

Seed Treatment during Germination in Linseed to Overcome Salt and Drought Stresses (*Linum usitatissimum* L.)

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Abstract—Evaluation of crop plants resistance to environmental stresses specially in germination stage is a critical factor in their selection in different conditions of cultivation. Therefore use of a procedure in controllable situation can help to evaluate plants reaction to stress quickly and precisely. In order to study germination characteristics of flax in water and salinity stress conditions were conducted two laboratories experimental. The two experimental were conducted in 4-replicant completing random design for salinity and water stress. The treatment, for salinity and water stress was three potential (zero, 40, 80 mM) of NaCl and three potential (zero, -2, -4 bar) of PEG respectively. Germination percentage and rate, in addition to Radical and plumule length and dry-weight and plumule/Radical ration were measured. All of characteristics reduce under water stress conditions. salinity stress significant reduce germination rate and Radical and plumule length of flax seeds. Hydropriming and osmopriming significant increased germination rate, plumule length and plumule/Radical ration of flax seeds. But germination percentage and Radical and plumule dry weight significant increased only in hydropriming treat. Hydropriming and osmopriming could not be used to improved germination under saline and drought stress. But has more tolerance in salinity and drought stress in flax by less reduce in Radical and plumule length under saline and drought stress.

Keywords—linseed, salt stress, water stress, seed treatment, Germination

I. INTRODUCTION

LINSEED is a one year plant and oil seed which is cultivated for oil use and fiber [25]. In most of the crop plants, seed germination and the primary growth of the seedling is one of the most sensible stages which is a reaction to the environmental stress. This stage of the plants life has a determining role on suitable and the final function of the plant [1]. Salinity of water and soil is one of the important in the dry and mid-dry regions, which can decrease the amount of production [28]. Salinity stress leads to decrease in germination and seedling establishment [1]. Being dry decrease the water absorption during ionic absorption process [19]. The sensibility to dry and salt stress differs during different stages of germination and Radical emergence [6]. Several reports indicates that the seeds which are able to show the suitable reaction to drought and salinity, during germination stage, have a better growth on seedling stage and will produce a more strong root system [17].

According to achieve improvement on germination of different seed product in stressful and non-stressful circumstances seed priming is used [2]. Different priming seed techniques is used, involving hydropriming, osmopriming with different hormones is reported, to facilitating the seedling emergence and resistance increasing to drought in different plants such as wheat [11], chickpea [12], sunflower [13], cotton [4]. Also the priming advantages is reported in other plants like; beet, corn, soybean and sunflower [9], [29], [26]. Rao et al [23] reported that canola primed seeds reduce the establishment risk of weak seedling in cold and unsuitable conditions. So priming improves the seedling growth under the stressful condition of water deficiency and salinity stress.

Salinity and drought are two increasing stress in the soil of the region and one of the ways to overcome with this, is selecting resistance cultivars. This research has studied germination reaction in the aspect of percentage age and germination percentage and rate, in addition to radical and plumule length and weight and plumule/radical ration linseed Genotype comparison on different levels salinity and drought stress, hydropriming and osmotic priming impact with KNO₃ upon linseed salinity and drought resistance will be studied on the germination stage. So, germination of this plant is recommended with more assuredness in the regions with salt soils and lack of water in first growth stages.

II. MATERIALS AND METHODS

This study has been done upon the linseed in 1388. For making drought and salinity stress, the soluble is used with osmotic potential zero, -2 and -4 bar of PEG-6000 [2]. Three grades of NaCl was respectively as: zero, 40 and 80 mM [5]. For hydropriming, linseed seeds were in a dark place for 6 hours in 25° temperture. For osmopriming, linseed seeds were in a dark place for 3 hours in 25° in 500 (ppm) KNO₃ soluble.

Germination test

For doing this test 50 primed seed was placed in four frequency in petri dish with watman paper, No 1, 10 mililitre in the prepared soluble. The papers in the soluble of NaCl were changed per 2 days. The time the radical length is 2 mililitre the seeds are in the germination stage.

The seeds were observed everyday and the number of the germinated seeds was registered. In the end of the last day plumule and radical length, and plumule/radical ration (R/S) and the dry-weight of the seedling was measured with 10 sample for each frequency. Germination percentage and rate were measured respectively with (1 and 2) relations, and

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plumule/radical ration (R/S) was measured according to soltane and colleague method [30] (3 relations).

$$\text{germination percentage} = S/T * 100 \quad (1)$$

$$\text{germination rate} = N1/D1+N2/D2+\dots+Ni/Di \quad (2)$$

$$\text{Allometry} = \text{plumule length} / \text{radical length} \quad (3)$$

In which S indicates the number of the germinated seeds, T indicates the total number of the seeds and Ni indicates the number of the germinated seeds on Di day. The total of the research were measured through SAS software. The averages were compared with the (LSD) test.

The experiment design

The experimental design on the factorial form (3×3) was performed on the frame of a completing random design with four replicant. The first factor, were the seed treatments including control, KNO₃ and hydropriming. The second factor was osmotic potential grades which is zero, -2 and -4 bar in PEG soluble or the salinity grades which is zero, 40 and 80 mm.

Data analysis is done through SAS and the differences between the averages is measured by LSD method (P<0.05).

III. RESULTS

The drought stress leads to the significant decrease of rate (42%) and germination percentage (12%), radical (87%) and plumule the (66%) length and plumule/radical ration (70%) and dry-weight of the Radical (70%) and plumule (73%) on the linseed seeds (table 1 and 2).

In the experience the drought stress, hydropriming and osmopriming leads to the significant increase in the plumule length and plumule/radical ration in the linseed seeds. But germination percentage and rate, radical and plumule dry-weight, shows a significant increase only in hydropriming treatment. It also should be mentioned that in hydropriming treatment the germination percentage and rate an increase had been detected, though it was not significant in statistical aspect (table 1 and 2).

The significant conjunction effect between drought stress and treated seeds in the features of radical and plumule length and plumule/radical ration showed that under drought stress condition radical and plumule length features and plumule/radical ration, because of the seed treatment showed less decrease with the increase of dry stress in comparison with the untreated seeds. There exists no mutual significant effect between drought stress and treated seeds for the other features under study (table 3). According to the positive and significant correlation ($r=0.97^{**}$) among radical and plumule length. In this study, if the seed is able to make a seedling with longer radical, under stress, it of course will have a longer plumule and as a result of this, through increasing the photosynthesis amount in the more resistance cultivars leads to keep and increasing the yield under stress condition. Salinity stress leads to the significant decrease of the germination rate (9%), plumule length (36%) and radical

length (32%) in the linseed seeds (table 4 and 5).

TABLE I
RESULT FROM ANALYSIS OF VARIANCE OF LINSEED GENOTYPE TESTED FOR THE GERMINATION PERCENTAGE AND RATE, RADICAL AND PLUMULE LENGTH AND WEIGHT AND PLUMULE/RADICAL RATION UNDER DROUGHT STRESS

Radical weight	plumule weight	plumule/ radical	Radical length	plumule length	Germination rate	germination percentage	Df	Source of variation
0.000003 6	0.000004	0.0016	0.26	0.17	2.38	0.11	3	replicant
0.00011**	0.00044*	0.065*	87.02*	85.0*	98.9**	296.9*	2	drought
0.000034*	0.00007*	0.089*	0.38	2.89*	10.0**	121.6	2	seed treatment
0.000008	0.000004	0.038*	3.02*	2.1*	1.07	28.06	4	seed treatment × drought
0.000003 7	0.000001	0.0076	0.92	0.55	1.21	39.5	24	Error

* $P < 0.05$, ** $P < 0.01$

TABLE II
EXPERIMENTAL FACTORS EFFECT ON THE GERMINATION PERCENTAGE AND RATE, RADICAL AND PLUMULE LENGTH AND DRY- WEIGHT AND PLUMULE/RADICAL RATION UNDER DROUGHT STRESS

Plumule weight (gr)	radical weight (gr)	plumule/ radical	plumule length (cm)	Radical length (cm)	Germination rate	germination percentage	Experimenta l factors
							grdrought
0.01 ^a	0.019 ^a	0.76 ^a	9.3 ^a	7.09 ^a	15.8 ^a	91.6 ^a	0
0.008 ^a	0.012 ^b	0.6 ^b	6.3 ^b	3.8 ^b	12.8 ^b	83.7 ^b	-2
0.003 ^b	0.005 ^c	0.23 ^c	3.1 ^c	0.94 ^c	9.10 ^c	80.5 ^b	-4
							seed treatment
0.006 ^b	0.012 ^b	0.42 ^b	6.0 ^a	3.3 ^b	11.5 ^b	81.5 ^b	control
0.009 ^a	0.015 ^a	0.55 ^a	6.4 ^a	4.2 ^a	13.6 ^a	88.9 ^a	Three hours KNO ₃
0.006 ^b	0.01 ^c	0.62 ^a	6.2 ^a	4.3 ^a	12.6 ^{ab}	85.5 ^{ab}	six hours hydropriming

The means with at least one common letter, according LSD test at 5%, probability have no significant differences

In a study which has been done on three cultivar of wheat under salinity stress a decrease on the germination percentage and rate, radical and plumule length and plumule weight in the three cultivars [11].

In the salinity stress experiment, hydropriming and osmopriming increased significantly the rate of germination, plumule length and plumule/radical ration in the linseed seeds. But radical dry-weight showed increase just in hydropriming treatment. It has to be mentioned that in each two seed treatment, the germination percentage, there exists increase, although it was not significant in statistical aspect (table 1 and 2). The significant conjunction effect between salinity stress and treated seeds in the features of radical length and radical dry-weight showed that under the salinity stress, the radical length feature had less decrease with salinity levels increase in comparison with the untreated seeds, because of seed treatment. However the radical weight in treated seeds was comparatively fixed with salinity stress increase. Significant

conjunction effect among salinity stress and treated seeds was not observed on the other features under study (table 6).

Some believes that in the stress condition, dry material storage in the radical and plumule tissues of resistance cultivars, is more probable than the sensitive cultivars [18]. In this experiment a positive and. significant correlation ($r=0.82^{**}$) was shown among plumule and radical length.

In the both experiments which had been done, the impact of the two seed treatment was not significant. But in both experiments a significant conjunction effect among salinity stress and the treated seeds was observed, also drought stress and treated seeds on the radical length feature was observed (table 1 and 4).

TABLE III
AVERAGES, LENGTH AND WEIGHT OF RADICAL AND PLUMULE OF LINSEED GENOTYPE UNDER DROUGHT STRESS

plumule/ radical	Radical length (cm)	Plumule length (cm)	drought stress	seed treatment
0.72 ^{ab}	9.8 ^a	6.9 ^a	0	control
0.49 ^{cd}	5.5 ^c	2.7 ^{cd}	-2	control
0.07 ^e	2.7 ^d	0.2 ^e	-4	control
0.84 ^a	8.7 ^{ab}	7.3 ^a	0	hydropriming
0.64 ^{bc}	7.7 ^b	5.0 ^b	-2	hydropriming
0.18 ^e	2.8 ^d	0.4 ^e	-4	hydropriming
0.73 ^{ab}	9.4 ^a	6.9 ^a	0	osmopriming
0.68 ^b	5.6 ^c	3.9 ^c	-2	osmopriming
0.46 ^d	3.7 ^d	2.1 ^d	-4	osmopriming

The means with at least one common letter, according LSD test at 5%, probability have no significant differences.

TABLE IV
RESULT FROM ANALYSIS OF VARIANCE OF LINSEED GENOTYPE TESTED FOR THE GERMINATION PERCENTAGE AND RATE, RADICAL AND PLUMULE LENGTH AND WEIGHT AND PLUMULE/RADICAL RATION UNDER SALINITY STRESS

Radical weight	plumule weight	plumule/radical	Radical length	plumule length	germination rate	germination percentage	Df	Source of variation
0.00000004	0.000005	0.020	0.306	0.807	0.474	11.32	3	replicant
0.000005 ^{**}	0.00024 ^{**}	0.057	27.91 ^{**}	15.84 ^{**}	6.17 ^{**}	44.7	2	salinity
0.000009 [*]	0.00019 ^{**}	0.191 ^{**}	0.55	5.59 ^{**}	11.3 ^{**}	33.4	2	seed treatment
0.000004 ^{**}	0.0001	0.034	3.18 [*]	1.95	0.25	11.51	4	seed treatment× salinity
0.0000008	0.00002	0.017	1.06	0.812	0.577	27.3	24	Error

* $P<0.05$, ** $P<0.01$

IV. DISCUSSION

As in this experiment, the drought stress results in decrease on the under studied features. The decrease in the germination as a result of drought stress can decrease the water absorption by seeds. If the water absorption has any problem or the absorption process was slow, germination metabolic actions will happen inside the seed slowly. So the radical emergence time will be increased and the germination rate will be decreased [22].

TABLE V
EXPERIMENTAL FACTORS EFFECT ON THE GERMINATION PERCENTAGE AND RATE, RADICAL AND PLUMULE LENGTH AND DRY- WEIGHT AND PLUMULE/RADICAL RATION UNDER SALINITY STRESS

Plumule weight(gr)	radical weight(gr)	plumule/radical	plumule (cm)	radical (cm)	germination rate	germination percentage	Experimental factors
salinity							
0.0083 ^a	0.019 ^b	0.76 ^b	9.3 ^a	7.0 ^a	15.7 ^a	97.1a	0
0.0073 ^b	0.029 ^a	0.81 ^a	6.2 ^b	5.0 ^b	14.3 ^b	89.1a	40
0.0088 ^a	0.023 ^b	0.65 ^b	6.3 ^b	4.5 ^b	14.3 ^b	87.2a	80
seed treatment							
0.008 ^b	0.029 ^a	0.57 ^b	7.1 ^a	4.6 ^b	13.5 ^b	87.2 ^a	control
0.009 ^a	0.024 ^a	0.84 ^a	7.1 ^a	6.0 ^a	15.7 ^a	91.0 ^a	Three hours Kno ₃
0.007 ^b	0.023 ^a	0.80 ^a	7.6 ^a	6.0 ^a	15.1 ^a	89.7 ^a	six hours hydropriming

The means with at least one common letter, according LSD test at 5%, probability have no significant differences

TABLE VI
AVERAGES, LENGTH AND WEIGHT OF PLUMULE OF LINSEED GENOTYPE UNDER SALINITY STRESS

weight of plumule(gr)	length of plumule(cm)	Salinity stress	seed treatment
0.008 ^{bcd}	9.8 ^a	0	grcontrol
0.008 ^{bcd}	6.1 ^e	-2	control
0.007 ^{cde}	5.6 ^e	-4	control
0.009 ^{ab}	8.7 ^{abc}	0	hydropriming
0.007 ^{de}	7.0 ^{cde}	-2	hydropriming
0.01 ^a	5.6 ^e	-4	hydropriming
0.007 ^{cde}	9.4 ^{ab}	0	osmopriming
0.006 ^e	5.6 ^e	-2	osmopriming
0.008 ^{bc}	7.7 ^{bcd}	-4	osmopriming

The means with at least one common letter, according LSD test at 5%, probability have no significant differences

The germination rate is one of the important aspects of the seed base which can be measured as a limiting factor in the plant establishment. the seeds which grow rapidly will have less exposure to the pest and disease attacks. So the establishment stability will be more in the farm. In this state the seedling is able to have a better and proper use of the environmental factors. In the survey safflower cultivars reactions against drought [97] showed that the radical length is a proper criteria to determining the resistance cultivars against drought. One of the reasons of plumule length decrease in the drought stress condition, is the decrease or the lock in transferring the nutritions from seed storage tissues to the embryo. In addition, reduction in water absorption through the seed in drought stress condition results in exudates hormones and enzyme reduction and at last it made problem with the growth of plumule, radical and seedling [32].

According to the obtained results on examining seed treatment and drought stress, significant hydropriming treat against osmopriming in relation with germination percentage and rate features and plumule length can be the result of quick water absorption in hydropriming seeds, and this process in

the seed will be done with a more speed. As a result of the water absorption cell dividing will be done with more speed and this will increase the germination percentage and rate and radical and plumule length. In most of the plants, pre-treatment the seeds make a better germination and seedling establishment [27]. Kaour et al [12] reported that hydropriming processes on the seeds under non-stressful condition in converse with stress, increases the radical and plumule length. During an experiment Fujikura et al [8] showed that the least amount of treated seeds with hydropriming was more than treated seeds with KNO₃. Morady et al [18] showed that hydropriming affect the corn seedling dry weight more, in comparison with the osmopriming method.

Salinity stress showed more delicacy of the aerial parts to salinity. Root with more length and consistency soil water absorption, will not face with the lack of water in critical stages of growth and prevents from decrease in yield under stressful conditions [33]. Actually the seeds which are able to growing a higher length root under salinity stress are more successful in comparison with the seeds which do not have this capability [14]. The reason of reduction germination percentage and rate with increased salinity is because of over-presenting of cation which not only are toxic but can decrease the water potential, in a way which the plant is unable to absorb water and has a kind of water deficit [29]. So more negative osmotic potentials from salt density increase and toxic ions prevents from enzyme hydrolysis of the seed storage materials and building new tissues with the use of hydrolysed material [24]. In addition, salinity hurts the cell membranes on germination stage, especially cytoplasm membrane and as a result of this the increase in membrane permeability will occur, because of the substitution of Ca⁺⁺ through Na⁺ which increases the K⁺ casualties [31]. The most effects of the stress of water its salinity, includes different patterns in proteins synthesis, make delay on emergence the basic tissues, and decrease in germination percentage and rate [21]. Existing of sufficient water for seeds and seedlings prevent these processes to be performed. In this experiment, salinity stress had not had a negative significant effect upon linseed germination percentage and the linseed is perhaps resistance in this feature against the salinity stress.

According to the results obtained from those two experiments it seems that in addition to this fact that linseed genotypes has relative tolerance to salinity, are in a high sensibility in comparison with drought stress. In other experiment the effect of PEG upon safflower germination was more than NaCl [3]. There exist some reports on the PEG to be toxic. its toxic effect can be of the high intensity of Al and Mg ions which is used in its synthesis. Also, it seems that PEG toxic is fundamentally because of under use oxygen reduction. So plant growth in a soluble which involves PEG will be damaged [10]. In both experiments the effect of both treatment seed on the radical length was not significant. But in both of the experiment there exist a significant conjunction effect among salt stress and the treated seeds, also the drought stress on the radical length feature was observed (table 1 and 4). As

it was stated before more length of the root and consistency of water absorption would prevent the lack of water in the critical period of growth and also prevent the reduction of yield under drought stress condition [33]. The seed which are capable of having a root with more length are in more consistent against drought and salinity [14]. In this experiment hydropriming and osmopriming leads to the higher tolerance in against drought in the linseed plant by less decrease of radical length.

V. CONCLUSION

According to the results of these two experiments, it seems that linseed genotypes has a relatively tolerance against salinity, also are very sensitive in relation with drought stress. Hydropriming and osmopriming caused recovery on germination under non-stress conditions. It has to be mentioned that also the seed treatment does not make any improvement in germination percentage and rate in salinity and drought condition. But through increase and decrease in radical and plumule length will be maintained by the capability of more able in absorbing water and protecting the photosynthesis bodies (as an effective factor in yield) leads to the yield increase and probably increase in the resistance of drought in the linseed plant.

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