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EFFECT OF SEED PRIMING ON THE GERMINATION, SEEDLING EMERGENCE, YIELD AND QUALITY OF FORAGE PRODUCTION IN TALL FESCUE (*FESTUCA ARUNDINACEA* SCHREB)

Ghasem Ali Dianati Tilaki [1], Behzad Behtari [2], Mohammad Ali Alizadeh [2], and Ali Ashraf Jafari [2]

[1] Faculty of Natural Resources of Tarbiat Modares University
P.O. Box 46414-356, Noor-Iran
E-mail: dianatitilaki@yahoo.com
[2] Research Institute of Forests and Rangeland
Tehran, Iran

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Effect of seed priming on the germination, seedling emergence, yield and quality of forage production in tall fescue (*Festuca arundinacea* Schreb). – **Tilaki Ghasem Ali Dianati, Behtari Behzad, Alizadeh Mohammad Ali, and Jafari Ali Ashraf**. – This study evaluated the effect of priming on germination, emergence, yield and quality of *Festuca arundinacea* in both laboratory and greenhouse conditions. Previous priming studies have evaluated only germination and seedling emergence for fescue but priming treatment effects on forage quality have not been assessed. The Seeds were treated by hydropriming (distiller water) and osmopriming in polyethylene glycol 6000(PEG) and KNO_3 solution for 1, 3 and 6 day with osmotic potential 1.5 and 2.2 MPa. The results from germination percentage test in laboratory showed that with osmotic priming by PEG 1.5 for 6 day germination was significantly higher than with the control ($p \geq 0.05$), while, in greenhouse conditions, PEG 2.2 MPa for 1 day increased the seedling emergence (76.7%) compared to control (42.5%). Primed seeds also had significantly higher fresh weights than the seeds by other treatment and the control. The maximum digestibility percentage of dry matter and crude protein percentage of forage was recorded in PEG 1.5 MPa for 6 day that which exhibited significant difference with untreated seeds. The present study showed that priming enhanced the performance of germination the parameters of emergence and the quality in both laboratory and greenhouse conditions. The results suggest that, the principle of management and decision about efficacy of priming is not suitable to be assessed from absolute measures of laboratory performance.

Key words: hydropriming, osmopriming, *Festuca arundinacea*, forage quality, dry matter digestibility, crude protein.

Влияние предпосевной обработки семян на их прорастание, начальный рост проростков, урожай и качество продукции зелёной массы у овсяницы тростниковой (*Festuca arundinacea* Schreb). – Тилаки Хасем Али Дианати, Бехтари Бехзад, Ализаде Мохаммед Али, Джафари Али Ашраф. – В лабораторных условиях и условиях теплицы исследовали влияние предпосевной обработки семян *Festuca arundinacea* на всхожесть, прорастание, урожай и качество. Предыдущие исследования замачивания позволили выявить особенности прорастания и начальный рост проростков овсяницы, но не было оценено влияние предпосевной обработки на качество зелёной массы. Семена замачивали в дистиллированной воде, в полиэтиленгликоле 6000 (ПЭГ), а также в растворе KNO_3 на 1, 3 и 6 сут. с осмотическим давлением 1.5 и 2.2 МПа. Прорастания семян в лабораторных условиях при замачивании в растворе полиэтиленгликоля (ПЭГ 1.5) на 6 сут. значимо выше, чем в контроле ($p \leq 0.05$), тогда как в условиях теплицы замачивание семян в ПЭГ 2.2 МПа в течение 1 сут.

увеличило всхожесть (76.7%) по сравнению с контролем (42.5%). Замоченные семена обладали также значимо более высокой массой, чем семена с другими способами обработки и контролем. Наибольшая пищевая ценность сухого вещества и содержание сырого белка в зелёной массе было обнаружено при обработке семян ПЭГ 1.5 МПа в течение 6 сут., что существенно отличается от необработанных семян. В общем предпосевная обработки семян значительно улучшает всхожесть, показатели прорастания и качество всходов как в лабораторных условиях, так и в условиях теплицы. Результаты свидетельствуют, что принцип управления и принятия решений об эффективности предпосевной обработки семян не подходит для применения на основе результатов лабораторных измерений.

Ключевые слова: замачивание в воде, замачивание в осмотическом растворе, *Festuca arundinacea*, качество зелёной массы, питательность сухого вещества, сырой белок.

INTRODUCTION

Tall fesuce (*Festuca arundinacea* Schreb) is one of important forage crops and used as a perennial cool-season turf and forage grass species (Buckner et al., 1979). Tall fescue is the most widely planted for reclaiming rangeland in Iran. One of the problems Tall fescue is the difficulty of raising seedlings from seeds for successful establishment under conditions of irregular rainfall and drought stress prevalent in Iran.

Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops and small seeded grasses, in particular under adverse temperature or moisture conditions (Heydechker, Coolbaer, 1977). A wide variety of priming treatments have been used to enhance seed germination. Hydropriming and osmopriming are commonly used methods to prime the seeds. Hydropriming is the simplest method to hydrating seeds and minimizes the use of chemicals (McDonald, 1999). Hydropriming consists in soaking seed in distiller water and re-drying before complete conducting germination. Osmopriming is a pre-sowing treatment that consists of the incubation of seeds in an osmotic solution, usually a salt or PEG, in order to control their water uptake (Pill, 1995).

Generally, all priming treatment provides a controlled hydration of seeds to a level that allows pre-germination metabolic activity to proceed, but prevents actual emergence of the radicle after priming, the seed can be dried back to the initial moisture content (Bradford, 1986).

Recently, many studies have been done to improve the germination and emergence of grass seeds, but still very limited information is available regarding the effect of priming technique on the quality and yield of forage production. On the other hand, most priming studies of grasses have been limited to laboratory tests (Hardegree, Emmerich, 1992; Frett, Pill, 1995; Pill, Korengel, 1997).

The objectives of this study was to evaluate the effectiveness of hydropriming and osmopriming on germination and seedling emergence of *F. arundinacea* in laboratory and greenhouse conditions.

The Specific objective was to determine the effects of seed priming technique on yield and quality of *F. arundinacea* in the greenhouse conditions.

MATERIAL AND METHOD

Germination test. This study was carried out in the seed technology laboratory of the Faculty of Natural Resources, Tarbiat Modares University (TMU) of Iran. 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, 3, and 6 days. The five priming media were: (i) distilled water; (ii) KNO_3 – 1.5 MPa; (iii) KNO_3 – 2.2 MPa; (iv) PEG 6000 – 1.5 MPa; (v) PEG 6000 – 2.2 MPa; all priming media were prepared in distilled water.

After treatment, the seeds were rinsed with distilled water for two minutes and lightly hand-dried. While still damp, the seeds were spayed with Thiram fungicide at a rate of 0.65 mL kg^{-1} of seed (Giri, Schillinger, 2003). Then seed were air dried until the moisture level come back to the original content. The fifty (50) seeds with four replications were placed on two layers of filter paper moistened with 5 ml of distilled water in covered 9 cm Petri dishes. Germination testes were conducted in a germinator maintained at 15 – 25ºC for a period of 8 hours of darkness and 16 hours of light with a light intensity of 38u $molm^{-2}s^{-1}$ 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 (Hardegree, Van Vactor, 2000). The complete randomized design (CRD) with 16 treatment combinations was used.

The germination percentage was calculated from according to the total number of seeds germinated. The mean germination time (MGT) was calculated using the formula of Cantliffe (1991) and 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*, shoot length (cm) and *GP*, the germination percentage.

Emergence test. The seeds used in the laboratory test were taken from the same lots used in the greenhouse study. The seeds were evaluated in a mix of field-soil and sand in a greenhouse at TMU. Ambient temperatures in the greenhouse ranged from 10 to 30°C, but averaged 25°C during the experiment. The complete randomized design (CRD) was used. Fifty (50) seeds from each of treatment combinations replicated four times were hand-planted a pots and covered with 1.5 cm of moist soil and gently pressed with fingers. All pots were irrigated with tap water. Emergence was measured by counting seedlings at 24-h intervals beginning 3 day after planting and continued until no further emergence occurred. From the total number of seeds emerged, emergence percentage (EP) and Mean Emergence time (MET) was calculated using the formula of Ellis and Roberts (1980):

$$MET = \sum Dn / \sum n$$

where, *n* is the number of seeds, which were emergence on day *D*, and *D* is the number of days counted from the beginning of emergence.

Forage quality and yield. Tall fescue in this greenhouse study was grown for three month. Then, Plants were cut to ground level with manual shears and separated by hand for determination of fresh weight as the forage yield (g/pot).

After the forage tissue was cut, it was rinsed briefly in de-ionized water to remove surface dust. The samples were dried in a forced-air oven at 60°C for 48 hour, and their dry weight were determined (Suyama et al., 2007). The dried tissues was ground to pass a Thomas – Wiley laboratory Mill. crude protein (CP) water soluble carbohydrates (WSC) acid detergent fiber (ADF) crude fiber (CF) dry matter digestibility (DMD) and

ash contents obtained using the near infrared spectroscopy (NIR) analyzer in Research Institute of Forests and Rangelands in Tehran.

Statistical analysis. All data were subjected to one-way analysis of variance using MSTAT-C (version 1.42) (1990). The LSD test at 5% level of probability was used to test the differences among the means values.

RESULTS

Seed priming in laboratory. The comparison of means (Table 1) indicates that the germination percentage, MGT and vigor index were significantly affected by osmo and hydropriming. A higher germination percentage was observed in PEG 1.5 MPa for 6 days which was significantly compared to control ($P > 0.05$). Hydropriming for 1 day (with distiller water) also increases the germination percentage significantly, but compared to the osmopriming with PEG 1.5 MPa for 6 days it was lower. The results showed that osmopriming with KNO_3 – 1.5 MPa for 6 days significantly decreased the MGT of *F.arundinacea* as compared with control and the maximum value of MGT was observed in PEG – 2.2 MPa for 6 day. Priming with KNO_3 – 2.2 MPa for 1 day had statistically significant effect on increasing the vigor index as compared with the control, while, between all the treatments, the control had the lowest vigor index value.

Table 1

Effect of priming treatment by distilled water (hydropriming), osmotic solution of potassium nitrate (KNO_3) and polyethylene glycol (PEG 6000) (osmopriming) for 1, 3 and 6 days in water potential – 1.5 and – 2.2 MPa on germination percentage, mean germination time (MGT) and vigor index of *Festuca arundinacea* in laboratory conditions

Treatments	Seed traits								
	Germination, %			MGT, days			Vigor index		
	1 day	3 days	6 days	1 day	3 days	6 days	1 day	3 days	6 days
Distilled water	94.0*a*	92.5*abc*	92.0*abcd*	4.23*ef*	3.87*efg*	4.49*de*	94.1*abc*	95.8*abc*	83.4*bcd*
KNO_3 – 1.5 MPa	84.5*bcde*	80.0*e*	84.5*bcde*	4.25*ef*	3.63*efg*	3.25*g*	78.2*cd*	84.1*abcd*	91.3*abcd*
KNO_3 – 2.2 MPa	93.5*ab*	90.0*abcd*	83.0*de*	3.58*efg*	3.46*fg*	4.10*efg*	104.4*a*	99.1*ab*	81.1*bcd*
PEG – 1.5 MPa	87.5*abcde*	90.5*abcd*	95.0*a*	5.95*bc*	5.27*cd*	6.42*b*	76.8*cd*	91.3*abcd*	88.2*abcd*
PEG – 2.2 MPa	88.5*abcde*	89.0*abcde*	84.5*bcde*	7.79*a*	6.67*b*	8.10*a*	93.7*abcd*	94.2*abc*	91.8*abcd*
Control	83.5*cde*			5.42*cd*			72.9*d*		

Means follwed by the same letter in a row do not differ significantly at $p \leq 0.05$ (LSD); *a*, *b*, *c*, *d*, *e*, *f*, *g* significant difference ($p < 0.05$).

Seed priming in greenhouse. Table 2 shows that PEG – 2.2 MPa for 1 day increased the seedling emergence percentage (76.7%) significantly, whereas the control had the mean seedling emergence percentage of 42.5%. Treatment by PEG -1.5 MPa for 1 day with value of 18.5% gave a lower seedling emergence percentage than by other primings. The maximum value of MET was recorded by hydroprimed seeds for 6 day which, was statistically significant compared to the control, and the minimum value of MET was recorded for priming by KNO_3 – 2.2 MPa for 6 day, which was not statistically significant compared to the control. Treatment by PEG 2.2 MPa for 1 day resulted in significantly higher fresh weight (g/pot) than by other treatment or the control. A lower

seedling fresh weight (g/pot) was recorded from PEG 1.5 MPa for 1 day as compared with other treatments.

Table 2

Effect of priming treatment by distilled water (hydropriming), osmotic solution of potassium nitrate (KNO_3) and polyethylene glycol (PEG 6000) (osmopriming) for 1, 3 and 6 days in water potential – 1.5 and – 2.2 MPa on emergence percentage, mean emergence time (MET) and fresh weight (g/plot) of *Festuca arundinacea* in greenhouse conditions

Treatments	Seed traits								
	Emergence, %			MET, days			Fresh weight, g/plot		
	1 day	3 days	6 days	1 day	3 days	6 days	1 day	3 days	6 days
Distilled water	44.0*bcd*	55.5*ab*	31.5*cde*	7.6*abcde*	7.5*abcde*	10.8*a*	37.8*bc*	40.3*bc*	34.5*bc*
KNO_3 – 1.5 MPa	56.5*ab*	51.0*bc*	61.0*ab*	6.3*bce*	6.0*cde*	5.8*de*	48.5*abc*	44.3*abc*	42.8*abc*
KNO_3 – 2.2 MPa	40.0*bcde*	54.5*abc*	61.0*ab*	8.6*abc*	6.7*bcde*	5.6*e*	34.6*bc*	3.8*bc*	44.3*abc*
PEG – 1.5 MPa	18.5e	62.0*ab*	25.0*de*	8.1*abcde*	6.2*bcde*	8.2*abcd*	24.7*c*	52.2*ab*	28.3*bc*
PEG – 2.2 MPa	76.7*a*	49.0*bc*	41.5*bcde*	6.8*abcde*	6.8*abcde*	8.8*ab*	6.73*a*	37.8*bc*	35.0*bc*
Control	42.5*bcde*			6.5*bcde*			34.0*bc*		

Means follwed by the same letter in a row do not differ significantly at $p \leq 0.05$ (LSD); *a*, *b*, *c*, *d*, *e* significant difference ($p < 0.05$).

Effect seed priming on Forage quality. Significant differences have been observed in quality parameters between untreated seeds and treated seed of *F.arundinacea.* The maximum DMD and CP percentage (Fig. 1, *a*, *b*) were recorded by PEG -1.5 MPa for 6 days, and the minimum observed were by KNO_3 – 1.5 MPa for 6 days, which exhibited significant difference with untreated seeds (the control). The maximum CF was recorded with the control seeds. The minimum and statistically significant CF as compared with the control was observed in PEG 1.5 MPa for 6 days (Fig. 1, *c*). The contents of ADF, WSC and Ash (Fig. 2, *a*, *b*, *c*) showed no significant difference between treated and control seed. However, a lower ADF and Ash in PEG 2.2 MPa for 6 days and a higher level of WSC compared to non-primed seed was clearly exhibited.

Table 3 presents correlation between quality parameters in *F. arundinacea*. The highest correlation coefficients were found between DMD and ADF (φ = -0.97). The DMD was positively correlated with CP and negatively correlated with ADF, ash and CF. The CP was positively correlated with WSC and negatively correlated with ADF, Ash and CF. Ash was negatively correlated with WSC, CF and positively correlated with ADF.

Table 3

Correlation analysis (Pearson coefficient) between quality parameters of *Festuca arundinacea* in greenhouse conditions

Quality parameters (%)	DMD	CP	WSC	ADF	Ash	CF
Dry matter digestibility (DMD)	1	–	–	–	–	–
Crude protein (CP)	0.62**	1	–	–	–	–
Water soluble carbohydrates (WSC)	0.2	0.3*	1	–	–	–
Acid detergent fiber (ADF)	-0.97**	-0.47**	-0.2	1	–	–
Ash	-0.5**	-0.52**	-0.62**	0.4**	1	–
Crude fiber (CF)	-0.04	-0.42**	0.1	-0.02	-0.46**	1

*,** – significant correlation at 0.01 and 0.05 level respectively.

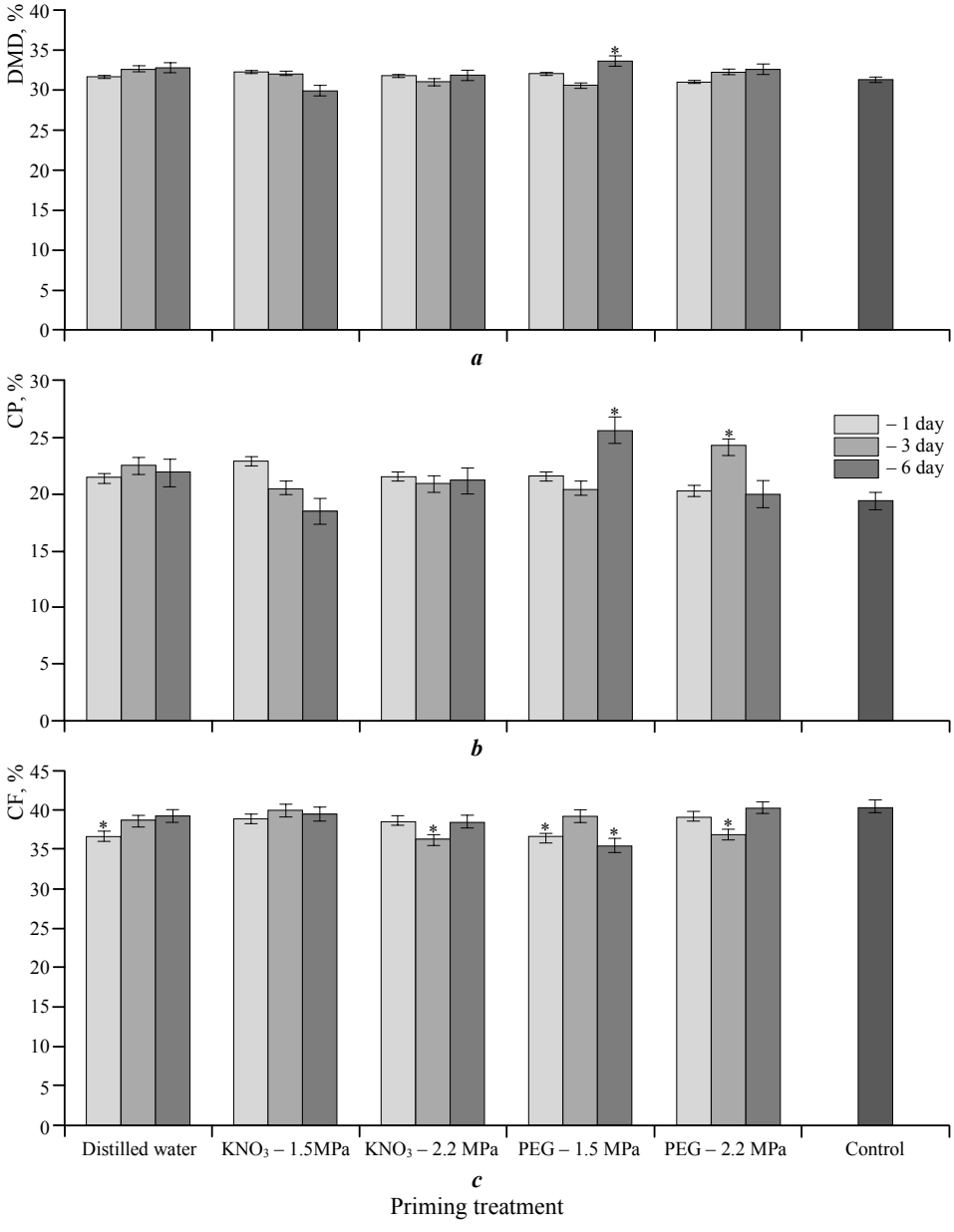

Fig. 1. Changes in percentage of dry matter digestibility (DMD) (*a*), crude protein (CP) (*b*) and crude fiber (CF) (*c*) of *Festuca arundinacea* under priming treatment by distilled water (hydropriming), osmotic solution of potassium nitrate (KNO_3) and polyethylene glycol (PEG 6000) (osmopriming) for 1, 3 and 6 day in water potential – 1.5 and – 2.2 MPa in greenhouse conditions. Marked column are significant versus control at $P \leq 0.05$ (*) by LSD test

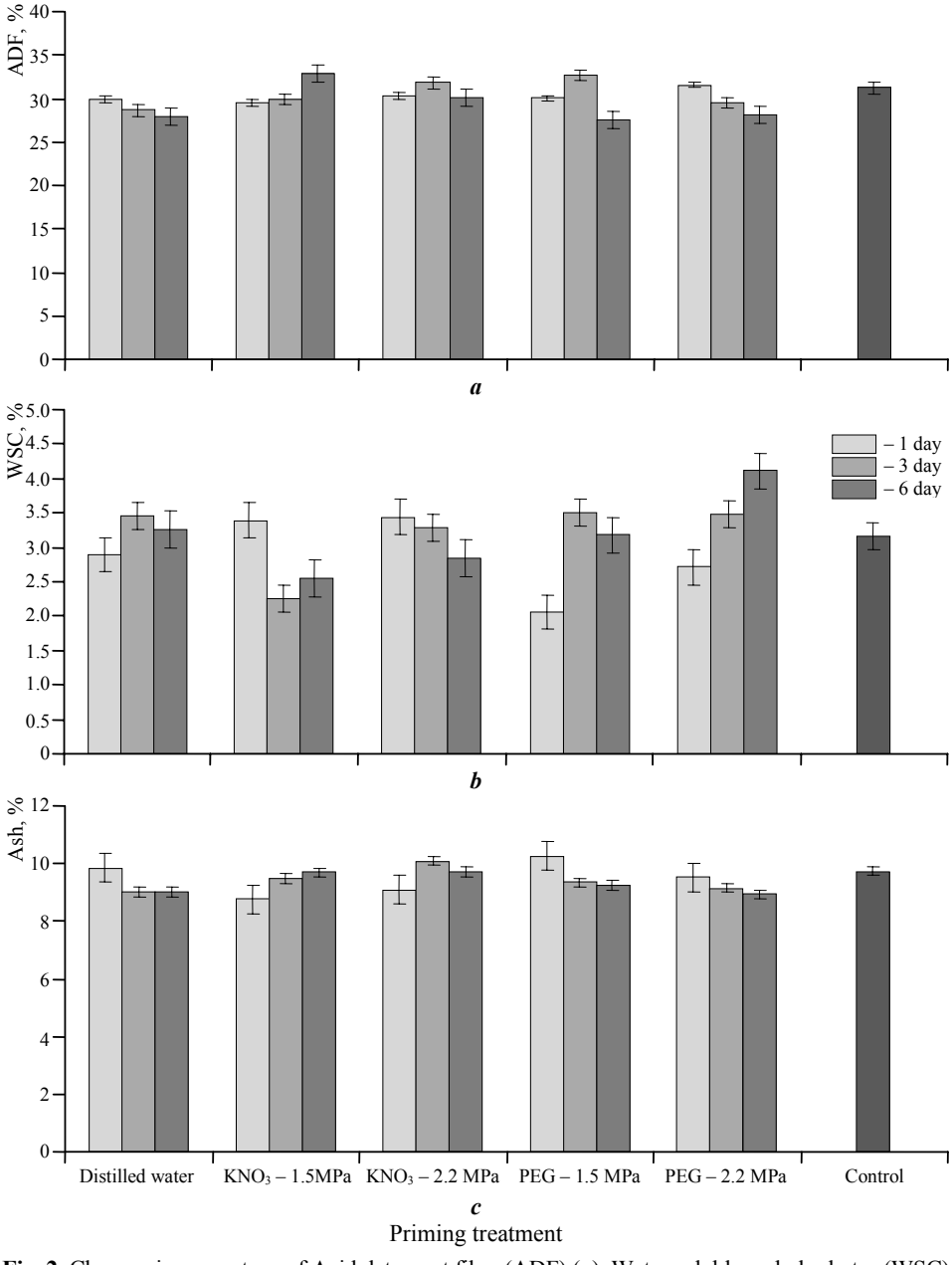

Fig. 2. Changes in percentage of Acid detergent fiber (ADF) (*a*), Water soluble carbohydrates (WSC) (*b*) Ash (*c*) of *Festuca arundinacea* under priming treatment consisting of potassium nitrate (KNO_3) and polyethylene glycol (PEG 6000) for 1, 3 and 6 days in water potential 1.5 and 2.2 MPa in greenhouse conditions

DISCUSSION

Seed germination. The results of this experiment indicated that seed priming had a positive effect on germination percentage, MGT and vigor index of *F. arundinaceous* in laboratory conditions. The results from the germination percentage tests showed that osmotic priming in PEG – 1.5 MPa for 6 days were generally higher than other priming. However, we cannot disregard the beneficial effect of hydropriming. Heydechker et al. (1973), remarked that hydropriming only broke seed dormancy, whereas treatment with PEG may imply additional physiological effects. It is probable that, in addition to a hydration stimulus on germination, osmotic priming by PEG inhibits radicle emergence, limits the rate of water absorption thus preventing membrane damage, and restores germinability to aged seeds more effectively. The resulted from the experimentation showed that osmotic priming by KNO_3 and hydropriming decreased the MGT of *Festuca arundinacea* significantly compared to treatment by PEG. The higher efficacy of MGT by PEG might be due to the state of slab and mucilage of PEG, so that water uptake was slower than with the other methods resulting in less progressed metabolic processes and slower germination (Badeck et al., 2006). Studies of Demir and Van De Venter (1999) and Chiu et al. (2006) suggest that Priming decreases MGT of various species.

In this research, all priming treatments increased the vigor index of seedlings. GongPing et al. (2000) suggested that the improvement of vigor index was associated with activated oxygen metabolism in seedlings.

Seedling emergence. The results from seedling emergence test in greenhouse conditions showed that osmotic seed priming by PEG – 1.5 MPa for 1 day increased emergence percentage (76.7%) so that, this priming was significantly ($p > 0.05$) increase seedling emergence upper 44.6% compared to the control. Our finding showed that the seedling emergence percentage for all priming treatments in greenhouse conditions were lower than the germination percentages in the laboratory. These results are in line with the work done by Foti et al. (2008). It was reported that laboratory conditions were optimal environment for seed germination, while, in the field, seeds are subjected to a lot of stress, which all adversely affects germination and consequent seedling emergence. Therefore, an increased germination percentage is not always due to an improved emergence of seedlings. This study confirmed that priming by PEG – 1.5 MPa for 6 days was not able to increase the emergence percentage despite that the germination percentage of this treatment was higher in laboratory conditions (95% germination versus 25% emergence). Also, the osmotic seed priming by PEG 1.5 for 1 day gave higher fresh weight. In fact, following the increase in the emergence percentage, the fresh weight was increased. These results are in line with the work done by, Frett and Pill (1995), Pill and Korengel (1997), Pill and Necker (2001) and Dissanayake et al. (2008), who reported that priming treatment increased the length and the fresh weight seedlings compared to non-primed seeds.

Forage quality. An increased forage quality is one of the fundamental factors in forage production. Generally, forage quality increases as the DMD and CP increase and as the NDF and CF decreases. The results of the present study showed that priming treatments increased DMD and CP contents and decreased the CF content in tall fescue forage. The interpretation of the role of priming in improvement of forage quality was difficult, because information about the influence of priming on forage quality is very scarce. A Similar study was conducted by Hussain et al. (2006) on hybrid sunflower.

They reported that the increased achene protein content might be due to an increased nutrient intake of sunflower.

Compared to the control, the treatment by PEG – 1.5 MPa 6 days gave significantly higher DMD (%) and CP (%) and lower CF (%), as indicated by the positive and negative correlations, respectively, (Table 3).

The crude protein content of forage is one of the most important factors for forage quality evaluation (Caballero et al., 1995; Assefa, Ledin, 2001), Crude fiber, the fibrous portions of a plant, such as cellulose, that are partially digestible and relatively low in nutritional value. This study showed that almost all priming treatments enhanced crude protein content and deterred crude fiber in fescue seedling as compared with the control.

CONCLUSION

The present study showed clearly that priming techniques enhanced the performance of germination, the emergence parameters and the forage quality of all fescue in both laboratory and greenhouse conditions. Moreover, priming improved quality characteristic such as DMD, CP and CF in greenhouse conditions. The findings in our study provide evidence that, for all priming treatments, the laboratory performance was better than that in the greenhouse. Therefore, laboratory evaluation of similar species should not be limited to laboratory tests. Finally, priming with PEG – 1.5 MPa for 1 day is a recommended method that is practical- to be applied for increased emergence and yield performance of tall fescue under sub-optimal conditions, and priming with PEG 1.5 MPa for 6 days is suggested for increasing quality characteristics based on greenhouse evaluation.

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