

*Full Length Research Paper*

# Changes in germination characteristics and seedling growth between storage and non-storage of primed tall fescue seed

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In seed priming studies, seeds usually are sown directly in the field or stored for short periods of time. The objective of this study was to determine the effects of storage on the primed seed of tall fescue. Hydropriming and osmopriming methods were used in this study to prime the seeds. The water osmotic potential for osmopriming was between -1.5 and -2.2 MPa. After drying, the seeds were first divided into two groups. For the first group, the seeds were sealed in aluminum foil bags and stored at 25°C for up to 1 year and the second group was used for un-stored germination test. Germination percentage for primed seeds stored at 25°C for 1 year decreased significantly compared to the un-stored primed seeds in most treatments, but the control seeds (stored non-primed seeds) in comparison with hydropriming for 1 day had the lowest germination value. The results reveal that there were significant decreased in the germination percentage in stored seeds. The mean germination times of seeds primed and un-stored were lower than those of stored primed seeds. Generally, results show that in order to improve seed germination performance without loss of longevity of tall fescue species, hydropriming can be advised.

**Key words:** Drying, hydropriming, KNO<sub>3</sub>, longevity, mean germination time, PEG 8000, seed priming.

## INTRODUCTION

Priming provides controlled hydration of seeds to a level that allows pre-germination metabolic activity to proceed, but prevents the actual emergence of the radicle after priming, the seeds can be dried back to the initial moisture content (Bradford, 1990). A wide variety of priming treatments are available to enhance seed germination, although hydropriming and osmopriming are commonly used methods to prime seeds (McDonald, 1999). Hydropriming consists of soaking seeds in pure water but drying them before complete germination. Osmopriming is a pre-sowing treatment which consists of incubation of seeds in an osmoticum solution (Pill, 1995). This technique has been applied for seeds of many crops and small seeded grasses to increase the germi-

nation rate, total germination and seedling uniformity particularly under adverse temperature or moisture conditions (Bradford, 1990; Ozbingol et al., 1998; Nascimento and West, 1998; Hardegree and Emmerich, 2000; Heydecker and Coolbaer, 1977). In many seed priming studies, seeds usually are sown directly in the field or stored for short period. Researches show that storing primed seeds reduces germination or seedling emergence and there are conflicting results on the effect of storage life of primed seeds (Pill et al., 1991). Priming can reverse some of the aging-induced deteriorative factors, and thus improve seed performance (Taylor et al., 1998). A critical factor determinant of primed seed performance is postpriming storage environment. The effects of priming on seed longevity may be species specific, depending on the nature of the storage and priming conditions (Chiu et al., 2002). For example, long-term priming (10-days) of leek (*Allium porrum* L.) and onion (*Allium cepa* L.) seeds stored at 10°C retained viability after 1 year (Drew et al., 1997). Carrot (*Daucus*

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*carota* L.) seed primed for 17 days lost some viability after 12 months of 10°C storage (Dearman et al., 1987). Primed tomato (*Lycopersicon lycopersicum* L.) seed (7 days priming) stored at 10°C retained viability for 12 months, but at 30°C viability was reduced (Alvarado and Bradford, 1988). In contrast, short-term priming decreased the storage life of lettuce (*Lactuca sativa* L.) seed (Tarquis and Bradford, 1992). Tomato seed receiving 1 day of priming, however, had better storability. Adverse effects of priming during storage were caused by decreased DNA repair activity resulting from progression in the cell cycle (Van Pijlen et al., 1996).

Recently, many studies have been performed to improve feed quality in grass seeds by priming method, but still very little information is available about the deterioration of tall fescue (*Festuca arundinacea* Schreb) primed seeds followed by storage for long term.

Tall fescue is one of the important forage crops used as a perennial cool-season turf and forage grass species (Buckner et al., 1979). Tall fescue is widely planted for reclaiming the rangeland in Iran.

The objective of this study was to determine the effects of storage and non-storage on the primed seed of tall fescue for duration 1 year in room (25°C) temperature and also the effectiveness of priming method on its germination parameters following in the storage and non-storage conditions.

## MATERIALS AND METHODS

Seed of tall fescue were placed in 16 individual nylon-net bags and immersed in liquid priming media at a temperature of 20°C for durations of 1 day, 3 days and 6 days. The five priming media were: (i) distilled water; (ii) KNO<sub>3</sub> -1.5 MPa; (iii) KNO<sub>3</sub> -2.2 MPa; (iv) PEG 8000 -1.5 MPa; (v) PEG 8000 -2.2 MPa; the osmotic potential (MPa) at 20°C of each solution was verified separately using a vapor pressure Wescor 5500 osmometer. All priming media were prepared in distilled water.

After treatment, the seeds were rinsed with distilled water for two minutes and lightly hand-dried in towel. While still damp, the seeds were sprayed with Thiram fungicide at a rate of 0.65 ml kg<sup>-1</sup> of seed (Giri and Schilinger, 2003). Then seeds were air dried in room temperatures (25°C) and 40% relative humidity (RH) to near original moisture level. The moisture level was monitored and periodically weighed. When weight of random samples of primed seeds decreased near the weight of non-primed seeds, air drying was stopped, after drying, the seeds were first divided into two groups. One group of the seeds were sealed in aluminum foil bags and stored at 25°C for up to 1 year while the remaining seeds were used for non-storage germination test. Then seeds were placed between two layers of filter paper moistened with 5 ml of distilled water in a covered 9 cm Petri dishes. Germination test were conducted in a germinator maintained at 15 to 25°C for a period of 8 h of darkness and 16 h of light with a light intensity of 38 μmol m<sup>-2</sup>s<sup>-1</sup> provided by cool-white fluorescent lamps (ISTA, 1985). The germination was monitored every other day for 21 days while the seeds were counted when they exhibited radicle extensions of ≥ 2 mm (Hardegee and Van Vactor, 2000).

The germination percentage was calculated according to the total number of seeds germinated. Mean germination time (MGT) which is an inverse measure of germination rate, was calculated according to the following formula (Cantliffe, 1991).

$$MGT = \frac{A_1 D_1 + A_2 D_2 + \dots + A_n D_n}{A_1 + A_2 + \dots + A_n}$$

Where, A is the number of seeds germinating per day; D is the time corresponding to A in days and n is the number of days to final count.

After 21 days, the lengths of roots and shoots of 10 randomly selected seedlings from each replication were measured. Vigor index (VI) of the seedlings was calculated according to the following formula Abdul-Baki and Anderson (1973):

$$VI = (RL + SL) \times GP,$$

Where, RL is the root length (cm); SL is the shoot length (cm) and GP is the germination percentage.

## Experimental design and statistical analysis

The applied experimental design was a two - factors factorial experiment arranged in a completely randomized design; with three replications and 50 seeds per replication. The first factor was priming treatments (16 treatment combinations) while the second was storage and non-storage. Result data (in percentage) were transformed to arcsine values before statistical analysis. Analysis of variance of the data was computed using the MSTAT-C Program (Michigan State University). The PLSD Fisher test at a 5% level of probability was used to evaluate the differences among the means.

## RESULTS

Analysis of variances showed that the main effect of storage/non-storage (A) on germination, MGT and shoot length was statistically significant, while there was no effect on root length and vigor index (Table 1).

Comparison of means showed that significantly higher germination percentage was observed in non-storage condition (P < 0.01), while MGT and shoot length increased in storage condition (Table 2).

Main effect of priming treatment (B) indicates that the germination percentage, MGT and vigor index were significantly affected by priming technique (Table 1). A higher germination percentage was observed in hydropriming for 1 day compared with the control (P < 0.05) (Table 3). The results show that osmopriming with KNO<sub>3</sub> -1.5 MPa for 3 days and KNO<sub>3</sub> -2.2 MPa for 1 day decreased the MGT of *F. arundinacea* seeds as compared with the control, although, this difference was not significant. The maximum value of MGT was observed in PEG -2.2 MPa for 6 days and 1 day. Maximum numerical value in vigor index was recorded for hydropriming (distilled water), which was not statistically significant compared with the control.

A significant two-way interaction (priming × storage/non-storage) was found for germination percentage and mean germination time (Table 1). The results show that the germination percentage for primed seeds stored at 20°C for 1 year decreased significantly compared to the un-stored primed seeds in most treatments, but when compared to the control seed (stored non primed seeds)

**Table 1.** F-values resulted from two-way analysis of variances of the effects of Storage/ non-storage (A), Priming (B) and A\*B on germination, MGT, Vigor index, Shoot and root length in tall fescue.

Source of variation	d.f	Vigor index	Germination (%)	Shoot length (cm)	Root length (cm)	MGT (day)
Main effect						
Storage/ non-storage (A)	1	2.24 ns	88.12**	243.40**	0.99ns	129.56**
Priming (B)	15	5.19**	5.11**	0.80 ns	0.75 ns	21.74**
Interaction effect						
A*B	15	5.78 ns	3.46**	1.35 ns	1.57 ns	4.17**

\*\*Significant at the 0.01 probability level, ns: Not significant; d.f: Degree of freedom; MGT: Mean germination time.

**Table 2.** Main effect of storage/non storage *Festuca arundinacea* seeds on vigor index, germination (%),shoot and root length and mean germination time.

Treatment	Seed trait		
	Germination (%)	Shoot length (cm)	MGT (day)
Non storage	88.3 ± 0.91 <sup>a</sup>	7.6 ± 0.09 <sup>b</sup>	5.03 ± 0.20 <sup>b</sup>
Storage	71.3 ± 2.75 <sup>b</sup>	10.6 ± 0.19 <sup>a</sup>	7.02 ± 0.31 <sup>a</sup>

\*Means ±SE followed by the same letters are not significantly different by ANOVA, test PLSD at P < 0.05 level of significance.

with hydropriming (distilled water) for 1 day, it showed the control had lower germination value (Figure 1).

Control stored seeds and control unstored seeds had almost similar germination percentage. The results show that priming (all treatments) for duration of 6 days significantly decreased the germination percentage in stored seeds.

The MGT of seeds primed and un-stored were lower than those of stored primed seeds (Figure 2). Maximum value of MGT in seeds primed and un-stored was 8 days, whereas it was 11 days for seeds primed and stored. Generally, MGT increased by storage at 20°C for 1 year, but apparently this trait was greater in seeds primed for 6 day.

## DISCUSSION

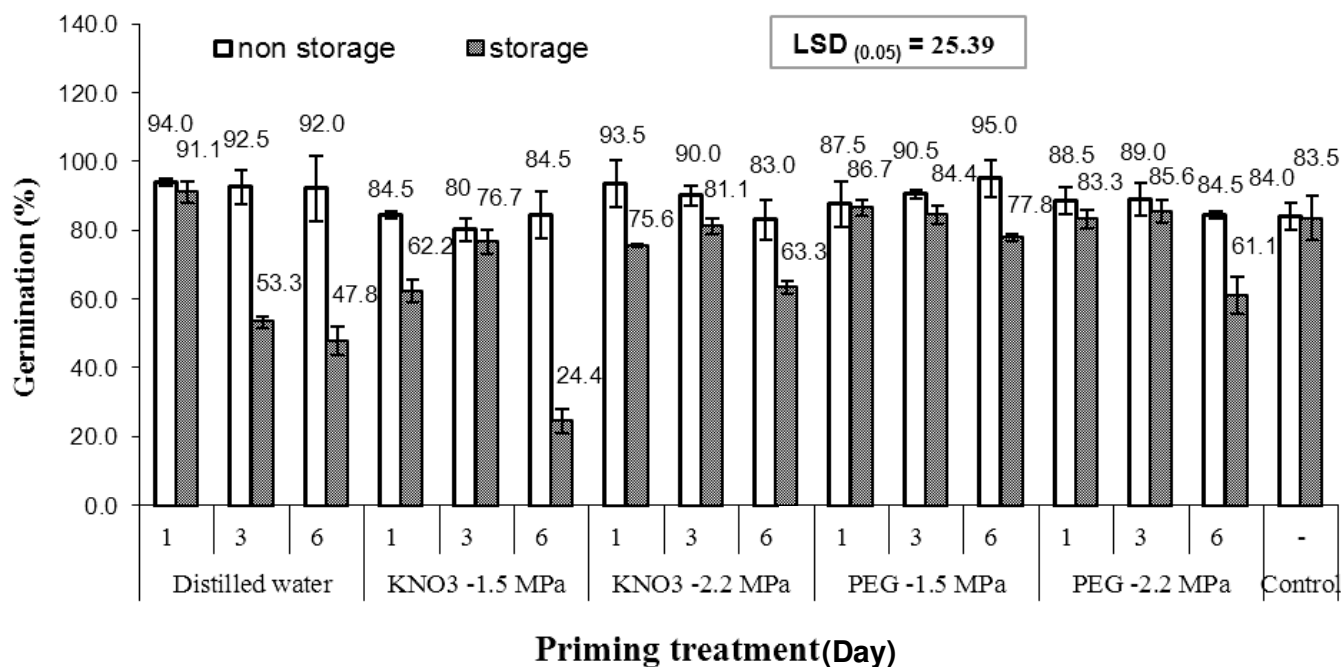
Seed priming had a positive effect on germination percentage, MGT and vigor index of *F. arundinacea*. While priming enhances seed germination performance, the longevity of primed seeds in storage is often reduced (Van Pijlen et al., 1996; Chang and Sung, 1998; Taylor et al., 1998). Our findings reveal that deterioration of *F. arundinacea* seeds primed resulted from storage were different while hydropriming improved both germination percentage and vigor index in storage conditions. Other priming treatments did not show such a trend. Fujikura and Karssen, (1995) indicated the beneficial effects of

hydropriming on aged or un-aged cauliflower seeds. The present study showed that hydropriming had the most effective method for improving germination of stored primed seeds in *F. arundinacea*, especially when the seeds were hydrated for 1 day. Heydecker et al. (1975) remarked that hydro-priming only broke seed dormancy, whereas treatment with PEG may imply additional physiological effects. They raised the possibility that osmotic priming by PEG inhibits radicle emergence, limits the rate of water absorption thus preventing membrane damage, and restores germ inability to aged seeds more effectively while, the results showed that osmotic priming decreased the germination percentage of *F. arundinacea* compared to treatment by distilled water (hydropriming). Parera and Cantliffe (1994) and McDonald (1999) mention that hydropriming is the simplest approach to hydrating seeds and minimizes the use of chemicals. Hsu et al. (2003) expressed that the exact causes of faster deterioration of primed seeds have not been well defined yet. Van Pijlen et al. (1996) hypothesized that the reduced longevity of primed seeds is caused by a decrease in DNA repair activity due to progression in the cell cycle during hydration. In general, the beneficial effects of priming were obvious even after long term storage. Between all priming treatments, hydropriming is known to improve germination performance without loss of longevity. Also hydropriming as physiological treatment caused an increase in the seed performance following storage conditions of *F.*

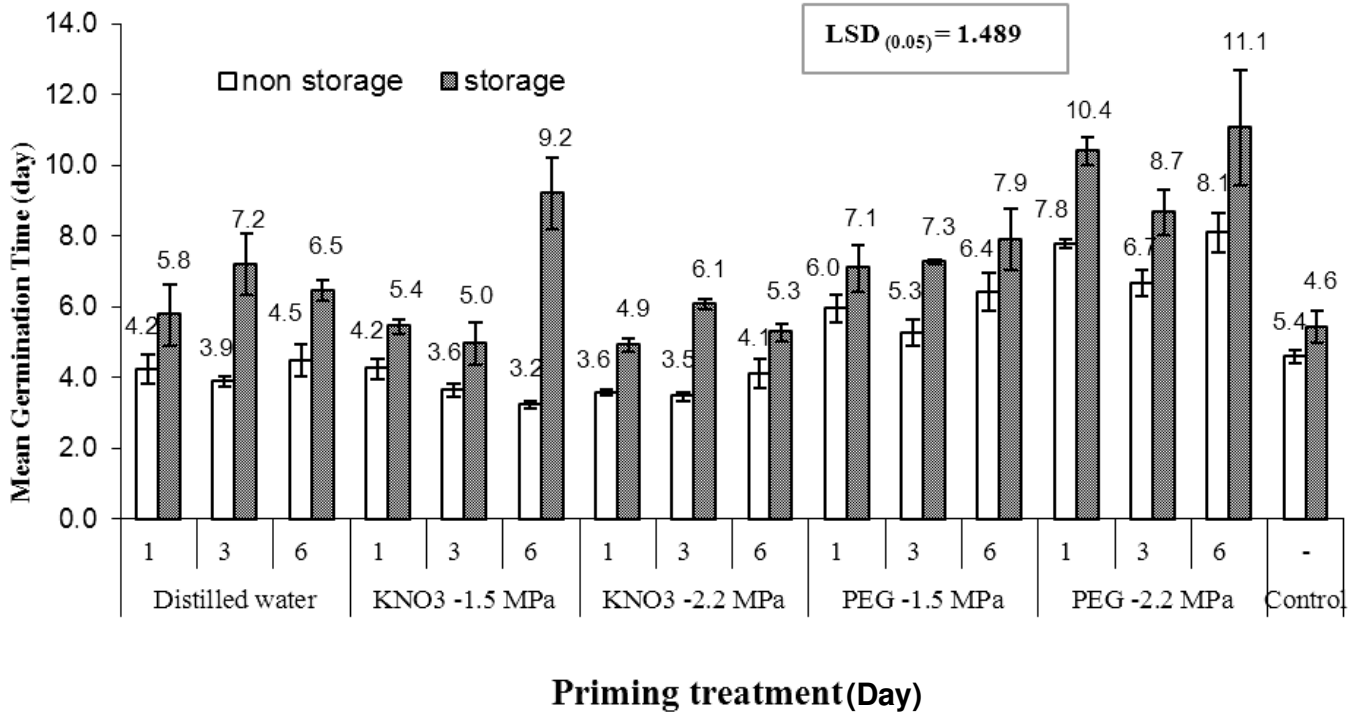
**Table 3.** Main effect of priming treatment on germination (%), mean germination time (MGT) and vigor index of *Festuca arundinacea*.

Priming media	Duration priming (Day)	Seed trait		
		Germination (%)	MGT(day)	Vigor index
Distilled water	1	92.7 ± 1.84 <sup>a*</sup>	4.89 ± 0.50 <sup>fgh</sup>	1095 ± 99.08 <sup>a</sup>
	3	75.7 ± 8.19 <sup>bcd</sup>	5.30 ± 0.75 <sup>efg</sup>	821 ± 75.30 <sup>d<sup>ef</sup></sup>
	6	73 ± 9.89 <sup>bcd</sup>	5.33 ± 0.48 <sup>efg</sup>	719 ± 90.07 <sup>fg</sup>
KNO <sub>3</sub> -1.5 MPa	1	74.9 ± 4.85 <sup>d</sup>	4.76 ± 0.30 <sup>gh</sup>	821 ± 31.35 <sup>cdef</sup>
	3	78.6 ± 2.34 <sup>cd</sup>	4.19 ± 0.37 <sup>h</sup>	897 ± 41.38 <sup>bcd<sup>ef</sup></sup>
	6	58.8 ± 12.55 <sup>d</sup>	5.80 ± 1.27 <sup>def</sup>	630 ± 139.41 <sup>g</sup>
KNO <sub>3</sub> -2.2 MPa	1	85.8 ± 4.44 <sup>ab</sup>	4.15 ± 0.28 <sup>h</sup>	1003 ± 57.60 <sup>abc</sup>
	3	86.2 ± 2.48 <sup>bc</sup>	4.58 ± 0.53 <sup>gh</sup>	1000 ± 61.61 <sup>abcd</sup>
	6	74.6 ± 4.63 <sup>d</sup>	4.60 ± 0.34 <sup>gh</sup>	787 ± 29.39 <sup>efg</sup>
PEG -1.5 MPa	1	87.1 ± 2.79 <sup>ab</sup>	6.44 ± 0.39 <sup>cd</sup>	984 ± 110.82 <sup>abcd</sup>
	3	87.9 ± 1.91 <sup>ab</sup>	6.13 ± 0.45 <sup>cde</sup>	963 ± 92.42 <sup>abcde</sup>
	6	87.6 ± 4.10 <sup>ab</sup>	7.05 ± 0.53 <sup>bc</sup>	948 ± 110.82 <sup>abcde</sup>
PEG -2.2 MPa	1	86.3 ± 2.28 <sup>bc</sup>	8.90 ± 0.55 <sup>a</sup>	1025 ± 61.92 <sup>ab</sup>
	3	87.5 ± 2.64 <sup>ab</sup>	7.53 ± 0.50 <sup>b</sup>	1019 ± 77.49 <sup>abcde</sup>
	6	74.5 ± 5.53 <sup>d</sup>	9.37 ± 0.91 <sup>a</sup>	854 ± 31.92 <sup>bcd<sup>ef</sup></sup>
Control	0	84.8 ± 3.77 <sup>bc</sup>	5.06 ± 0.26 <sup>fgh</sup>	934 ± 116.9 <sup>abcde</sup>

\*Means ±SE followed by the same letters are not significantly different by ANOVA, test PLSD at P < 0.05 level of significance.



**Figure 1.** Interaction effects of priming treatment and storage duration on germination percentage in *Festuca arundinacea* seeds. Vertical bars represent ±SE of means.



**Figure 2.** Interaction effects of priming treatment and storage duration on mean germination time in *Festuca arundinacea* seeds. Vertical bars represent  $\pm$ SE of means.

*arundinacea*.

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