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A Study Effects of Drought Stress on Germination and Early Seedling Growth of Flax (*Linum Usitatissimum* L.) Cultivars

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ABSTRACT

Water deficit is one of the most common constraints to crop productivity in the world. Therefore it is of prime necessity to try to find suitable genotype resistant to this limiting factor. In order to study the effect of drought stress on germination and early seedling growth of four cultivars of flax namely Legina, Linda, Sevenade and Bavaria by using three concentrations of PEG 6000 (0, -6 and -12 bar) an experiment was designed by using a Completely randomized design with four replications in Biotechnology Laboratory, Islamic Azad University- Karaj Branch. After 12 day of germination, shoot length, root length, seedling length, seedling fresh weight, germination percentage, germination rate, seed vigor and root to shoot ratio measured. Results of ANOVA showed significant differences among cultivars, stress levels and its interaction for most investigated traits, which demonstrates high diversity among cultivrs that enabled us to screen drought tolerant cultivar. On the basis of the results of this study, flax cultivar can be classified into two groups, Sevanade and Bavaria the best performing under drought conditions, Legina and Linda the cultivars having medium tolerance to water stress at germination stage and early seedling growth.

Key words: Germination percentage, Polyethylene glycol, Seed vigor.

Introduction

Among various environmental stresses, drought stress has become a critical problem worldwide due to its dramatic effects on plant physiology and performance [19].

[35,37,38] It can be said that it is one of the most devastating environmental stresses. Iran, with an annual 240 mm of rainfall, is classified as a dry region of the world [18].

Water deficit, extreme temperatures and low atmospheric humidity lead to drought, which is one of the most limiting factors for better plant performance and higher crop yield [30,16]. Therefore, plants that use water more efficiently can produce higher yields in water limited environments [11]. However, depending upon plant species, certain stages such as germination, seedling or flowering could be the most critical stages for water stress [1].

Germination is a critical stage of the plant life and resistance against drought during the germination makes a plant stable [18]. The adverse effects of water shortage on germination and seedling growth had also been well reported in different crops such as wheat [10,] sorghum [14] and sunflower [24]. Water availability of the soil is considered one of the principal causes of low germination in seeds [23]. Water stress acts by decreasing the percentage and rate of germination [8]. The study of the influence of the drought using osmotic solutions is one of the methods in the study of resistance during the germinal phase [27]. Polyethylene Glycol solution is commonly used to control water potential in seed germination studies.

Linseed (*Linum usitatissimum* L.) is one of the most important oil crops for the extraction of oil and fibres. About 80 % of the linseed oil goes for industrial purpose and the remaining 20 % is used for edible purposes [34]. it is a multi-purpose crop with benefits extending to both human and animal nutrition [29]. This reflects its very high content of essential fatty acids (EFAs), a high percentage of dietary fiber, and the highest level of “lignans” from any plant or seed products used for human food [25]. However, little is known about the response of linseed to osmotic stress. Experiments showed that the germination rate, plant fresh weight, hypocotyl and radicula length for all Flax genotypes were decreased at the increase of osmotic stress. The evaluated flax variety was significantly different for germination speed and radicula length [34].

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The present study was therefore, conducted with the objectives to determine the response of flax genotype to drought stress at germination and seedling stages under controlled conditions. Moreover, PEG was used for drought stress induction in Flax.

Materials and Methods

In order to study the effects of water stress on germination indices in flax cultivars, an experiment was conducted in factorial form, using a completely randomized design with four replications. In this experiment, four flax cultivars (Sevenade, Linda, Legina and Bavaria) were evaluated in three levels of drought treatment (distilled water as control,-6 and -12 bar). This experiment was carried out at Biotechnology Laboratory, Islamic Azad University-Karaj Branch, Iran.

Osmotic solutions are used to impose water stress reproducibly under in vitro conditions [26]. Polyethylene glycol molecules with a Mr_6000 (PEG 6000) are inert, non ionic and virtually impermeable chains that have frequently been used to induce water stress and maintain a uniform water potential throughout the experimental period [21]. Molecules of PEG 6000 are small enough to influence the osmotic potential, but large enough to not be absorbed by plants [28]. Because PEG does not enter the apoplast, water is withdrawn from the cell. Therefore, PEG solution mimic dry soil more closely than solutions of low Mr osmotica, which infiltrate the cell wall with solutes [33]. PEG 6000 was prepared by dissolving the required amount of PEG in distilled water and placed in a shaker bed (at 25°C) for 16 h.

The seeds were sterilized by soaking in a 5% solution of hypochlorite sodium for 5 min. After the treatment, the seeds were washed several times with distilled water. 25 seeds were put in each Petridish (with 9cm diameter) on filter paper moistened with respective treatment in 4 replications. The petridishes were covered to prevent the loss of moisture by evaporation. The petridishes were put into an incubator for 12 days at 20 centigrade degrees temperature. Every 24 hours after soaking, germination percentage and other traits were recorded daily. After 12 days of incubation, shoot length, root length, seedling fresh weight, seed vigor and root to shoot ratio of germinated seeds was measured. Seeds were considered germinated when the emergent radical reached 2 mm length. Rate of germination and coefficient of velocity of germination were calculated using the following formulas:

Formula 1: $GP = S_{NG}/S_{N0} \times 100\%$

Where GC is germination percentage, S_{NG} is the number of germinated seeds, and S_{N0} is the number of experimental seeds with viability [6,7].

Formula 2: $GR=\sum N/\sum(n\times g)$

Where: GR: Germination race; n: number of germinated seed on g^{th} day and g: Number of total germinated seeds

Formula 3: Seed Vigor = [seedling length (cm) × germination percentage]

The data were subjected to analysis of variance (ANOVA) using SAS release 9.1 software package. Means were compared using LSD at 5% probability level.

Results and Discussions

Analysis of variance showed that, there were significantly difference between cultivars, drought stress levels and them interaction. The results of this study reveal that various concentrations of PEG had a significant effect on the all measured traits (Table 1).

The differences between the means (Cultivars and drought stress levels) were compared by LSD and are shown in Table 2. It observed that, in all of cultivars there was a decrease in germination percentage due to drought stress increment and maximum germination percentage was delayed. While in this experiment different cultivars had different response to the drought stress. Among the flax cultivars, Sevenade had the highest germination percentage of 53.33% for the control treatment while Bavaria had the highest germination percentage of 8.33% at -6 bar drought level. At drought levels of 0 and -6 bar, the highest germination rate were attained from cultivars Linda and Legina respectively (Table 3). In contrast, the highest germination percentage in increasing drought levels was measured on Bavaria.

Under conditions of the highest drought stress that is -12 bar all cultivars had not any germination after 12 days. The maximum fall in germination percentage and germination rate was detected in Sevenade with 93.75% and 82.85% respectively, while the minimum was observed in Linda and Legina cultivars for germination percentage and germination rate respectively. Some studies referred that drought stress can contribute to improve germination rate and seedling emergence in different plant species by increasing the expression of aquaporins [13], enhancement of ATPase activity, RNA and acid phosphathase synthesis [12], also by increase of amylases, proteases or lipases activity [3].

The rate of reduction in germination percentage in comparison with the control was detected from Bavaria as 82.52%, Legina as 82.35%, Linda as 75.83% and Sevenade as 93.75%, which means that

Legina and Linda were more tolerant to drought stress in terms of germination percentage.

According to [4], decrease of seed germination under stress conditions is due to occur of some metabolically disorders. It seems that, decrease of germination percentage and germination rate is related to reduction in water absorption into the seeds at imbibitions and seed turgescence stages [15].

Root length is one of the most important characters for drought stress because roots are in contact with soil and absorb water from soil. For this reason, root length provides an important clue to the response of plants to drought stress. Among the cultivars, the longest root length was determined in cultivar Bavaria at -6 bar level while Legina gave the shortest root length at this level.

Generally, increasing drought levels decreased root length, and Linda cultivar exhibited the greater performance in respect of root length. The rate of decline in root length for the cultivar was more marked in Legina cultivar with 100% and sevenade cultivar with 98.69%. Mean shoot length varied between 0.18 and 0.85 cm in the cultivars and 1.35 and 0 cm for drought levels (Table 3). Mean shoot length was 1.35 cm at control level while it decreased linearly to 0 cm at -12 Bar drought stress.

In general, shoot length diminished with increasing salinity levels in all cultivars (Table 2). The highest and the lowest seedling fresh weight were observed in Sevenade and Legina cultivars, respectively (Table 2). Among the cultivars, Linda was affected the least by drought stress because it gave the lowest reduction rate for seed vigor. Although our cultivars showed different responses to each drought level, the highest values in control level were usually obtained from Bavaria cultivar except for germination rate trait. In addition, it was clearly determined that there were no statistical differences between measured cultivars at high salinity levels for all traits (Table 2).

Table 1: Analysis of variance of measured traits of flax cultivars under drought stress.

S.O.V	df	Germination Percentage	Root Length (cm)	Shoot Length (cm)	Seedling Length (cm)	Seedling Fresh Weight (gr)	Germination Rate	Seed Vigor	Root to Shoot Ratio
Cultivars	3	260.36**	0.38**	1.11**	2.77**	0.00006**	0.03*	10787.23**	0.08**
Stress	2	6943.21**	3.77**	9.82**	25.77**	0.00091**	0.58**	52914.62**	2.61**
Cultivars*stress	6	271.67**	0.37**	1.11**	2.74**	0.00006**	0.01ns	10781.00**	0.08**
Error	36	9.13	0.02	0.03	0.09	0.000009	0.007	128.51	0.01
Means		14.75	0.29	0.45	0.74	0.0043	0.19	33.29	0.23

*, **, ns: significant at 5%, 1% level and not significant, respectively.

Table 2: Mean comparison of main effects using Least Significant Difference (at 5% probability level).

	Germination Percentage	Root Length (cm)	Shoot Length (cm)	Seedling Length (cm)	Seedling Fresh Weight (gr)	Germination Rate	Seed Vigor	Root to Shoot Ratio
cultivars								
Sevenade	18.88	0.51	0.85	1.37	0.007	0.12	72.37	0.19
Bavaria	18.66	0.35	0.51	0.86	0.004	0.22	41.23	0.21
Legina	11.11	0.17	0.18	0.36	0.001	0.24	10.39	0.35
Linda	10.34	0.12	0.24	0.37	0.004	0.19	9.17	0.16
LSD 5%	2.50	0.11	0.14	0.25	0.002	0.07	9.38	0.10
drought stress								
0	38.58	0.85	1.35	2.20	0.01	0.38	99.65	0.70
-6	5.67	0.02	0.00	0.02	0.00	0.21	0.18	0.00
-12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LSD 5%	2.16	0.10	0.12	0.21	0.002	0.06	8.12	0.09

Table 3: Interaction mean comparison of measured traits using Least Significant Difference (at 5% probability level).

Cultivar	Drought Level	Germination Percentage	Root Length (cm)	Shoot Length (cm)	Seedling Length (cm)	Seedling Fresh Weight (gr)	Germination Rate	Seed Vigor	Root to Shoot Ratio
Sevenade	0 (Control)	53.33	1.52	2.57	4.09	0.02	0.33	216.97	0.60
Sevenade	6 bar	3.33	0.02	0.00	0.02	0.00	0.06	0.17	0.00
Sevenade	12 bar	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bavaria	0 (Control)	47.66	1.02	1.55	2.57	0.01	0.39	123.42	0.66
Bavaria	6 bar	8.33	0.03	0.00	0.03	0.00	0.29	0.27	0.00
Bavaria	12 bar	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Legina	0 (Control)	28.33	0.51	0.57	1.08	0.01	0.40	31.18	1.07
Legina	6 bar	5.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00
Legina	12 bar	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Linda	0 (Control)	25.00	0.35	0.74	1.09	0.01	0.41	27.23	0.48
Linda	6 bar	6.04	0.03	0.00	0.03	0.00	0.18	0.30	0.00

Linda	12 bar	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LSD 5%		4.33	0.20	0.25	0.43	0.004	0.12	16.25	0.18

It seems that, PEG concentration (drought stress) affects on seed germination via limitation of water absorption by seeds [11], excessive use of nutrient pool [5] and creation of disorders in protein synthesis.

PEG concentrations resulted in a decline of both shoot length and root length. In all drought levels, Legina gave the highest root to shoot ratio while the lowest values were determined on Linda. The decline in seedling length between the control and final drought levels was the lowest in Linda with 97.48% while the highest reduction was determined on Legina with 100%.

Generally, all measured traits declined with increasing drought levels, which showed that a greater reduction in shoot length occurred than that in root length. On the other hand, it means that the shoots were more adversely affected than roots by PEG concentration. [2] found that, resistance to stress at germination stage and primary growth of seedling is independent from next growth stages and evaluation of stress tolerance need more experiment at next growth stages.

Discussion:

Polyethylene glycol (PEG) causes osmotic stress and could be used as a drought simulator [31]. In the present experiment PEG-6000 was used to create the osmotic stress, as most of the researchers [17] utilized it for the development of water deficit environment in laboratory studies. The variation among cultivars showed that germination percentage decreased with the increase in PEG-6000 concentration in all the cultivars. However Sevenade performed better than others. Many reports indicated that germination percentage and seed vigor can be utilized as screening criteria for stress tolerance.

In present study the findings are very similar to the former case, in which germination decreased due to the increase in PEG-6000 concentration. Present study strongly supports that germination percentage and seed vigor can be utilized to screen sunflower germplasm for drought tolerance. There are many reports which are in agreement with the present findings indicating that drought stress severely reducing the seed germination and early seedling growth. But the varieties having genetic potential to maintain the higher growth under stress conditions are drought tolerant. On the basis of the results of this study, flax cultivar can be classified into two groups, Sevanade and Bavaria the best performing under drought conditions, Legina and Linda the cultivars having medium tolerance to water stress.

Conclusion:

Results of this experiment showed that, different levels of drought stress have significant effect on flax seed germination and early seedling growth. Many researchers have been reported similar results [9,22]. Obviously, acceptable growth of plants in arid and semiarid lands which are under exposure of drought stress is related to ability of seeds for best germination under unfavorable conditions, so necessity of evaluation of drought resistance cultivars is important at primary growth stage. To find the best tolerant cultivar to such conditions, taking all traits into account in this study, we found that Sevenade is the most resistant and Linda is the most sensitive varieties.

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