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The Evaluation of the Responses of Pinto Bean (*Phaseolus vulgaris* L.) Seedling Emergence and Growth to Different Seed Priming

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Abstract

The overall yield and yield component of leguminous crop largely depends on high rate of seed emergence, adequate seedling establishment and growth and development. The experiment was carried out in the experimental field research of Faculty of Agriculture Shiraz University. The aim of this research was to evaluate the effect response of pinto bean (*Phaseolus vulgaris* L.) seedling emergence and growth to different seed priming. The experiment was arranged in a completely randomized design in split plot with three replications. The treatment were Pinto bean varieties at three levels (E9, E10, and Khomain), pretreatments (priming) at seven levels: 1) polyethylene glycol (PEG), 5 bar at 6 hours; 2) salicylic acid (SA), 500 mg per liter at 12 hours; 3) potassium chloride (KCl), 20 mM at 6 hours; 4) calcium chloride ($CaCl_2$), 15 at 3 hours; 5) sodium chloride (NaCl), 15 mM at 6 hours; 6) water (H_2O), at 12 hours; 7) control (without pretreatment). The results showed that the effect of pretreatments on seedling traits was significant. The highest percentage, rate, emergence index, seventh and fourteenth day energy, height and total dry weight seedling was related to the pre-treatment the H_2O at 12 hours and $CaCl_2$ at 3 hours. According to the results it can concluded that pretreatment H_2O and $CaCl_2$ had been effective on the seedling emergence components. Principle component analysis showed that Seedling emergence rate was determined as the main component. Seed pretreatment improved the establishment and uniformity of seedling emergence on bean.

Keywords: emergence, pretreatment, principal component analysis, seedling

1. Introduction

Seedling establishment is a sensitive stage in crop growth. Uniformity and high emergence percentage has prominent effects on yield (Thakur et al, 1998). Speed and uniformity of emergence in the field is essential for achieving high performance with good quality (Parera & Cantliffe, 1994; Subedi & Ma, 2005). In this field, many research studies have shown that seed pretreatment is an effective technology to enhance speed and uniformity of emergence (Basra et al., 2004) to achieve high early vigor (Taghvaei et al., 2012; Giri & Schillinger, 2003; Farooq et al., 2006) and better yields in vegetables (Parera & Cantliffe, 1994) and field crops (Chiu et al., 2002). Germination begins with water uptake and imbibition. Early phase of germination included (i) imbibition, (ii) the lag phase and (iii) protrusion of the radicle through the testa (Hampton & Tekrony, 1995). In fact, this technique for the treatment of seed before germination can be used in specific environments and allows some metabolic processes necessary for germination to occur without the occurrence of germination (Taylor et al., 1998). Pretreatment is affected by some factors such as plant species, pretreatment media type and concentration, pretreatment duration, temperature, vigor and seed primed storage condition (Rezaei et al., 2009). Time of sowing to seedling establishment, has a large impact on the performance of field crops. Rate emergence, percentage emergence and seedlings establishment are important, too (Bradford, 1986). When seeds are planted in soil, they spend a lot of time to absorb water, if this time is reduced by pretreatment; the germination will be faster (D. C. Nielson & N. O. Nelson, 1998). Increasing the activity of hydrolytic enzymes such as alpha and beta amylase, iso-citrate lyaz and asteroase phosphatase and 3-phospho glycerate dehydrogenase on seeds pretreatment could justifie the improving emergence percentage and rate. Pretreatment by increasing the activity of the enzyme ATPase and increased RNA synthesis and acid phosphatase (Fu et al., 1988) and increased activity of amylase and protease and lipase (Ashraf & Foolad, 2005) improves the emergence rate in different plant

species. The effect of hydro and Osmo priming were useful on germination and growth factors of two commercial cultivars of peas under saline conditions and significantly increased indices of germination and seedling length (Khodabakhsh et al., 2010). The effects Osmo-priming of bean are improved seedling establishment, seed vigor, increased rate and percentage of seedling emergence thereby increased thousand grain weight and the yield and yield components (Jahanbakhshee et al., 2012). The effect Priming of salicylic acid on characteristics of cowpea (*Vigna unguiculata* L.) seeds under water stress at booting stage could increase emergence index and percentage, leaf area on the plant, reduce the number of days to flowering and day to booting causes Increasing grain yield in both irrigation and drought stress conditions (Shekari et al., 2010). Taghvaei and Ghaedi (2014) reported the effect of osmtic conditions on germination of *Haloxylona phyllum* L. sees and recovery under water had a significant on seedling length and seedling dry weight.

2. Material and Methods

The soil texture was clay and result soil analysis is shown in Table 1.

Table1. Soil properties of experimental field

Textured Soil				N ppm	P ppm	K ppm	PH	EC
Clay	Sandy%	Clay%	Silt%					
	18.84	42.44	38.72	119	12	330	6.92	0.631

The experiment was carried out in the experimental field research of Faculty of Agriculture Shiraz University. The aim of this research was to evaluate the effect response of pinto bean (*Phaseolus vulgaris* L.) seedling emergence and growth to different pretreatments. The experiment was arranged in a completely randomized design in split plot with three replications. The treatments were Pinto bean varieties at three levels (E9, E10, and Khomain), pretreatments at seven levels: 1) polyethylene glycol (PEG), 5 bar at 6 hours; 2) salicylic acid (SA), 500 mg per liter at 12 hours; 3) potassium chloride (KCl), 20 mM at 6 hours; 4) calcium chloride ($CaCl_2$), 15 at 3 hours; 5) sodium chloride (NaCl), 15 mM at 6 hours; 6) water (H_2O) at 12 hours and 7) control. In this study, Cultivars studied E9, E10 and Khomeini was prepared of Fars Research Center for agriculture and Natural Resources Eghlid. The seeds was maintained in plastic packages with minimum Permeability at temperature 5 °C to start the experiment. Before planting, the seeds were primed in the laboratory. The seeds were planted in the spring. The fields were ploughed, harrowed and divided into three replications each with 63 plots per replication. Each plot consisted of 6 rows planting with 2 m length. Inter and intra-row spacing were 30 cm × 10 cm. Seeds were sown in suitable depth. In this study daily counting to 14 were done. Different growth characteristics including emergence percentage, Emergence Index, Emergence rate, Energy of emergence (EE) Seedling height, and Leaf Area Index (LAI), total dry weight for pinto bean were measured under different treatment. Emergence percentage (EP) (Penalosa & Eira, 1993), Emergence Index (Shekari et al., 2010) was calculated by the following formula

Emergence Percentage:

$$EP = 100\ (ni/N) \tag{1}$$

Where, ni: number of emerged seeds per time; N: total number of planted seeds.

Emergence Index:

$$EI = (E1 \times 14) + (E2 \times 13) + \ldots + (E14 \times 1) \tag{2}$$

Where, E = emergence seedling number was counted from the first to the last count.

Mean time to full emergence and emergence rate were calculated according to the equation of Ellis and Roberts (1981).

$$MTE = \sum(ni \cdot ti)/\sum n \tag{3}$$

$$ER = 1/MTE \tag{4}$$

Where, ni: number of seeds newley emerged; ti: the number of time counted from the beginning of emergence.

Emergence Uniformity (Soltani et al., 2001):

$$GU = D90 - D10 \tag{5}$$

Where, D10 = the time takes to emergence reach to 10% their maximum; D90 = the time takes to emergence

reach to 90% their maximum.

Whatever number of emergence was less, was more uniformity.

Energy of Emergence (Agarwal, 2003):

$$EE = EP \text{ on determine day/total number of planted seeds} \quad (6)$$

At the end of the days 14th the leaf area of each treatment was recorded by leaf area meter model (DELTA-T). To determine the dry weight, samples were placed inside the oven for 24 hours at a temperature of 75 °C (ISTA, 1985.) then the sensitive balance with an accuracy of 0.0001 was measured. Principal component analysis was done by using SPSS. These Data were analyzed using SAS software version 9/1 and the differences between the mean compared using Duncan's at the 5% level.

3. Results and Discussion

3.1 Emergence Percentage

Pretreatment had a significant effect on emergence percentage (Table 2). The pretreatment with H_2O at 12 hours and KCL at 6 hours accounted for the highest and lowest effect on emergence percentage respective (Table 3). Pretreatment with water increased the emergence percentage. As the pretreatment of H_2O at 12 hours from 83.61 (control) reached to 92.40 and in primed with pretreatment KCL at 6 hours from 83.61 (control) reached to 72.68. There was no a significant difference in pretreatment calcium chloride 3 at hours, H_2O at 12 hours and control. In pretreatment PEG, NaCl, KCl at 6 hours and SA at 12 hours no significant difference was observed.

Table 2. Table experience variance (mean square) studied traits

S.O.V	Df	Emergence Percentage (Ep)	Emergence Index (EI)	Emergence Rate (ER)	Emergence Uniformity (EU)	Energy of Emergence (EE) seventh day	Energy of Emergence (EE) fourteenth day	Seedling Height	Leaf Area Index (LAI)	Total dry weight (TDW)
Block	2	259.81	2280950.78	3.53	0.05	0.01	0.02	124.33	1.91	150.67
Prime(P)	6	563.02**	5454954.2**	1.09*	0.81**	0.05**	0.04**	185.38**	193.23**	0.007**
Sub Error	14	162.3	2E+06	6.03	0.48	0.01ns	0.01	55.55	25	0.001
Cultivars©	2	15.86 ns	1841095.44*	3.06 ns	0.47 ns	0.01 ns	0.01ns	6.04ns	31.44 ns	0.001 ns
C *P	12	49.18ns	52469.52ns	4.8 ns	0.37 ns	0.007ns	0.003ns	34.36ns	72.04**	0.002*
Error	28	87.73	685291	1.2	0.26	0.21	0.17	746.88	26.67	0.001
Cv		11.67	16.75	11.29	7.25	17.37	11.71	4.86	11.7	10.47

Note. *, ** and ns, significant differences in levels 1%, 5%, and no significant respectively.

Table 3. Pretreatment on mean of EP, EI, MTE, ER, The seventh and fourteenth day of emergence energy, seedling height, LAI and Total dry weight

Treatment	Emergence Percentage (Ep)	Emergence Index (EI)	Emergence Rate (ER)	Emergence Uniformity (EU)	Energy of Emergence (EE) seventh day	Energy of Emergence (EE) fourteenth day	Seedling Height	Leaf Area Index (LAI)	Total dry weight (TDW)
PEG 6 hours	70.83c	41.44 b	0.0019ab	7.43a	0.42b	0.59c	110.88a	47.39a	0.31ab
Nacl 6 hours	78.42bc	47.23b	0.0018ab	7.09ab	0.48ab	0.65bc	106.33abc	41.51bc	0.28bc
$Cacl_2$3 hours	87.31ab	57.03ab	0.0017ab	6.74ab	0.56ab	0.72ab	110.55a	47.94a	0.33a
AS12 hours	76.11bc	44.10 b	0.0018ab	7.04ab	0.44b	0.63bc	101.33bc	40.23bc	0.27bc
Kcl 6 hours	72.68c	44.06b	0.0020a	7.36ab	0.45b	0.60c	107.55ab	45.23ab	0.30ab
H_2O 12 hours	92.40a	62.84a	0.0017ab	6.61b	0.62a	0.77a	109.11ab	49.62a	0.33a
Control	83.61abc	49.09ab	0.0016b	7.18ab	0.52ab	0.69abc	98.89c	37.04 c	0.26c
Cultivars									
E9	81.86a	52.15a	0.001a	7.21a	0.52a	0.68a	106.85a	44.24a	0.30a
E10	81.70a	49.79ab	0.001a	6.95a	0.50a	0.68a	105.95a	42.86a	0.29a
Khomain	77.02a	46.27b	0.001a	7.01a	0.47a	0.64a	105.90a	45.30a	0.30a

Note. Means with similar letters in each column are not significantly different according to Duncan's multiple range tests at the 5% level.

3.2 Emergence Index

Emergence index was significantly affected by pretreatment (Table 2). Average emergence index in H_2O at 12 hours pretreatment was 62.84 but it decreased by PEG at 6 hours 41.44 (Table 3). Pretreatment in H_2O at 12 hours, $cacl_2$ at 3 hours and control not a significant difference was observed. Also between pretreatments, NaCl, KCl, PEG at 6 hours and SA at 12 hours, were not observed difference. Cultivars had a significant effect on the emergence index (Table 2). The higher emergence index was 52.15 in E9 cultivar and the lowest emergence index was 49.27 in khomain cultivar. Between cultivars E9 and E10 no significant difference was observed (Table 3). Similar results have been reported about the rape seed by Ramezami and Rezaee (2013). Ghassemi Golezani et al. (2010) reported that primed beans seeds with hydro priming at 7 hours increased the emergence index, establishment of seedling, and plant biomass. Treatment priming significantly changed seedling emergence and establishment in rice (Farooq et al., 2006).

3.3 Emergence Rate

Emergence rate was significantly affected by pretreatment (Table 2). Average emergence rate in seed primed with KCl at 6 hours reached to 0.0020 while in seeds primed with control decreased to 0.0016 respectively (Table 3). Pre-treatment with KCl at 6 hours was significantly different from control. Other pretreatments didn't reveal significant difference from control. The results obtained in this study, were consistent with the results of Abdul Rahmani et al. (2007) and Ghassemi-Golezani et al. (2008). Pretreatment of seeds enhanced the speed of water uptake in the first phase of germination (Drew et al., 1997). The second phase of germination process is associated with a reduced rate of water up take over time. The start of the third phase and emergence after than is signaled by an increase in rate of water uptake, which in final. The accelerated experiment could be explained by more water uptake in the first and third phases of germination process germination (D. C. Nielson & N. O. Nelson, 1998).

3.4 Emergence Uniformity (EU)

Pretreatment had a significant effect on the emergence uniformity (Table 2). Average emergence uniformity in PEG at 6 hours pretreatment was 7.43 but it decreased by H_2O at 12 hours 6.61 (Table 3). Pretreatment of PEG, NaCl, at 6 hours, $CaCl_2$ at 3 hours, SA at 12 hours and control didn't reveal a significant difference. Similar results have been reported by Soltani et al. (2007). These traits due to coincidence in emergence are important at the field management and final yield (Soltani et al., 2007). The results indicate that the use of pretreatment at the appropriate time can increase the uniformity of emergence

3.5 Energy of Emergence (EE)

3.5.1 The Seventh Day Energy of Emergence (EE)

The seventh day energy of emergence was significantly affected by pretreatment (Table 2). Pretreatment on the seventh day energy of emergence was highest 0.62 and lowest 0.42 in H_2O at 12 hours and PEG at 6 hours, respectively (Table 3). Pretreatment H_2O at 12 hours with $CaCl_2$ at 3 hours, NaCl at 6 hours and control did not show a significant difference.

3.5.2 The Fourteenth Day Energy of Emergence (EE)

Pretreatment had a significant effect on the fourteenth day energy of emergence (Table 2). Pretreatment with H_2O at 12 hours 0.77 and PEG at 6 hours 0.59 were highest and lowest influence the fourteenth day energy of emergence respectively (Table 3). Between pre-treatment $CaCl_2$ at 3 hours and H_2O at 12 hours there was not a significant difference. Also KCl and PEG treatment had no significant difference.

3.6 Seedling Height

Pretreatment had a significant effect on Seedling height (Table 2). Pretreatment with $CaCl_2$ at 3 hours 110.55 and control 98.89 was highest and lowest cause on seedling height respectively (Table 3). Minimum height of the seedlings were observed in the control treatment with a significant difference with other treatments. Pretreatment of PEG, NaCl, KCl at 6 hours, $CaCl_2$ at 3 hours and H_2O at 12 hours showed no significant difference. Also between pretreatment SA at 12 hours and control no significant difference was observed. The results obtained in this study, were consistent with the results of Taghvaei and Ghaedi (2014) and Ghassemi-Golezani et al. (2010).

3.7 Leaf Area Index

Leaf area index was significantly affected by pretreatment (Table 2). Average pretreatment with H_2O at 12 hours 49.62 and control 37.04 had highest and lowest effects on leaf area index respectively (Table 3). Between pretreatment PEG, KCl at 6 hours, $CaCl_2$ at 3 hours, and H_2O at 12 hours no significant differences was observed. Pretreatment of SA at 12 hours and NaCl at 6 hours showed no significant difference. The results obtained in this

study, were consistent with the results of Shekari et al. (2010). Pretreatment increased the leaf area index. Soughir et al. (2013) reported that leaf area index plants derived from primed seeds were higher compared with non-primed seeds.

3.8 Total Dry Weight Seedling

Pretreatment had a significant effect on total dry weight seedling (Table 2). Pretreatment with H_2O at 12 hours 0.33 highest and control 0.26 showed the lowest total dry weight seedling (Table 3). Between control, SA at 12 hours and NaCl at 6 hours no significant difference was observed. Pretreatments PEG, KCl at 6 hours, $CaCl_2$ at 3 hours, and H_2O at 12 hours showed no significant difference. The efficiency of seed pretreatment for better seedling emergence and dry weight seedling is also reported by Abdul Rahmani et al. (2007) and Ghassemi-Golezai et al. (2008).

3.9 principal Component Analysis and the Correlations between Traits

The results of principal component analysis are presented on the basis of 9 characters. According to the eigenvalues of the main components (emergence rate), a main component was selected which explained 81.68% of the total variation (Table 4). The emergence rate was the largest contribution in the total variance was explained. Characteristics of emergence percentage, energy fourteenth day, leaf area index and total dry matter had the highest correlation coefficient with the first principal component (Table 5). Thus this component (emergence rate) as a main component establishment plant is introduced. Because changes are mainly related to the plant by the component justified. The second principal component (emergence index) explains 68.85% of total variance (Table 4). Leaf area index and total dry weight of the component have a high correlation coefficient with Component (Table 5). 46.29% of total variance explained by the third component (emergence percentage) (Table 4). The characteristics of speed, uniformity emergence and total dry weight have the largest coefficient in the component (Table 5). Correlations between traits (Table 5) showed that the emergence rate with characters such as emergence energy seventh and fourteenth days showed a significant positive correlation. It demonstrate the fact that emergence rate increases with increasing energy.

Table 4. Eigen values, variation explained (%), cumulative variance (%) and total variance explained of the principal components based on correlation matrix of emergence

Component	Eigen values	Variation explained (%)	Cumulative variance (%)	Cumulative %
Emergence Percentage(EP)	4.295	47.722	47.722	46.299
Emergence Index(EI)	2.054	22.824	70.546	68.858
Emergence Rate(ER)	1.002	11.139	81.684	81.684
Emergence uniformity(EU)	0.899	9.99	91.674	
Seventh day Energy of emergence(EE)	0.681	7.569	99.243	
Fourteenth day Energy of emergence(EE)	0.041	0.454	99.698	
Seedling Height	0.023	0.251	99.948	
Leaf Area Index	0.005	0.052	100	
Total dry weight(TDW)	-1.00E-13	-1.01E-13	100	

Table5. Coefficients of determination of the first three principal components related to the studied traits

Plant characteristics	Emergence Rate(ER)	Emergence Index (EI)	Emergence Percentage (EP)
Emergence Percentage(EP)	0.249	0.01	-0.08
Emergence Index(EI)	0.189	0.032	0.204
Emergence Rate(ER)	-0.234	0.036	0.59
Emergence uniformity(EU)	0.018	-0.054	-0.685
Seventh day Energy of emergence(EE)	0.18	0.01	0.256
Fourteenth day Energy of emergence(EE)	0.249	0.01	-0.08
Seedling Height	-0.076	0.141	0.076
Leaf Area Index	0.022	0.497	0.075
Total dry weight(TDW)	0.027	0.491	0.03

4. Conclusion

Pretreatment improves early vigor of bean seeds in field. The result showed that pretreatment of H_2O at 12 hours and $CaCl_2$ at 3 hours improve seedling height, leaf area index and total dry weight in the beans. In other words, primed seeds with of H_2O at 12 hours and $CaCl_2$ at 3 hours emerged faster than other treatments, so seedlings were withdrawn from the soil faster and quickly established. This action causes the seedling to be exposed to pests and pathogens soil for a shorter time. As well seedling competition with weeds on food sources will be more successful. Because the pre-treatment of seeds emerges more quickly, it produces more dry matter at a given time. Leaf area and height, thereby increase the plant's photosynthetic capacity. Principal components analysis showed that emergence rate (the first component) explains characteristics emergence percentage, energy fourteen days, leaf area index and total dry weight. The correlations between traits (Table 6) showed that Emergence rate (the first component) with traits such as emergence of energy, seventh and fourteenth days showed significant negative correlation but emergence index (the second component) with attributes such as emergence of energy, seventh and fourteenth days showed significant positive correlation. Demonstrate the fact that seedling height, leaf area index and total dry weight increases with increasing emergence rate. Pretreatment increased seedling height, leaf area index, total dry weight and establishment plants. Increasing the activity of hydrolytic enzymes such as alpha and beta amylase can be in primed seeds justified on the seedling height, leaf area index, total dry weight and establishment plants.

Table 6. Correlation of EP, EI, MTE, ER, the seventh and fourteenth day of emergence energy, seedling height, LAI and Total dry weight

	(EP)	(EI)	(ER)	(EU)	Seventh day (EE)	Fourteenth day (EE)	Seedling Height	LAI	(TDW)
Emergence Percentage (EP)	1								
Emergence Index (EI)	.905**	1							
Emergence Rate (ER)	-.655**	-.284*	1						
Emergence uniformity (EU)	-.278*	-.304*	0.026	1					
Seventh day Energy of emergence (EE)	.888**	.960**	-.288*	-.409**	1				
Fourteenth day Energy of emergence (EE)	1.000**	.905**	-.655**	-.278*	.888**	1			
Seedling height	-.254*	-0.189	0.159	0.163	-.275*	-.254*	1		
Leaf Area Index	-0.032	-0.074	-0.058	0.133	-0.131	-0.032	0.147	1	
Total dry weight (TDW)	-0.037	-0.101	-0.098	0.158	-0.147	-0.037	0.175	.970**	1

Note. *, **And respectively, significant differences in levels 1%, 5%.

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