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Effects of Priming Techniques on Seed Germination and Early Growth Characteristics of *Bromus tomentellus* L. and *Bromus inermis* L.

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Abstract

Seed priming is known as a seed treatment which improves seed performance under environmental conditions. Objective of this study was to evaluate the effects of different priming treatments on seed germination behaviour of two genotypes of *Bromus* under laboratory conditions to find out the most effective priming treatment. Seeds were treated with the following seed-soaking media: (i) unsoaked seed (control); (ii) hydropriming with distilled water for 4, 8, 12 and 16 hours, (iii) osmopriming treatments with PEG 6000 for *Bromus tomentellus* were -0.6, -0.8, -1.0 and -1.2 MPa osmotic potentials with duration of 12, 24 and 36 hours and for *Bromus inermis* osmoticpotentials were the same as *B. tomentellus* but duration of priming treatments was soaking seeds for 12, 24, 36 and 48 hours. Seeds of both genotypes were placed in liquid priming media at 25°C. Priming treatments significantly affected germination vigour of both genotypes. The response of both genotypes to different priming techniques approximately was similar. Osmopriming treatment (-0.6 MPa and 12 h) increased final germination percentage of *B. tomentellus*. Priming treatments increased coleoptile length significantly comparing to control but hydropriming for 4 h exhibited longer coleoptile than others. Higher vigour index was observed in hydropriming for 12 h but this difference was not significant with osmoprime treatment (12 h-0.6 Mpa). *B. inermis* produced more germinated seeds and vigourous seedlings than *B. tomentellus* but germination rate was higher in *B. tomentellus*.

Keywords: *Bromus inermis*, *Bromus tomentellus*, germination, priming

Introduction

Seed priming is known as seed treatment wich improves seed performance under environmental conditions. In fact seed priming is a procedure that partially hydrates the seed, 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 (Fu *et al*., 1988, faster embryo growth (Dahal *et al*., 1990) and reduced leakage of metabolites (Styer and Cantliffe, 1983) compared with control. Seed priming has been found a doable technology to enhance rapid and uniform emergence, high vigour, and better yields in vegetable and flower species (Dearman *et al*., 1987; Parera and Cantliffe 1994; Bruggink *et al*., 1999), small seeded grasses (Heydecker and Coolbaer, 1978; Bradford, 1986) and some field crops (Hartz and Caprile, 1995; Chiu *et al*., 2002; Giri and Schillinger, 2003; Murungu *et al*., 2004; Basra *et al*., 2005, 2006; Kaur *et al*., 2005; Kaya *et al*., 2006; Farooq *et al*., 2006 a, b; 2007 a, b; Janmohammadi *et al*., 2009).

Seed priming is commonly used to reduce the time between seed sowing and seedling emergence (Parera and Cantliffe, 1994). 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 vigour and dehydration, and storage conditions of the primed seed (Parera and Cantliffe, 1994). Although, the previous studies indicate that some benefits are associated with pre-sowing treatments for seed vigour enhancement, but there is dearth of information about the germination performance of primed seeds of *Bromus* spp. Therefore, the present study was carried out with the objective of evaluating the effects of different priming treatments on seed germination behaviour of two genotypes of *Bromus* under laboratory conditions to find out the most effective priming treatment.

Materials and methods

The study was conducted in the seed laboratory of Natural Resources Faculty, University of Tehran, Iran. Seeds of two *Bromus* genotypes including *B. tomentellus* and

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B. inermis subjected to seed priming. Seeds were treated with the following seed-soaking media: (i) unsoaked seed (control); (ii) hydropriming with distilled water for 4, 8, 12 and 16 hour; (iii) osmopriming treatments with PEG 6000 for *B. tomentellus* were -0.6, -0.8, -1.0 and -1.2 MPa osmotic potentials with duration of 12, 24 and 36 hours while for *B. inermis* osmotic potentials were the same as *B. tomentellus* but duration of priming treatments was soaking seeds for 12, 24, 36 and 48 hours. Seeds of both genotypes were placed in liquid priming media at 25°C. Seeds were covered with plastic bags to refuse moisture loss.

After soaking, seeds were washed with distilled water, then redried in the incubator at 25°C in the dark. Germinaton test was conducted by placing 25 seeds from each of the treatments in 90 mm diameter Petri dishes on Whatman filter paper that was moistened with 5 ml distilled water. Seeds were kept in germinator at 25°C in dark condition. A completely randomized design with three replications was used. Radicle protrusion of 2 mm was scored as germination (Kaya *et al*., 2006). Germination was counted in 24 hours intervals and continued until no further germination occurred. The seedlings were evaluated as described in Seedling Evaluation Handbook (AOSA, 1991).

Final germination percentage (%), coleoptile and radicle length (cm) and seedling length (cm) was recorded after 14 days of planting on filter paper. For statistical analysis, the data of germinating percentage was transformed to $\arcsin\sqrt{(100/X)}$. Experimental data was analyzed by a statistical packet SAS, version 6.12. Treatments means were compared using Duncan's multiple comparsion test at 5% level of probability. The vigour index was calculated according to the following formula:

Vigour index (VI) = [seedling length (cm) × germination percentage]

Mean germination time (MGT) was calculated based on the Ellis and Roberts equation (1981).

Results

Priming treatments significantly affected germination vigour of both genotypes. The response of both genotypes to different priming techniques approximately was similar. Speed of germination was recorded for hydro and osmoprimed seeds as indicated by lower value of MGT or by higher germination rate (Tab. 1 and 2).

Osmopriming treatment (-0.6 Mpa and 12 h) increased final germination percentage of *B. tomentellus* by 22% while hydropriming (4 h) increased germination percentage about 12% compared to control. So, *B. tomentollus* germination percentage exhibited posetive response to osmopriming than hydroprimig (Tab. 1). But for germination rate, osmopriming treatment could not exhibit significant effect while hydropriming treatment (4 and 8 h) had significant effect and hydroprimed seed for 4 h germiated faster than non-primed treatment. All priming treatments increased coleoptile length significantly compared to control but hydropriming for 4 h exhibited longer coleoptile than the others.

Radicle length of osmoprimed seeds increased due to priming treatment of (-0.6 Mpa, 12 h) significantly while hydropriming showed no significant result. All priming treatments increased seedling length of *B. tomentellus*

Tab. 1. Effect of priming treatments on the germination and seedling characteristics of *Bromus tomentellus*

Treatments			Final germination (%)	Germination rate	MGT[1] (day)	Coleoptile length (cm)	Radicle length (cm)	Seedling length (cm)	Vigour
Control			56.8 C	0.395 B	2.582 B	4.185 I	4.4 CDE	8.585 F	487.8 H
Osmopriming	12 h	-0.6 MPa	69.65 A	0.4061 B	2.469 BC	9.012 BCD	6.5 A	15.51 A	1087 A
		-0.8 MPa	66.77 AB	0.3681 B	2.739 B	8.448 CDE	5.36 BC	13.81 B	927 BC
		-1.0 MPa	58.15 C	0.4046 B	2.475 BC	8.273 DE	5.733 AB	14.01 B	815 CD
		-1.2 MPa	56.17 CD	0.4021 B	2.499 BC	8.212 DEF	4.677 CD	12.89 BC	723.8 DEF
	24 h	-0.6 MPa	55.57 CD	0.4046 B	2.472 BC	5.633 H	4.79 BCD	10.42 E	579.5 GH
		-0.8 MPa	55.57 CD	0.2999 C	3.375 A	7.1 FG	4.887 BCD	11.99 CD	666.5 EFG
		-1.0 MPa	57.42 C	0.3154 C	3.172 A	7.78 EFG	3.05 F	10.83 DE	621.5 FGH
		-1.2 MPa	57.47 C	0.3178 C	3.222 A	8.548 CDE	4.552 CDE	13.1 BC	749.8 DE
	36 h	-0.6 MPa	50.77 D	0.3947 B	2.533 BC	5.65 H	5.287 BC	10.94 DE	555.5 GH
		-0.8 MPa	53.79 CD	0.3757 B	2.685 B	6.867 G	3.025 F	9.892 E	532.3 H
		-1.0 MPa	54.44 CD	0.3961 B	2.526 BC	7.588 EFG	3.075 F	10.66 DE	580.5 GH
		-1.2 MPa	55.72 CD	0.3756 B	2.667 B	7.4 EFG	3.65 EF	11.05 DE	619.3 FGH
Hydropriming	4 h		63.4 B	0.5037 A	1.994 D	11.27 A	4.4 CDE	15.67 A	993 AB
	8 h		57.3 C	0.4648 A	2.158 CD	9.532 BC	4.432 CDE	13.97 B	798.3 D
	12 h		45.1 E	0.4023 B	2.492 BC	9.533 BC	4.133 DE	13.67 B	615 FGH
	16 h		42.84 E	0.3899 B	2.582 B	9.733 B	3.133 F	12.87 BC	551.3 GH

* Figures not sharing the same letters in the same column differ significantly at $p < 0.05$; 1: Mean germination time

Tab. 2. Effect of priming treatments on the germination and seedling characteristics of *Bromus inermis*

Treatments			Final germination (%)	Germination rate	MGT (day)	Coleoptile length (cm)	Radicle length (cm)	Seedling length (cm)	Vigour
Control			61.59 C	0.2361 D	4.238 A	8.068 FGH	5.3 ABCDEF	13.37 DEF	822.5 FG
Osmopriming	12 h	-0.6 MPa	72.29 B	0.316 A	3.172 D	10.45 AB	7.003 A	17.45 A	1262 ABC
		-0.8 MPa	67.12 BC	0.3002 AB	3.354 CD	10.27 ABC	6.588 AB	16.86 AB	1136 BCDE
		-1.0 MPa	71.54 B	0.295 ABC	3.392 BCD	11.01 A	6.78 A	17.79 A	1275 ABC
		-1.2 MPa	69.44 B	0.287 ABC	3.492 BCD	10.21 ABC	6.533 AB	16.75 AB	1165 ABCDE
	24 h	-0.6 MPa	41.54 EF	0.2959 ABC	3.384 BCD	7.633 H	5.968 ABCDE	13.6 DEF	563.8 I
		-0.8 MPa	41.54 EF	0.2959 ABC	3.384 BCD	9.1 CDEF	6.44 ABC	15.54 ABCD	646 GHI
		-1.0 MPa	68.44 BC	0.2802 ABC	3.576 BCD	9.78 BCD	4.8 CDEFG	14.58 BCDE	1005 DEF
		-1.2 MPa	67.81 BC	0.2699 BCD	3.744 BC	10.55 AB	6.802 A	17.35 A	1177 ABCD
	36 h	-0.6 MPa	21.37 G	0.2599 CD	3.857 AB	7.65 H	4.483 EFG	12.13 F	261 J
		-0.8 MPa	38.32 F	0.2808 ABC	3.593 BCD	8.868 DEFG	4.912 BCDEFG	13.78 CDEF	530 I
		-1.0 MPa	48.52 DE	0.2823 ABC	3.576 BCD	9.587 BCD	3.608 G	13.19 DEF	646.5 GHI
		-1.2 MPa	60.99 C	0.2833 ABC	3.563 BCD	9.4 BCDE	3.928 FG	13.33 DEF	817.3 FG
	48 h	-0.6 MPa	43.85 EF	0.2849 ABC	3.563 BCD	6.185 I	3.85 FG	10.03 G	439.5 IJ
		-0.8 MPa	48.33 DE	0.2859 ABC	3.504 BCD	7.74 GH	4.707 DEFG	12.45 EF	600.5 HI
		-1.0 MPa	43.02 EF	0.2839 ABC	3.549 BCD	7.22 HI	5.313 ABCDEF	12.53 EF	542.3 I
		-1.2 MPa	53.15 D	0.294 ABC	3.408 BCD	8.387 EFGH	6.573 AB	14.96 BCD	797.5 FGH
Hydropriming	4 h		65.53 BC	0.239 D	4.188 A	9.717 BCD	4.932 BCDEFG	14.65 BCDE	962 EF
	8 h		82.31 A	0.2869 ABC	3.509 BCD	9.8 BCD	6.332 ABCD	16.13 AB	1334 AB
	12 h		80.68 A	0.2845 ABC	3.55 BCD	9.868 ABCD	6.832 A	16.7 AB	1353 A
	16 h		67.81 BC	0.2755 BC	3.663 BCD	9.392 BCDE	6.55 AB	15.94 ABC	1082 CDE

*Figures not sharing the same letters in the same column differ significantly at $p < 0.05$

but it was more clear in hydropiriming for 4 h and osmopriming (-0.6 Mpa, 12 h). Finally, osmoprimed seeds of *B. tomentellus* exhibited higher vigour index than hydro primed seeds. Germination percentage of *B. inermis* was increased due to seed priming, and hydropriming seeds for 8 h exhibited higher germination percentage than the others (Tab. 2). Germination rate was also affected by seed priming and osmopriming (12 h and -0.6 Mpa) exhibited higher speed of germination compared to control and other priming treatments. No significant increse was obtained for radicle length of *B. inermis* due to seed priming. All osmopriming treatments for 12 h increased coleoptile length significantly but there was no significant difference between all concentrations of PEG in this duration for seed priming. Higher seedling length was observed in osmopriming treatment (12 h, -0.6 Mpa) compared to other treatments including control. Higher vigour index was observed in hydropriming for 12 h but this difference was not significant with osmoprime treatment (12 h, -0.6 Mpa) (Tab. 2). *B. inermis* produced more germinated seeds and vigourous seedlings than *B. tomentellus* but germination rate was higher in *B. tomentellus* (Fig. 1).

Discussion and conclusions

Seed germination and seedling growth are critical for seedling at the first life stages and often subject to high mortality rates. The three early phases of germination are: (i) imbibition, (ii) lag phase, and (iii) protrusion of the radical through the testa (Simon, 1984). Priming is a procedure that partially hydrates seed, followed by drying of seed, therefore germination processes begin, but radicle emergence does not occur. There are reports that hydration of seed up to, but not exceeding, the lag phase with priming permits early DNA replication (Bray *et al*., 1989), increased RNA and protein synthesis (Fu *et al*., 1988; Ibrahim *et al*., 1983), greater ATP availability (Mazor *et al*., 1984), faster embryo growth (Dahal *et al*., 1990), repair of deteriorated seed parts (Karssen *et al*., 1989; Saha *et al*., 1990). These help radicle protrusion through the seed coat and shorten the time to seed germination.

In this research, seed priming increased germination characteristics of under study *Bromus* geynotyps. This might be due to faster water uptake by primed seed comparing to the control treatment. Similarly, Khan (1993) reported that osmo-conditioning with seed hydration treatments, seed hardening and moisturizing on vermiculite improved the performance of sweet corn seed. The pre-

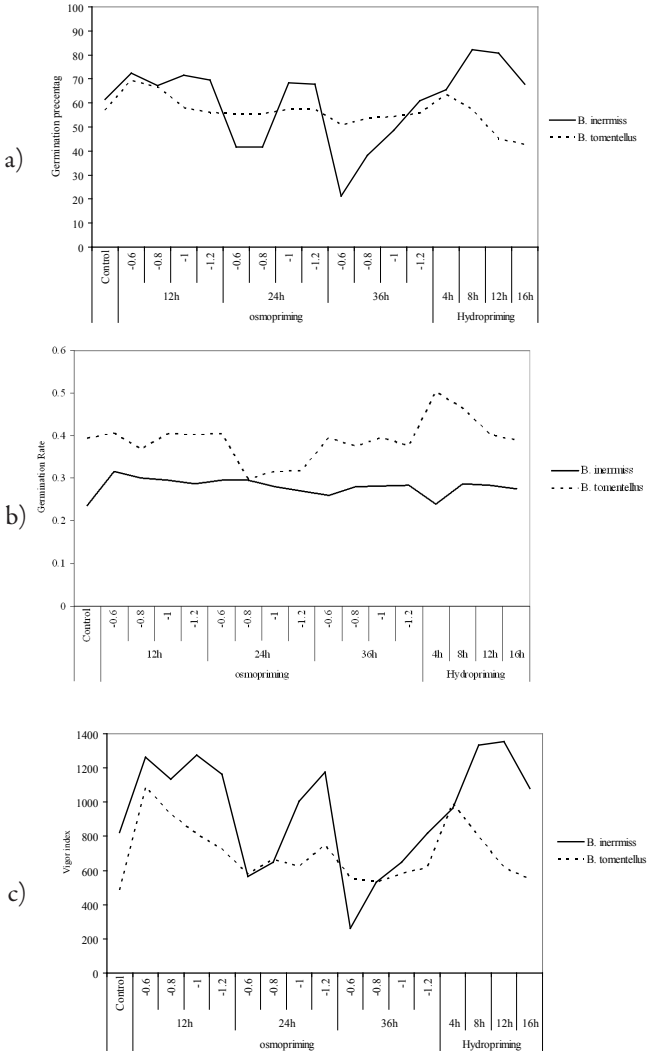

Fig. 1. Germination characteristics of two *Bromus* species under different priming treatments: a) Germination percentage; b) Germination rate; c) Vigour index (seedling length × germination percentage)

soaking of seeds allows the hydration of membranes and proteins, and the initiation of various metabolic systems. These are arrested when the seeds are dried or moisture is withheld, but recommence when the seeds imbibe water for the second time (Bewley and Black, 1982). Ashraf and Rauf (2001) reported that final germination percentage, fresh and dry weight of corn seed increased by seed priming significantly. So due to all this information and present study it is clear that most effects of seed priming is due to seed hydration. So, optimization of priming technique is very important to achive the best time and concentration combination. Higher and faster germination will increase uniformity and final yield.

According to *B. tomentellus* and *B. inermis* values in view point of livestocks forage in Iranian rangelands, also considering their natural habitats soil condition (relatively high moisture and low salinity), findings of this research help us to prepare *Bromus* seeds through priming techniques when their sowing, as high quality forage, is necessary in arid and semi arid areas of Iran. Additionally, it is suggested to work on how could seed priming of *Bromus* seeds affects tolerance to environmental stress.

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