	4.92**	19.65**	0.02**	0.97**	1.25**	0.02**	0.97**	1.27**
Acidity (D)	2	2594.2**	1.27**	0.51**	3.12**	0.53**	1.85**	4.25**	0.22**	1.85**	3.27**
ABC	4	2079.2**	3.95**	7.65**	22.08**	0.08**	0.49**	0.83**	0.04**	0.50**	0.68**
ABD	4	2016.7**	2.43**	7.81**	18.60**	0.13**	0.21**	0.41**	0.06**	0.21**	0.29**
ACD	8	11054.2**	1.80**	2.83**	8.57**	0.02**	0.13**	0.21**	0.006**	0.13**	0.17**
BCD	4	383.3**	0.37**	1.47**	2.82**	0.001^{ns}	0.00002ns	0.001ns	0.002*	0.00002ns	0.002ns
ABCD	8	72.3**	1.14**	2.46**	6.53**	0.08**	0.38**	0.70**	0.03**	0.38**	0.54**
Error	108	25	0.002	0.002	0.0017	0.0022	0.0022	0.0064	0.0005	0.0018	0.004
Coefficient of variations		8.45	1.99	1.24	0.78	20.13	3.32	5.53	24.8	5.01	6.83
R^2		0.97	0.99	0.99	0.99	0.94	0.98	0.97	0.96	0.98	0.98

Table 2: Correlation results of traits in process of germination and seedling growth of three red bean cultivars

Germination Percentage		Root Length	Seedling Length	Plant Length	Fresh Root Weight	Fresh Seedling Weight	Fresh Plant Weight	Dry Root Weight	Dry Seedling Weight	Dry Plant Weight
Germination percentage	1	0.054	0.16	0.12	0.27	-0.28	-0.14	0.25	-0.28	-0.18
Root length	0.054	1	0.86	0.96	0.29	-0.08	0.09	0.32	-0.008	0.069
Seedling length	0.16	0.86	1	0.97	0.19	-0.13	-0.04	0.25	-0.13	-0.05
Plant length	0.12	0.96	0.97	1	0.25	-0.078	0.02	0.29	-0.078	0.0008
Fresh Root weight	0.27	0.29	0.19	0.25	1	0.39	0.66	0.96	0.39	0.58
Fresh Seedling weight	-0.28	-0.08	-0.13	-0.078	0.39	1	0.95	0.42	0.99	0.97
Fresh Plant weight	-0.14	0.09	-0.04	0.02	0.66	0.95	1	0.67	0.95	0.99
Dry Root weight	0.25	0.32	0.25	0.29	0.96	0.42	0.67	1	0.42	0.60
Dry Seedling weight	-0.28	-0.008	-0.13	-0.078	0.39	0.99	0.95	0.42	1	0.97
Dry Plant weight	-0.18	0.069	-0.05	0.0008	0.58	0.97	0.99	0.60	0.97	1

Numbers larger than 0.27 are significant.

was used with a combination of other factors compared to when second level of light was used with a combination of other factors. The best germination level for KS31169 variety was factor combination B1C2D3 (85%) and the lowest percentage of germination was factor combination B2C2D3 (15%) which showed 82 percent reduction. The best germination level for Akhtar variety was factor combination B2C1D1 (85%) and the lowest percentage of germination was factor combination B1C2D3 (15%). It was also observed for this variant that germination rate decreased with increasing salinity levels. For Goli variety the treatment combinations of 1111, 1212 and 1223 (85%) showed the highest percentage of germination. The trend of germination for this treatment with different factor combinations were highly different which reflects the severe interactions of factors. In general, for Goli variety higher germination rate would be observed if the second level of light, the second and third levels of salt and different levels of acidity are used; probably because dormancy will be the most affected (Figure 1).

There is a positive relationship between light intensity and photosynthetic rate. In theory, photo-synthesis is done in any light intensity, but in practice Respiration takes precedence over photosynthesis in low light intensities. Potential of environment's water has direct impact on the water absorption rate and thus germination (Koochaki *et al*., 1367). Reduction in germination under drought stress can be linked to a reduction in water uptake by seeds. If the water absorption by seeds is impaired or water absorption is slowed, germination metabolic activities inside the seeds will be slower. As a result, root's emergence from the seed will need longer time and hence germination rate and percentage will be reduced (Katrgy *et al*., 1994). Reduction in water absorption by the stressed seeds reduces the secretion of hormones and enzymes, and consequently disorders seedling growth (Kulkarni and Deshpande, 2007). Also compared to other germination components, germination percentage is a better factor to evaluate drought resistance (Kulkarni and Deshpande (2007).). Germination and seedling emergence requires a lot of energy which is provided through the release of energy in chemical bonds of stored materials in seeds including carbohydrates, fats and proteins during the process of respiration (Shalhevet, 1993). Thus the high level of stored materials causes rapid and uniform seed germination and production of a stronger seedling (Lopez *et al*., 1995).

Root and shoot length: in terms of root length, the highest length was for treatment combinations 2221 (3.16), 1121 (3.61) 1122 (3.51) and 2222 (3.51) and the lowest root length was for treatment combinations 3113 (1.03) and 1213 (1.03). As for shoot length, treatment combinations 2221 (5.36) and 1121 (5.36) showed the highest and 2131 (2.01) and 3231 (2.01) showed the lowest results. In general, for seedling length the treatment combination with maximum and minimum length of root and shoot showed highest length for seedling. For Goli variety if a combination of factors as the second level of light (B2), the third level of salt concentration (C3) and different levels of third factor is used, response of plant is heightened and its root length further increases. For Akhtar variety if the combination of factor levels B2C2D1-2-3 are used maximum elongation can be seen in the plant root. If the combination of factor levels B1C3D1-2-3 are used for KS31169 variety, plant's response to environmental conditions will be interesting. For Goli variety combination B1C2D1-2-3 demonstrated to have more effect compared to other combinations on stem elongation. For Akhtar variety when combination B2C2D1-2-3 was used, the highest plant stem elongation was observed. KS31169 variety's maximum length of the stem was observed when combination of factors B1C3D1 and B1C2D1 were used (Figure 2). Root system characteristics can be used as one of the important parameters while selecting a plant for drought resistance. Among the evaluated parameters such as germination percentage and root and shoot growth, root length is considered as the best indicator for evaluation of the response of cultivars to drought stress (Michel and Kaufman1973). In fact, with increasing of drought stress, water availability for seed germination is reduced and thus the metabolic activities are slowed and root length is reduced. Reduction in root and shoot length under drought stress is natural. Reduced or no transfer of nutrients from the seed storage tissue to the fetus can be one of the reasons (Krishramorty *et al*., 1998). In general, the germinated seeds in environments that are under stress have shorter stems and roots (Katergy *et al*., 1994). Katrgy *et al*. (1994) evaluation of effects of salt and drought stress on root length of eleven varieties of cotton showed that this trait was influenced more compared to the shoot length. They concluded that the most sensitive part of the plant against this tension is the root length. They also found out that when under stress, Cytokininhormone's function in root halts. Belaqziz *et al.* (2009) reported that root and shoot length in medicinal plant was decreased with increasing of drought stress.

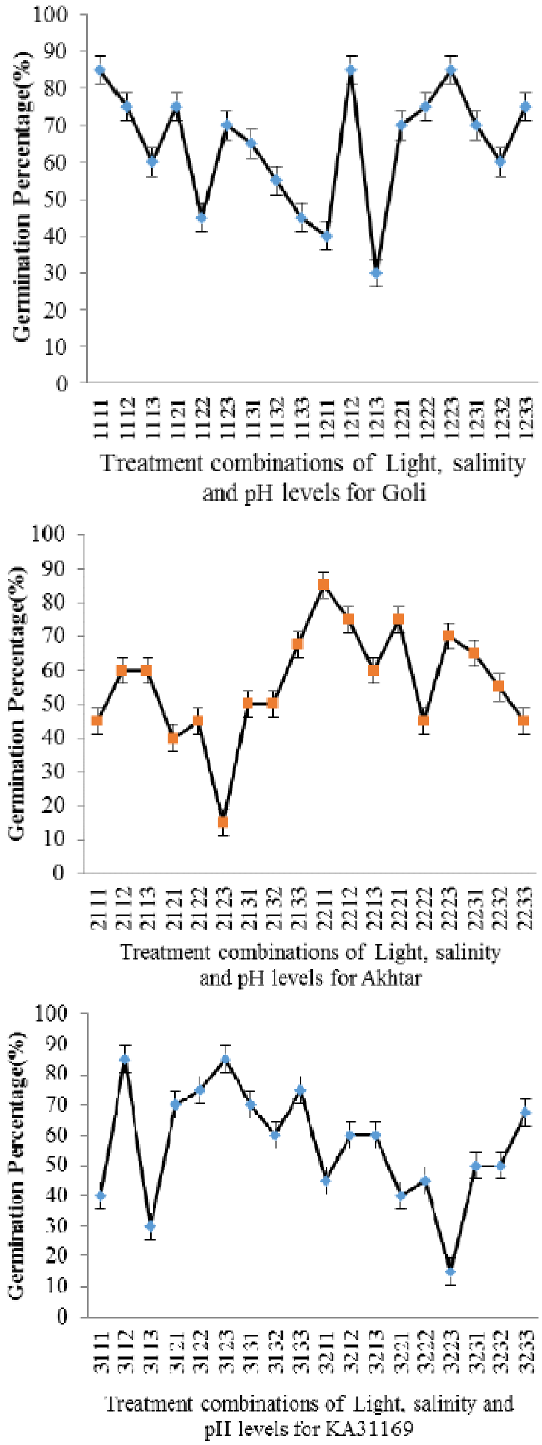

Fig. 1: The effect of different combinations of treatments on seed germination rates of different varieties (first, second, third and fourth numbers are different levels of treatment of light, salinity and pH, respectively).

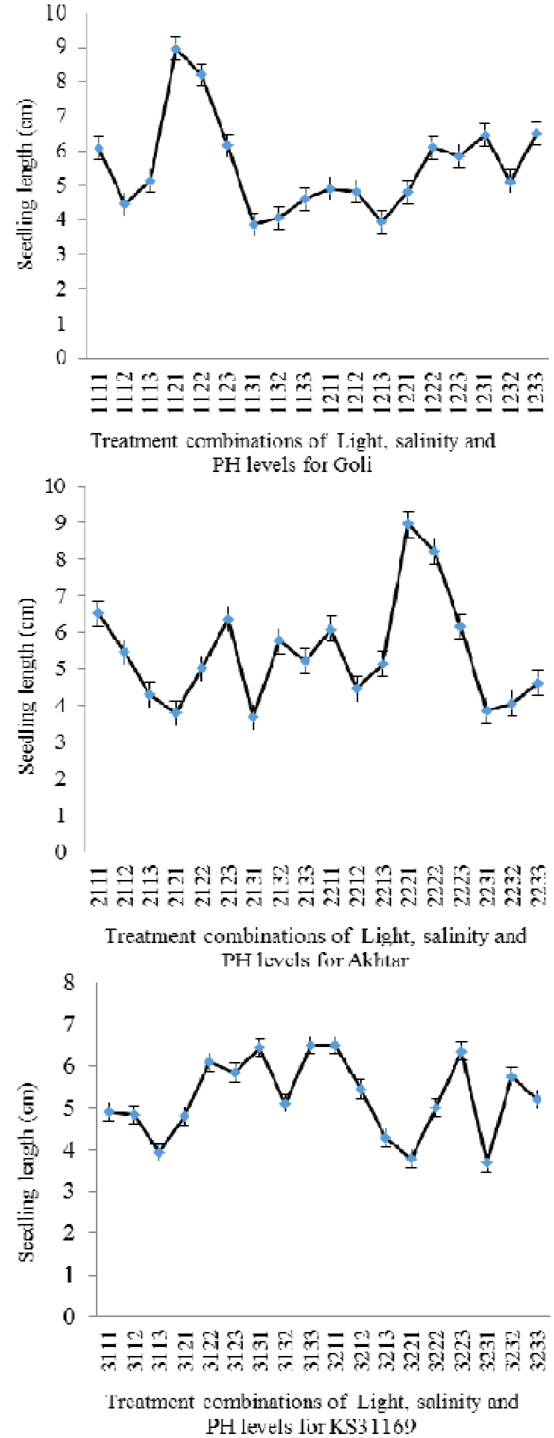

Fig. 2: The effect of different combinations of treatments on seed length of different varieties (first, second, third and fourth numbers are different levels of treatment of light, salinity and pH, respectively).

Root dry weight and seedling dry weight: two treatment combinations, 2232 (0.091) and 2132 (0.091), and two treatment combinations 3132 (0.025) and 1232 (0.025) showed the highest and the lowest root dry weight respectively. The Highest and the lowest dry weight of shoot were for treatment combinations of 2122 (0.9), 3222 (0.9) and 3132 (0.37), 1232 (0.37) respectively. Treatment combinations for seedling dry weight 3221 (1.41) and 2121 (1.41) showed the highest and treatment combinations 1232 (0.40) and 3132 (0.40) showed the lowest results for dry seedling weight. For Goli variety, treatment combination 1133 showed maximum and 1232 showed minimum seedling dry weight. This demonstrated that the third level of salt concentration, third level of

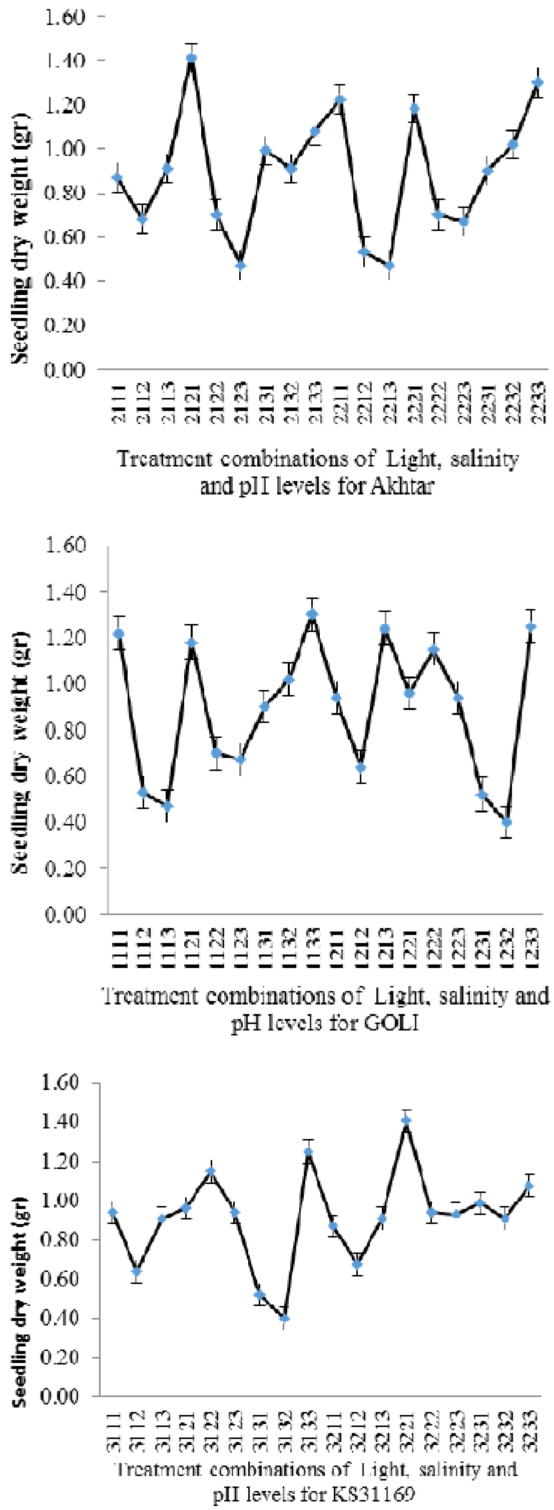

Fig. 3: The effect of different combinations of treatments on seedling dry weight of different varieties (first, second, third and fourth numbers are different levels of treatment of light, salinity and pH, respectively).

acidity and first level of light is the best treatment combination for this variety and resulted in highest seedling dry weight. For Akhtar verity these treatment combinations demonstrated in best results: third level of acidity with first level of concentration and second level of light, third level of acidity with second level of concentration and first level of light, third level of acidity with third level of concentration and second level of light. For KS31169 variety combination 3221 gave best response to environmental conditions and had the highest dry weight while combination 3132 showed the lowest dry weight. Combination of the second level of light with other factorial levels, except for 3221 and 3212, showed no difference (Figure 3). In a study on Milk thistle Kulkarni and Deshpande (2007) reported that reduction in root and shoot dry weight with increasing drought stress is natural. The results showed that by increasing the levels of drought stress, root and shoot dry weight of wheat has declined. High percentage, high speed and vigorous germination of seeds are suitable properties for crops planting in arid and semi-arid areas (Sosa *et al*., 2005).

The correlation between traits: The correlation index is used to measure and determine the correlation between two random variables. The correlation coefficient clarifies strength, weakness and direction of variations in two variables (Rezai, 1374). Relationship between traits (Table 2) showed that there is no significant relationship between seedling dry weight and germination percentage, root length, shoot length and seedling length. In general, germination percentage did not have high and significant correlation with any of the traits.

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