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Annals of Biological Research, 2012, 3 (5):2479-2485  
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## The effects of Osmo and Hydrothermal priming on germination traits of Sheep Fescue (*Festuca ovina* L.)

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### ABSTRACT

For investigating the effects of osmopriming and hydropriming treatments on the germination indexes and seed vigor on Sheep Fescue (*Festuca ovina*), two experiments were done. In the first experiment which was fulfilled based on randomized complete block design with four replicates, effect of polyethyleneglycol 6000 solution was investigated in five osmotic potentials (-8, -10, -12, -14 and -16 bar), two temperatures (15 and 25° C) and four times (12, 24, 36 and 48 hours). Second experiment was fulfilled by using distilled water in two temperatures (15 and 25° C) and four times (12, 24, 36 and 48 hours) with four replicates on the above mentioned. Then, based on the maximum germination rate and vigor indexes, the best treatment composition was determined for osmopriming and hydropriming solutions from the view point of osmotic potential, temperature and priming duration. In osmopriming experiment, treatment composition of 12 hours in 15° C and -8 potential had the most significant effect on the percent and speed of germination, length of radicle, coleoptile and seedling as well as vigor indexes in Tall Fescue. In hydropriming effect on this plant, the same condition as osmopriming was observed and in the majority of evaluated traits, treatment composition of 12 hours prime and 15° C had the maximum numerical value in comparison to control and other treatments. Finally, it is inferred that favorite effects of osmo and hydropriming in 15° C temperature are increased in comparison with the control group (none prime seeds).

**Key words:** Osmopriming, hydropriming, temperature, seed, germination.

### INTRODUCTION

Rapid seed germination and stand establishment are critical factors to crop production under stress conditions. In many crop species, seed germination and early seedling growth are the most sensitive stages to stresses. Seed priming is known as seed treatment which improves seed performance under environmental conditions. In fact seed priming is a procedure that partially hydrates the seed, and then seeds are dried, so that germination processes begin, but radicle emergence does not occur. Methods of seed priming have been described comprehensively by Bradford (1986) and Khan (1992) which include soaking seed in water or osmotic solution, and intermixture with porous matrix material. Lots of information are available which show hydration of seeds up to, but not exceeding, the lag phase with priming increased RNA and protein synthesis [16], faster embryo growth [11] and reduced leakage of metabolites [39] compared with control group. Seed priming has been found a doable technology to enhance rapid and uniform emergence, high vigor, and better yields in vegetable and flower species [12,33,8] small seeded grasses [20,7] and some field crops [19,9,17,4, 27, 28, 22].

Seed priming is commonly used to reduce the time between seed sowing and seedling emergence [33]. Earlier works showed that the success of seed priming is influenced by the complex interaction of factors including plant species,

water potentiality of the priming agent, duration of priming, temperature, seed vigor and dehydration, and storage conditions of the primed seed [33]. Although, the previous studies indicate that some benefits are associated with pre-sowing treatments for seed vigor enhancement, but there is dearth of information about the germination traits of primed seeds of Sheep Fescue. Therefore, the present study was carried out with the objective of evaluating the effects of different priming treatments on seed germination manner of Sheep fescue under different temperature to find out the most effective priming composition treatments.

## MATERIALS AND METHODS

### *Sample preparation*

This study was carried out at the Department of Agronomy, Faculty of Agriculture, University of Tehran, Iran. Sheep Fescue seeds were used as seed material obtained from the Natural Resources Organization of Hamedan Farm (from Hamedan province, Iran).

### *Seed treatments*

For osmopriming, Sheep Fescue seeds were immersed in osmotic potentials of -8, -10, -12, -14, -16 bar, for polyethylene glycol (PEG 6000) at 15 °C and 25 °C for 12, 24, 36 and 48h under dark conditions [31]. Thereafter, the seeds were rinsed with distilled water three times. The treated seeds were surface-dried and dried back to their original moisture content via experience at room temperature. osmoprimed treated seeds was equilibrated at room temperature (about 25°C) for 24 hours.

For hydropriming, seeds were immersed in distilled water at 15 °C and 25 °C for 12, 24, 36 and 48h under dark conditions. The treated seeds were surface-dried and dried back to their original moisture content at room temperature (about 25°C) for 24 hours.

### *Germination tests*

Four replicates of 50 seeds were germinated top of double layered papers with 5 ml of water in 9cm Petri dishes. These Petri dishes contained seeds were put into sealed plastic bags to avoid moisture loss. Seeds were allowed to germinate at 15-25±1°C in 14 days (ISTA, 1996). Germination was considered to have occurred when the radicles were 2mm long. Germination percentage was recorded every 12 h until final mentioned days. Germination rate was calculated as described in following formula (ISTA, 1996):

$$GR = \frac{\text{No of germinated seed at first count}}{\text{days of first count}} + \dots + \frac{\text{No of germinated seed at final count}}{\text{days of final count}}$$

Seedling length, seedling dry weight were measured after the 14th days. Vigor indexes 1&2 were calculated according to the following formula:

$$\text{Vigor index 1 (VI1)} = [\text{seedling length (cm)} \times \text{germination percentage}]$$
$$\text{Vigor index 2 (VI2)} = [\text{seedling dry weight (gr)} \times \text{germination percentage}]$$

### *Statistical analysis*

The Statistical analysis was based on a randomized completely block design (RCBD); with four replications and 50 seeds per replicate. Data for germination percentage were subjected to arcsine transformation before analysis of variance was carried out with SAS software. Mean comparison was performed with Duncan's test if F-test was significant at ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

### *Optimization of Sheep Fescue seed priming*

In order to determine the effects of priming treatments on germination characteristics, first analysis of variance was performed for germination data without control group. Significant three-way interactions (time, temperature and osmotic potential) were found for all investigated characters (data not shown).second, analysis of variance was performed for germination data with control group.

In the first experiment, which was done in order to evaluate of the best osmotic potential level as well as osmopriming duration and temperatures, variance analysis of data showed that all of the germination traits of Sheep Fescue seeds are effected by osmopriming treatments (Table 1 and Table 3).

Variance analysis of second experiment was investigated in order to study hydropriming effect on Sheep Fescue seed in different times (12, 24, 36 and 48h) and two temperatures (15 and 25°C) showed that, germination percentage and some of measured traits are effected by hydropriming in comparison with control group (Tab. 2 and Tab. 4).

#### *Final Germination Percentage (FGP)*

In osmopriming treatment, the maximum amount of final germination percentage was in the time of 36h, potential of -10bar and temperature of 15°C which did not have significant differences with the time of 12 and 24h in the same temperature. But it was different with the time of 48h and other potentials in this time and in the same temperature (Table 3). The recommended time treatment is 12h. Superior treatment (12h\*-8bar\*15°C) was more than control group. Demir and Van de Vanter (1999) reported that, osmopriming of watermelon seeds caused the decrease mean germination time and increase of its percentage

In hydropriming treatment, the maximum amount of this trait was in the time of 12h, and temperature of 15°C (Table 4). Moradi *et al.* (2008) showed that, for most evaluated germination parameters of corn seeds, hydropriming was the effective treatment.

#### *Germination Rate (GR)*

In osmopriming treatment, the maximum amount of germination rate was in the potential of -8bar in 12h with 15°C and it did not have significant differences with treatment combination of 36h, potential of -10bar in the same temperature. So, potential of -8bar in the time of 12h is recommended because of lower time and potential. Moreover, superior treatment was more than control group (Table 3). Hur (1991) showed that in Italian ryegrass and sorghum, germination percentage as well as germination rate were increased in response to osmopriming.

In hydropriming treatment, all of time treatments in 15°C were higher than 25°C and the maximum amount of germination rate was in the time of 12h, and temperature of 15°C (Table 4). Harris *et al.* (1999), Giri and Schilinger (2003) and Finch-Savage *et al.* (2004) reported that the priming effect on seed germination rate was positive in comparison with control group.

#### *Coleoptile Length (CL)*

In osmopriming treatment, the longest coleoptile length was observed in the time of 36h, potential of -8bar and temperature of 15°C. This temperature had higher amount of time and potential in comparison with 25°C. So, it can be said that the most suitable treatment combination is in 15°C, potential of -8bar and 12h which was more than control group (Table 3). In a study with Turkish pine (*Pinus brutia*) var. *eldarica*, Khalil *et al.* (1997) determined that plants raised from seed preconditioned at room temperature in aerated solution of PEG 8000 for different time periods exhibited faster germination and higher shoot length compared to plants raised from untreated seed.

In hydropriming treatment, the longest coleoptile length was observed in the time of 12h and temperature of 15°C which is higher than control group (Table 4). Tomato seed primed resulted in significant increases in stem length, shoot weight, leaf area, number of flowers, fruit set, and final yield [24].

#### *Radicle Length (RL)*

In osmopriming treatment, in this trait, because of the existence of several superior treatment combinations, the first treatment which is higher than control group and includes 15°C, 12h, potential of -8bar is recommended (Table 3). osmoconditioning also promoted the rates of radicle extension, seedling emergence, and expansion of the cotyledons and the first leaf of cucumber [34].

In hydropriming treatment, the maximum amount of Radicle length was in the time of 24h with 15°C and it did not have significant differences with treatment combination of 12h in the same temperature. So, it seems the time of 12h is recommended because of lower time (Table 4). Priming has been shown to induce nuclear DNA synthesis in the radicle tip cells in tomato [30].

#### *Seedling Length (SL)*

In osmopriming treatment, the analysis of treatments as well as control group, the temperature of 15°C showed the higher amount which regarding to the economic aspect can be introduced as the superior treatment beside the potential of -8bar and time of 12h (Table 2). osmoconditioning of Bermuda grass (*Cynodon dactylon*) seed using PEG followed by immediate sowing improved germination and seedling growth under saline conditions [1].

In hydropriming treatment, all of the amount of seedling length in temperature of 15°C were higher than 25°C but the time of 12h and 24h with 15°C did not have significant differences. So, the time of 12h is recommended because

of lower time and preserve seeds from electrolyte leakage (Table 4). In snap beans (*Phaseolus vulgaris*), hydropriming resulted in improved germination and seedling emergence and growth [40].

*Seedling Dry Weight (SDW)*

In osmopriming treatment, In this trait, regarding to optimal use of time and material, it was the time of 12h, temperature of 15°C and potential of -12bar that show higher amount in comparison with control group and other treatments (Table 3). Khalil *et al.* (1997) showed that plants raised from osmoprimed seed exhibited higher dry weight in comparison with control group. Similar positive effect of osmopriming on dry weight in Italian ryegrass (*Lolium multiflorum*) and sorghum (*Sorghum bicolor*) was observed [21]. Dabrowska *et al.* (2001) determined that both solid matrix priming and osmopriming significantly increased the speed and capability of emergence and mean dry weight of hot pepper seedlings.

In hydropriming treatment, all of maximum amount of this trait were observed in temperature of 15°C. the time of 12h was better time than other treatments in same temperature because it takes higher dry weight than other treatments and control group (Table 4). In field experiments, hydropriming of safflower (*Carthamus tinctorius*) seed for 12h resulted in higher capitula per plant, grains per capitulum, 1000-seed weight, grain yield, and oil content compared to untreated seed [6]. Similar improvements were observed in maize, rice, chickpea [18], and pearl millet [29] grown under dry land conditions.

*Vigor Index 1*

In osmopriming treatment, From treatment combination in comparison with control group, time of 36h, potential of -10bar, treatment of 15°C, can be regarded as superior treatment which showed higher amount compared with control group (Table 3). Osmopriming may contribute to rapid seed germination by affecting active oxygen metabolism. In wild rye (*Leymus chinensis*) seed, for example, priming with 30% PEG for 24 h resulted in increases in the activity of superoxide dismutase (SOD) and peroxidase (POD) and a rapid increase in the respiratory intensity, which were associated with an increase in germination vigor [23].

In hydropriming treatment, the maximum amount of this trait was in the time of 12h and temperature of 15°C which has significant differences with the other treatments and control group (Table 4). Hydropriming resulted in a significant improvement in germination and seedling vigor and a decrease in leakage of electrolytes from germinating seed [38].

*Vigor Index 2*

In osmopriming treatment, In this trait, superior treatment showed higher amount (15°C\*12h\*-8bar) compared with control group (Table 3). Fu *et al.* (1988) determined that soaking peanut (*Arachis hypogaea*) seed in 20–25% PEG for 48 h greatly increased phosphate uptake and RNA synthesis in embryonic axes while improving seed vigor. In hydropriming treatment, same as vigor index1 the maximum amount of this trait was in the time of 12h and temperature of 15°C which has significant differences with the other treatments and control group (Table 4). Thornton and Powell (1992) determined that for seeds of cauliflower (*Brassica oleracea*) and Brussels sprouts (*Brassica oleracea*), an 8h hydropriming treatment at 25°C was the most effective for improving the rate and uniformity of germination, root growth, and seed vigor.

**Table 1. The ANOVA table showing the osmopriming treatment in comparison with control on germination traits of Sheep Fescue**

S.O.V	df	Mean of Squares (MS)							
		FGP (%)	GR (1/day)	CL (cm)	RL (cm)	SL (cm)	SDW (gr)	VII	VI2
Block	3	202.2 <sup>ns</sup>	0.0000073 <sup>ns</sup>	2.41 <sup>ns</sup>	0.047 <sup>ns</sup>	3.01 <sup>ns</sup>	0.0000012 <sup>ns</sup>	571 <sup>ns</sup>	0.10 <sup>ns</sup>
Treatment	40	1089.98 <sup>**</sup>	0.000052 <sup>**</sup>	5.9 <sup>**</sup>	6.1 <sup>*</sup>	22.01 <sup>**</sup>	0.0000041 <sup>**</sup>	3241 <sup>*</sup>	0.56 <sup>**</sup>
Error	120	43.44	0.0016	0.4	1.98	2.2	0.0000011	161	0.028

*ns, \*\*, \* Respectively non significant and significant of 1 and 5 percent of probability*

*FGP : Final Germination Percentage, GR: Germination Rate, CL: Coleoptile Length, RL: Radicle Length, SL: Seedling Length, SDW: Seedling Dry Weight, VII: Vigor Index1, VI2: Vigor Index2*

**Table 2. The ANOVA table showing the hydropriming treatment in comparison with control on germination traits of Sheep Fescue**

S.O.V	df	Mean of Squares (MS)							
		FGP (%)	GR (1/day)	CL (cm)	RL (cm)	SL (cm)	SDW (gr)	VII	VI2
Block	3	4.91 <sup>ns</sup>	0.0000074 <sup>ns</sup>	0.35 <sup>ns</sup>	0.1 <sup>ns</sup>	0.061 <sup>ns</sup>	0.00000032 <sup>ns</sup>	27 <sup>ns</sup>	0.028 <sup>ns</sup>
Treatment	8	4312.22**	0.00062*	40.9**	12.21**	81.02*	0.0000081**	9985**	2.43**
Error	24	4.42	0.00000089	0.12	0.12	0.42	0.0000002	33	0.005

ns, \*\*, \* Respectively non significant and significant of 1 and 5 percent of probability

FGP : Final Germination Percentage, GR: Germination Rate, CL: Coleoptile Length, RL: Radicle Length, SL: Seedling Length, SDW: Seedling Dry Weight, VII: Vigor Index1, VI2: Vigor Index2

**Table 3. Effect of osmoprime treatments on the germination and seedling characteristics of Sheep Fescue**

Temperature (Celsius)	treatments		traits								
	Time (hours)	Potential (bar)	FGP (%)	GR (1/day)	CL (cm)	RL (cm)	SL (cm)	SDW (gr)	VII	VI2	
15	12	-8	69.01bc	0.0162a	7.71a-f	5.14 a-f	12.9abc	0.025a	885.4cd	1.74bc	
		-10	65.03bcd	0.0151bc	7.12d-h	5.85abc	12.96abc	0.021e-k	848.2de	1.36b-e	
		-12	69.61bc	0.015bc	7.14d-h	4.69 a-h	11.83b-f	0.024ab	813.8ef	1.65bcd	
		-14	65.33bcd	0.0144c	8.36ab	4.22c-k	12.62a-d	0.021e-k	826.1e	1.35b-e	
		-16	69.61bc	0.0151bc	7.31c-g	5.12 a-f	12.42a-e	0.023a-d	857.5d	1.57bcd	
	24	-8	62.13b-e	0.014bcd	8.22abc	5.48 a-e	13.73ab	0.022a-g	851de	1.35b-f	
		-10	63.56b-e	0.014bcd	7.54b-f	4.1 c-k	11.64b-f	0.021e-k	739.2gh	1.34b-e	
		-12	67.14bc	0.0151bc	8.37ab	6.19ab	14.61a	0.021e-k	977.6b	1.41b-e	
		-14	65.14bcd	0.014bcd	7.8a-e	5.85abc	13.61ab	0.021e-k	888.6cd	1.35b-e	
		-16	67.28bc	0.015bc	7.98a-d	5.13 a-f	13.12ab	0.021e-k	881.3bcd	1.4bcd	
	36	-8	62.07b-e	0.0129b-e	8.58a	4.68a-h	13.27ab	0.02f-k	823.6ef	1.24b-f	
		-10	92 a	0.016ab	7.69a-f	5.57a-e	13.26ab	0.022a-g	1193.4 a	1.98a	
		-12	68.18bc	0.0151bc	7.54b-f	6.24a	13.78ab	0.021e-k	938.4bc	1.43b-e	
		-14	67.74bc	0.0153bc	7.47b-f	5.7a-d	13.17ab	0.021d-k	892.1c	1.42bcd	
		-16	71.08b	0.015b	7.98a-d	5.7a-d	13.68ab	0.0216a-i	972.3b	1.53bcd	
	48	-8	61.96b-e	0.013 b-e	7.69a-f	5.27a-e	12.97abc	0.0213c-j	803.6f	1.31b-f	
		-10	64.90bcd	0.014bcd	6.81e-i	4.88a-g	11.69b-f	0.021e-k	758.7g	1.36b-e	
		-12	63.18b-e	0.0131b-e	6.81a-d	4.53a-j	11.34b-f	0.023a-d	716.5h	1.45f-i	
		-14	65.82bcd	0.014bcd	7.98a-d	5.21a-f	13.19ab	0.021e-k	868.1cd	1.38b-e	
		-16	68.93bc	0.0151bc	7.72a-f	5.11a-f	12.84abc	0.0214b-j	884.6cd	1.47bcd	
	25	12	-8	66.21bcd	0.0141bcd	6.76f-i	5.54a-e	12.3a-f	0.021e-k	814.0ef	1.38bcd
			-10	50.35fg	0.0115fg	6 i-l	4.31b-j	10.32d-h	0.021e-k	520m	1.05e-h
			-12	62.53b-e	0.013b-e	6.18h-k	4.29b-j	10.49c-h	0.0195g-k	645j	1.19bc
			-14	24.14kl	0.01kl	4.03p	2.67j-m	6.71k	0.021e-k	162u	0.50k
-16			48.95fg	0.011fg	5.32k-o	3.31f-l	8.615g-j	0.02f-k	424.2o	0.98d-g	
24		-8	59.12c-f	0.0124 c-f	6.21h-k	3.8d-l	10.05e-i	0.021e-k	596.8k	1.24b-e	
		-10	55.55def	0.012d	5.93i-l	3.96c-k	9.89f-i	0.021e-k	549.3l	1.16c-f	
		-12	67.13bcd	0.014bcd	6.38g-j	3.96c-k	10.34d-h	0.019h-k	683.9i	1.25bcd	
		-14	32.79ijk	0.0114ijk	4.86m-p	1.46m	6.323k	0.018k	200.3t	0.57jk	
		-16	30.11jk	0.0111jk	5.45j-n	4.43a-j	9.89f-i	0.02f-k	310r	0.62jk	
36		-8	39.31hij	0.0115hij	4.69nop	2.9h-m	7.59ijk	0.02f-k	291s	0.76hij	
		-10	54.32ef	0.0121ef	6.36g-j	3.73f-l	10.09f-i	0.02f-k	537.7lm	1.06b-f	
		-12	42.02gh	0.012ghi	5.03l-o	2.36klm	7.39e-h	0.0185jk	307rs	0.77g-j	
		-14	19.11l	0.0093l	4.42op	2.74i-m	7.17jk	0.019h-k	138.9v	0.36k	
		-16	24.15kl	0.01kl	5.52j-n	3.14g-m	8.65g-j	0.0182ijk	203.8t	0.42k	
48		-8	38.78hij	0.0115hij	4.88m-p	4.6a-i	9.475g-j	0.02f-k	357.4p	0.76hij	
		-10	43.01gh	0.0151gh	5.71j-n	2.64j-m	8.35g-j	0.02f-k	358.1lp	0.84b-e	
		-12	53.04ef	0.0121ef	5.43j-n	3.14g-m	8.58g-j	0.0182ijk	454.5n	0.95b-f	
		-14	18.74l	0.0094l	4.79m-p	1.98lmn	6.783k	0.02f-k	128.8w	0.38k	
		-16	33.49hij	0.015hij	6.1ijk	3.78d-l	9.89f-i	0.019h-k	350.4pq	0.65ij	
Control			53.92 def	0.0114hij	7.18c-g	3.29f-l	9.98ghi	0.02f-k	585.1k	1.1b	

In each column means followed by the same letter are not significantly different at the P < 0.05 level

FGP : Final Germination Percentage, GR: Germination Rate, CL: Coleoptile Length, RL: Radicle Length, SL: Seedling Length, SDW: Seedling Dry Weight, VII: Vigor Index1, VI2: Vigor Index2

**Table 4. Effect of hydropriming treatments on the germination and seedling characteristics of Sheep Fescue**

Temperature (Celsius)	treatments		traits						
	Time (hours)	FGP (%)	GR (1/day)	CL (cm)	RL (cm)	SL (cm)	SDW (gr)	VII	VI2
15	12	78.01a	0.018a	9.1ab	5.71a	14.42a	0.026a	1115a	1.95a
	24	66.11b	0.017ab	8.77bc	5.69 a	15.2a	0.024ab	931.2b	1.55b
	36	62 c	0.016bc	8.53c	3.91 c	13.31 b	0.024ab	761.1c	1.43c
	48	54.81e	0.010e	8.5 c	4.3 b	13.77b	0.022 b	700.4d	1.23d
25	12	41.01 f	0.009f	5.85e	3.82 c	10.07 c	0.021 c	387.5f	0.86f
	24	26.6 g	0.006g	3.4 f	1.59 e	5.85 e	0.0211 c	137.4f	0.57g
	36	6.08 h	0.002h	1.59 h	0.31 f	2.94 g	0.018 e	13h	0.13h
	48	6.09 h	0.002h	2.5 g	1.4 e	4.87 f	0.02 d	26g	0.13h
Control		55.2 d	0.013d	7.11 d	2.11d	10.1 d	0.019 d	522.4e	1.15e

In each column means followed by the same letter are not significantly different at the  $P < 0.05$  level

FGP: Final Germination Percentage, GR: Germination Rate, CL: Coleoptile Length, RL: Radicle Length, SL: Seedling Length, SDW: Seedling Dry Weight, VII: Vigor Index1, VI2: Vigor Index2

### CONCLUSION

During osmopriming experiment in this plant, time treatments of 12h and 36h allocated above amounts to themselves and were superior than control group in the majority of investigated traits. Priming's temperature had outstanding effect on treatment, since in all of the investigated traits, temperature of 15°C was the best. It seems that temperature of 15°C is near the optimum temperature of germination in this plant. So, it has significant effect on germination factors and indexes. Between times which are candidates of superior treatment, 12h can be introduced because it can save time as well as prevent possible damages like infection of seeds (because of being in the solution for the long time).

Researches explain that priming is practical technique to increase germination rate and consistence as well as vigor increase and better performance in vegetables, flower plant and crops [8, 9,14].

In hydropriming, time of 12h and temperature of 15°C were superior than other treatments and control group. Zheng *et al.* (2002) reported that, in lower temperature, priming had significant effect on the germination rate of rice seeds. Shivankar (2003) explained that, hydropriming can increase germination rate and consistency in the seed with low viability. Singh *et al.* (1999), also, conveyed the similar results. Effects of hydropriming on water potential, the driving force for water uptake during imbibition, and the activity of  $\alpha$ -amylase were examined in wheat and rice kernels [2]. Amylases are key enzymes that play a vital role in hydrolyzing the seed's starch reserve, thereby supplying sugars to the developing embryo [3]. Hydropriming of cereal rye and perennial ryegrass can significantly increase the rate percentage of germination in them [37]. Giri and Schilinger (2003) showed that, the effect of hydropriming with water is equal and even more significant than other priming environments. Regarding positive effects of seed priming on germination characteristic of Tall Fescue, it could be used as pre-sowing treatment in field conditions. In order to maintain high quality in primed seeds for extended storage periods, seeds should be stored at low temperature and low moisture content or after seed priming, seeds should be planted. Finally it is recommended the results of this study to be investigated in the farm condition in order to confirm the fulfilled experiments of this project.

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