Original Article

Effects of Osmo-Hydropriming and Drought Stress on Seed Germination and Seedling Growth of Rye (*Secale Montanum*)

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Abstract

Germination and seedling establishment are the major and critical steps in establishment of the crops in the arid and semi-arid environments. In this study an experiment was conducted to evaluate the effects of different time seed priming (osmo-hydropriming) on germination and seedling growth of Secale montanume seeds under drought conditions. Seeds were immersed in water with osmotic potentials of -0.5, -1 and -1.5 MPa. PEG600 was employed to induce the required levels of osmotic potentials. Induction of hydropriming was obtained by immersing the seeds in distilled water with aeration and no aeration conditions. Both two priming treatments were conducted at 3 time duration (6, 12 and 24 hours). These primed seeds were germinated and grew at osmotic potentials of 0 (distilled water), -0.5, -1, and -1.5 MPa by PEG6000. Results showed that the seed germination and seed growth were inhibited by increasing drought stress as the germination percentage of hydroprimed seeds with aeration was reduced from 74.02% to 60.45%. Both shoot and root growths were inhibited by increasing osmotic potential. Hydroprimed seeds with aeration caused reduction shoot and root length, while root/shoot length ratio was increased by hydroprimng without aeration. The least priming time (6 hr) had more effects on germination percentage. Result also showed that seed treatment at difference levels of PEG significantly affected germination characteristics. The highest germination percentage was obtained from seed priming with PEG induced -0.5 MPa osmotic potential. The highest root/shoot length ratio was related to osmopriming with -0.5 MPa. The time of osmopriming didn't show significant effect on germination characters and seedling growth. However 6 h osmopriming was more effective.

Keywords: osmotic potential, priming, seed, *Secale montanum*.

1. Introduction

Rye (*Secale cereale* L.) is known as one of the most recalcitrant species in tissue culture and genetic transformation. Rye is one of the most recently domesticated cereals. Its likely origin is the Caucasus region from where it spread out as a weed in wheat fields representing a so called "secondary crop" [26].

There are five species within the genus *Secale*: *S. silvestre*, *S. vavilovii*, *S. montanum*, *S africanum* and *S. cereale*, with all cultured forms seeming to be originated from the latter [26]. Rye was also used to develop the most important artificial amphidiploid cereal crop, *Triticale*, a hybrid between *Triticum* and *Secale*. Rye is mainly used as bread cereal and is prized due to the high nutritional value of its proteins, the characteristic taste and its long fresh-keeping properties compared to wheat bread. Compared to other cereal crops rye is the most adaptable. Due to its high cold resistance it can be grown at higher latitude and altitude than

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any other winter cereal. It well tolerates adverse soil conditions like acidity or alkalinity, can be grown on poor and marginal soils and gives reasonable yield even in regions where no other cereals can grow. In arid and semi-arid environments, the water needed for germination is available only for a short period, In addition successful crop establishment depends not only on the rapid and uniform germination of the seed, but also on the ability of the seed to germinate under low water availability [15]. However, if the stress effect can be alleviated at the germination stage, chances for attaining a good crop with economic yield production would be high [6].

Seed priming is a technique of seed enhancement which improves germination or seedling growth and rate or uniformity of the seedling establishment [38]. It improves seed performance by rapid and uniform germination, normal and vigorous seedlings, which resulted in faster and better germination and emergence of different crops [33, 10]. This also helps seedlings to grow under stress conditions [5, 11].

Priming allows some of the metabolic processes necessary for germination to occur without germination take place. In priming, seeds are soaked in different solutions with high osmotic potential. Thus the seeds were prevented from absorbing enough water for radicle protrusion, and suspending the seeds in the lag phase [38]. Seed priming has been commonly used to reduce the time between seed sowing and seedling emergence and to synchronize emergence [29].

These effects of priming are associated with repairing and building up of nucleic acids, increased synthesis of proteins as well as the repairing of membranes [24]. Priming also enhances the activities of anti-oxidative enzymes in treated seeds [19, 40]. Moreover, priming increases the activities of glyoxysome enzymes in primed bitter gourd seeds [23].

Among the abiotic stresses, drought is a major limiting factor for crop productivity all over the world. Drought affects almost every aspect of the physiology and biochemistry of plants which in turn significantly reduces yield [27, 30].

In osmopriming treatment which is also known as osmoconditioning, seeds incubated in polyethylene glycol (PEG), sorbitol, mannitol solution and let to uptake water in order to primary metabolic activities of germination process is started and radicle emergence happen [5]. Past studies revealed that PEG had no toxic effect since all seeds germinated. Mehra *et al.* [25] indicated that PEG molecules do not enter to seed and there was no toxicity of PEG.Osmopriming and hydropriming of

wheat seeds may improve germination and emergence [4] and may promote vigorous root growth [12].

Different results have been achieved as the yield of primed tomato seeds, in several cases priming led to earlier field emergence which could result in earlier maturation of the crop [22]; while in one case priming resulted only *in* earlier development prior to flowering [3]. For all crops the growth advantage caused by priming could be attributed entirely to the earliness of emergence of the primed seeds. Since hydro-priming is a very simple, economical and environmental friendly type of seed priming [17, 39].

Lot of information is 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 [13] and reduced leakage of metabolites [36] compared with control. Many recent studies (e.g. Tajbakhsh *et al*, 2004 and Sharafzadeh *et al,* 2006) showed that seed priming of crops specially rye might improve seedling establishment and growth [35, 37].

The main objectives of this study were to select sensitive ecotype of rye to drought stress among 10 ecotypes, and investigate the effects of hydro-priming (with aeration and not aeration) and osmo-priming at different duration on seed germination and seedling growth of *secale muntanom* seeds.

2. Material and Method

2.1. Seed collection

This experiment was conducted at Research Laboratory of Faculty of Agriculture, Ferdowsi university of Mashhad in 2010. Seeds of rye (*secale montanum*) were collected from Torogh research station of Mashhad. 10 ecotypes of rye were used in this research including 264 (Ghazvin), 12640, 3857 (Semnan), 8425 (Arak), 14947 (Arak), 1587 (Alborz), 941 (Esfahan), 591 (Golestan), 20560 (Karaj), 4811 (Sharekord). Seed lots were sealed in aluminum foil packets and stored in a cool temperature after collection.

2.2. Sensitive ecotype

For selecting one drought sensitive ecotype among 10 ecotypes of secale, a preliminary experiment was carried out. Inthis preliminary experiment, 10 ecotypes were exposed to different osmotic potential (0, -0.5, -1 and -1.5 MPa) for 14 days and germination characteristic and seedling growth were monitored. Three replicates of 25 seeds from each ecotype were placed between two

Whatman filter papers in a 9 cm Petri-dishes, after added 5 milliliter of solutions to the Petri-dishes.

A wet cloth placed in plastic trays and Petri-dishes were placed in trays, and trays covered with a moisture proof bag to minimize water evaporation. Seeds were then allowed to germinate at a constant temperature of 20°C in the dark in an incubator. Germination was recorded daily for 9 days. Seeds with 2 mm radical length were considered as germinated seeds. Root length, shoot length, root dry weight and shoot dry weight measured after 14 days. Cluster method was used for sensitive ecotype selection within 10 study ecotypes. This method was performed based on the means of germination character.

2.3. Priming treatments

2.3.1. Hydro-priming

The selected ecotype was exposed to seed priming. Hydro-priming (HP) carried out in distilled water with and without aeration (HP+O_2 and HP-O_2, respectively) for 6, 12 and 24 hours, at 25 °C in dark. Then, seeds dried on erasing paper and left overnight at room temperature to their original dry weight.

2.3.2. Osmo-priming

Seeds were pre-treated with different levels of osmotic potential (-0.5, -1 and -1.5 MPa) with PEG6000 for 6, 12 and 24 hours. Priming treatments were performed at 25°C under dark conditions. After different times of priming, samples of seeds were removed and rinsed with distilled water and then dried to the original moisture level and left overnight at room temperature to their original dry weight.

2.4. Germination tests

Germination and early seedling growth of hydro-primed and osmo-primed mixed seeds were studied for 14 days at distilled water (0 MPa) and solutions with osmotic potential of -0.5, -1 and -1.5 MPa, that prepared using either 192.6, 284.0 and 354.4 g/L PEG6000, as described in section 2 sub-section 2 (2.2.).

Petri dishes contained double layered filter paper with 5 ml test solutions and they were put in plastic bags to avoid moisture loss. Afterward they were placed in incubator at 20±1 °C for 14 days. Germination was considered when the radicles were 2 mm long.

Germination percentages was recorded every 24 h for 14 days. At the end of experiment, the final germination percentage and mean germination time (MGT) were calculated to evaluate

seed germination characteristics. The rate of germination was calculated as MGT, using the formula below:

$$MGT = \Sigma\, fx\, /\, \Sigma\, x$$

Where 'f' is the number of days from the beginning of germination test and 'x' is the number of seeds newly germinated on that day. Root length, shoot length, root dry weight and shoot dry weight measures after the 14 days.

2.5. Data analysis

A completely randomized design was used in the experiments. All data analyzed by MSTAT-C. Analysis of variance (ANOVA) was used to compare priming treatments effect, and significance differences of means using Duncans multiple range test ($P < 0.05$). Cluster method was used for sensitive ecotype selection.

3. Results and Discussions

3.1. Sensitive ecotype based on germination characteristics

Based on average values of morphological traits, the 10 ecotypes were classified into three clusters as, low, medium and high in responses to drought stress (fig.1). The representative ecotype after clustering was 4811 as sensitive ecotype to drought stress.
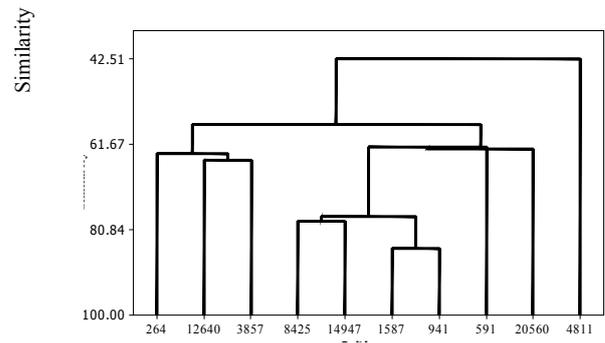
Figure 1. Cluster analysis dendrogram for 10 different ecotypes of rye using average of germination traits

3.2. Effect of hydropriming and drought stress

Both of unprimed and hydroprimed seeds germinated in solutions with different osmotic potentials (0, -.5, -1 and -1.5 MPa), within a time window of 1-9 days with a linear response during the first 3 days, leveling off after that (fig. 2). The number of days to first germination increased with the decrease of osmotic potential in PEG solutions, especially in -1 and -1.5 MPa.. Cumulative

germination percentage increased with increasing in imbibitions days in all of the treatment. In distilled water (0MPa) and -0.5 MPa there were no significantly difference between different treatment levels. Most significantly difference was showed in

osmotic potential of -1 MPa. In osmotic potential of -1 MPa the most and least cumulative germination percentage was related to 6 h hydroprimed and unprimed seeds respectively (fig. 2). In -1.5 MPa osmotic potential was obtained the same results.
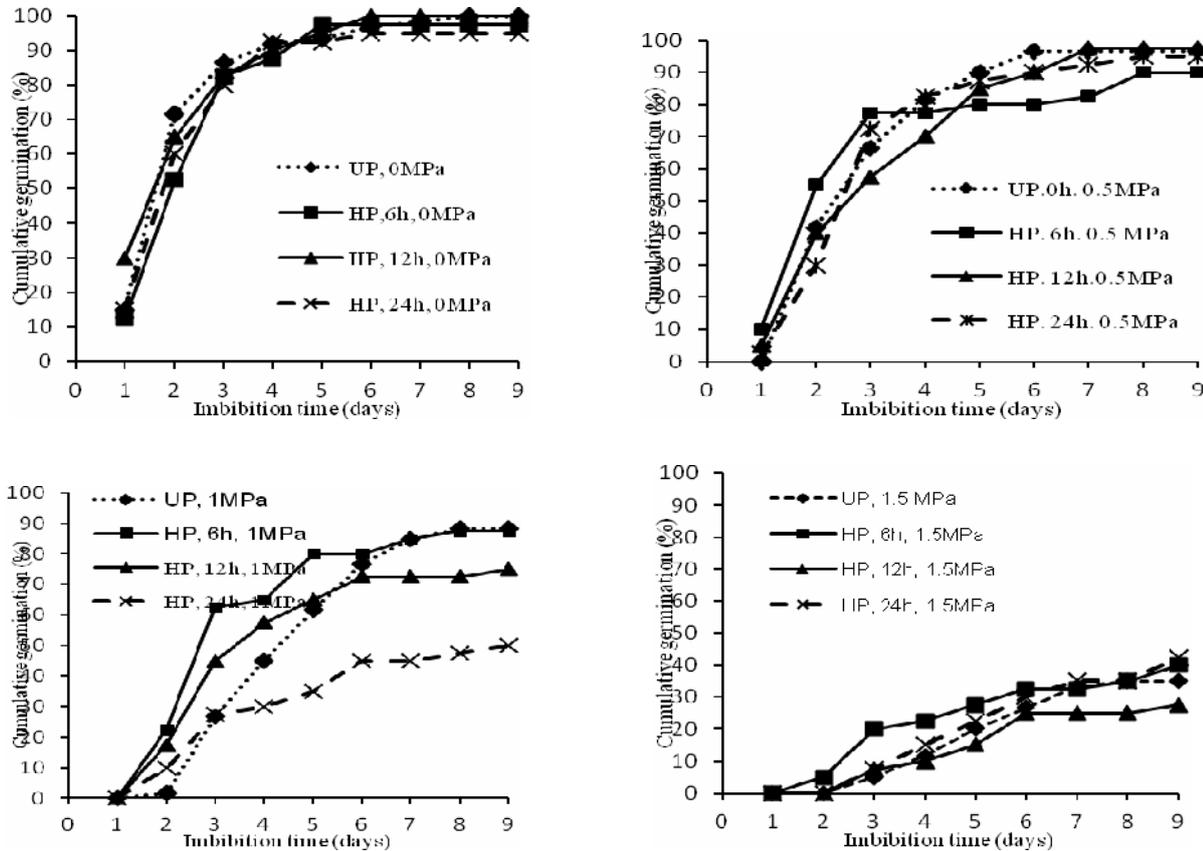
Figure 2. Cumulative seed germination time-courses of unprimed seeds (UP) and hydroprimed mixed seed (HP) at different osmotic potential in PEG solutions. The seeds were germinated under continuous dark condition. Different symbols represent the observed germination percentages against time at different osmotic potentials

In PEG solutions, seed germination percentages significantly declined with decreasing osmotic potential, finally from 88.4 % in distilled water to 27.9% at -1.5 MPa. No significant difference on germination percentage was observed at osmotic potential between 0 and -0.5 MPa (table 1). The MGT gradually increased with decrease of osmotic potential. Shoot and root length were significantly affected by osmotic potentials in PEG solutions. Both of them decreased with refusing osmotic potential from 0 to -1.5 MPa. At -1.5 MPa in PEG regularly remained enclosed by the seed coat and hypocotyls growth stopped after emergence of the radicles (table 1).

Root/shoot ratio significantly increased with decreasing osmotic potential until -1 MPa. At -1.5 MPa it was 0 due to inhibition of root growth at -

1.5 MPa. Root and shoot dry weight had the same tendency with root and shoot length (table 2). Significant difference was not observed between 0 and -0.5 MPa in both of parameters but they were larger at -0.5MPa osmotic potential.

Compared to hydroprimed without O_2 Seeds, hydroprimed with O_2 resulted in significantly higher germination percentages (74.0% vs. 60.4%). Germination rate and then MGT did not show significant difference in HP+O_2 and HP-O_2 (table 1). Root and shoot length had the same way between HP+O_2 and HP-O_2 treatments. In both of them, HP-O_2 primed seeds significantly performed higher lengths (table 1).

Root/shoot ratio also was increased at HP-O_2 seeds. Root and shoot dry weight had the same result with length (table 2).

Table 1. Effects of osmotic potential (Ψ) of PEG solutions and seed treatment (HP+O$_2$ = hydroprimed seeds with O$_2$; HP-O$_2$ = hydroprimed seeds without O$_2$) on germination percentage, mean germination time (MGT), shoot and root length of secal

Ψ(MPa)	Germination (%)			MGT(days)			Shoot length(mm)			Root length(mm)			Root/Shoot length ratio		
	HP+O$_2$	HP-O$_2$	Mean	HP+O$_2$	HP-O$_2$	Mean	HP+O$_2$	HP-O$_2$	Mean	HP+O$_2$	HP-O$_2$	Mean	HP+O$_2$	HP-O$_2$	Mean
0	79.3	97.5	88.41a	2.87	2.46	2.66d	121.16	120.6	120.88a	126.1	137.7	131.9a	1.03	1.13	1.08c
0.5	80/0	94.2	87.08a	3.82	3.15	3.48c	52.18	52.97	52.57b	92.60	101.0	96.8b	1.80	1.90	1.85b
1	63.3	67.7	65.5b	4.16	3.79	3.97b	5.70	3.36	4.53c	45.60	34.97	40.28c	4.80	7.13	5.80a
1.5	19.2	36.8	27.96c	4.89	5.06	4.97a	0.00	0.00	0.00d	0.00	4.50	2.25d	0.00	0.00	0.00d
Mean	60.5b	74.0a		3.93a	3.61a		44.76b	44.23a		66.07b	69.54a		1.90b	2.54a	
Signif. Ψ	***			*			**			***			***		
Seed treatment	***			**			*			*			***		
Interaction	***			ns			ns			*			***		

Values represent means, and different letters indicate significant difference p < 0.05 level.
n.s., not significant, * significant at P < 0.05 level, ** Significant at P < 0.01 level, *** significant at P < 0.001 level

Table 2. Effects of osmotic potential (Ψ) of PEG solutions and seed treatment (HP+O$_2$= hydroprimed seeds with O$_2$; HP-O$_2$ = hydroprimed seeds without O$_2$) on shoot and root weight

Ψ(MPa)	Root dry weight (g)			Shoot dry weight (g)			Root/Shoot weight ratio		
	HP+O$_2$	HP-O$_2$	Mean	HP+O$_2$	HP-O$_2$	Mean	HP+O$_2$	HP-O$_2$	Mean
0	3.44	4.05	3.75a	6.16	5.11	5.63a	0.55	0.19	0.37b
0.5	4.83	4.28	4.55a	5.94	6.55	6.24a	0.81	0.65	0.73a
1	2.62	2.90	2.76b	3.04	3.59	3.31b	0.59	0.84	0.71a
1.5	1.48	1.28	1.38d	0.16	3.95	2.05c	0.16	1.17	0.66c
Mean	3.09b	3.13a		3.82b	4.8a		0.52b	0.71a	
significance Ψ	**			*			ns		
Seed treatment	*			ns			**		
Interaction	ns			**			***		

Values represent means, and different letters indicate significant difference p < 0.05 level.
n.s., not significant, * significant at P < 0.05 level, ** Significant at P < 0.01 level, *** significant at P < 0.001 level

A significant two-way interaction (seed treatment and osmotic potential) was found for germination percentage (P < 0.001), root length (P < 0.05), root/shoot length ratio (P<0.001), shoot dry weight (P < 0.01) and root/shoot weight ratio (P < 0.001) but insignificant for MGT, root length and root dry weight (tables 1 and 2).

3.3. Effect of difference times of hydropriming and drought stress

Different times of priming on germination percentage showed that increase in hydropriming time caused a very significant reduction of the final germination percentage from 77.2% (6 h) to 51.7% (24 h) and there was not significant difference in the MGT from 6h to 24 h hydropriming (table 3). Other germination characteristics such as shoot and root length was not clearly different between different times of priming. The highest root/shoot ratio obtained from 12h hydropriming (table 3). Results also showed that shoot and root dry weight had the

same trend. Both of them didn't exhibit significantly difference between 6 and 12h prime and there was also declining in shoot and root dry weight at 24h hydropriming (table 4). A significant two-way interaction (seed hydropriming times and osmotic potential) was obtained for germination percentage (P < 0.001), MGT (P < 0.05), root length (P < 0.01), root/shoot length ratio (P<0.001) and root/shoot weight ratio (P < 0.05) but insignificant for shoot length and shoot and root dry weight (tables 3 and 4).

3.4. Effect of osmopriming and drought stress

Increasing drought stress caused decreasing of germination percentage and increasing of MGT the highest (96.8%) and lowest (28%) germination percentage obtained from 0 and 1.5 MPa, (table 5).

Shoot and root length and shoot and root dry weight had the same trends with germination percentage as decreased by increasing osmotic potential (tables 5 and 6).

Table 3. Effects of osmotic potential (Ψ) of PEG solutions and seed treatment time (hydropriming) on germination percentage, mean germination time (MGT), shoot and root length of secale

Prime time (hr)	Germination (%)				MGT(days)				Shoot length(mm)				Root length(mm)				Root/Shoot length ratio			
	6	12	24	Mean	6	12	24	Mean	6	12	24	Mean	6	12	24	Mean	6	12	24	Mean
Ψ(MPa)																				
0	93.75	100.0	71.50	88.41a	2.62	2.66	2.71	2.66d	123.9	125.3	114.1	121.1a	134.0	132.9	128.8	131.9a	1.05	1.02	1.17	1.08c
0.5	87.50	97.50	76.25	87.08a	3.12	3.19	4.16	3.49c	61.95	58.43	37.35	52.57b	106.8	110.9	72.68	96.79b	1.95	1.67	1.92	1.84b
1	92.50	67.50	36.50	65.50b	3.51	4.23	4.19	3.97b	12.65	0.15	0.80	4.53c	73.35	23.60	23.90	40.34c	6.22	10.43	0.17	5.60a
1.5	35.00	26.25	22.50	27.91c	4.35	5.66	4.91	4.97a	0.00	0.00	0.00	0.00c	6.75	0.00	0.00	2.25d	0.00	0.00	0.00	0.00d
Mean	77.18a	72.81b	51.68c		3.4a	3.93a	3.99a		49.62a	45.97a	38.06b		80.22a	66.85a	56.34a		2.30b	3.28a	0.81c	
significance Ψ		***				***				***				***				***		
Seed treatment		***				**				**				***				***		
Interaction		***				*				ns				**				***		

Values represent means, and different letters indicate significant difference p < 0.05 level.
n.s., not significant, * significant at P < 0.05 level, ** Significant at P < 0.01 level, *** significant at P < 0.001 level

Table 4 Effects of osmotic potential (Ψ) of PEG solutions and seed treatment time (hydropriming) on root and shoot weight and root/shoot ratio

Prime time (hr)	Root dry weight (g)				Shoot dry weight (g)				Root/Shoot weight Ratio			
	6	12	24	Mean	6	12	24	Mean	6	12	24	Mean
Ψ(MPa)												
0	3.16	4.25	3.83	3.74a	6.50	6.33	4.08	5.63a	3.18	2.68	2.95	2.93a
0.5	5.00	4.83	3.83	4.55a	6.33	7.16	5.25	6.24a	3.75	3.46	1.43	2.89a
1	3.10	3.27	1.91	2.76b	3.72	3.86	2.36	3.31b	4.40	2.19	1.44	2.67b
1.5	1.45	1.06	1.64	1.38c	0.98	0.35	0.34	0.55c	1.99	2.67	1.63	2.09c
Mean	3.17a	3.35a	2.80b		4.27a	4.42a	3.00b		3.33a	2.75b	1.86c	
significance Ψ		***				***				ns		
Seed treatment		ns				**				**		
Interaction		ns				ns				*		

Values represent means, and different letters indicate significant difference p < 0.05 level.
n.s., not significant, * significant at P < 0.05 level, ** Significant at P < 0.01 level, *** significant at P < 0.001 level

Table 5. Effects of osmotic potential (Ψ) of PEG solutions and seed treatment with PEG on germination percentage, mean germination time (MGT), shoot and root length and root/shoot length ratio of secale

Ψ(MPa)	Germination (%)				MGT(days)				Shoot length(mm)				Root length(mm)				Root/Shoot length ratio			
	0.5	1	1.5	Mean	0.5	1	1.5	Mean	0.5	1	1.5	Mean	0.5	1	1.5	Mean	0.5	1	1.5	Mean
0	100.0	98.33	97.50	98.61a	2.73	2.62	2.76	2.70d	140.50	128.4	140.8	136.6a	150.20	139.0	143.30	142.2a	1.07	1.08	1.02	1.06c
0.5	94.17	96.67	96.67	95.83b	3.27	3.44	3.44	3.30c	61.28	60.57	71.45	64.43b	115.1	115.4	114.1	114.9b	1.87	1.95	1.60	1.81b
1	86.67	80.00	85.83	84.17c	4.15	4.56	4.61	4.44b	8.95	12.60	14.67	12.07c	44.12	50.00	60.98	51.70c	5.69	4.66	4.60	4.98a
1.5	31.50	29.17	23.33	28.00d	5.51	5.55	5.28	5.54a	0.00	0.00	0.00	0.00d	7.40	7.71	8.26	7.79d	0.00	0.00	0.00	0.00d
Mean	78.08a	76.04b	75.83b		3.29b	4.04a	4.03ab		56.74a	50.40b	52.69b		79.22a	78.03a	81.66a		2.16a	1.92ab	1.80b	
significance Ψ		***				***				***				***				***		
Seed treatment		*				*				***				ns				*		
Interaction		**				**				**				ns				**		

Values represent means, and different letters indicate significant difference p < 0.05 level.

n.s., not significant, * significant at P < 0.05 level, ** Significant at P < 0.01 level, *** significant at P < 0.001 level

Table 6. Effects of osmotic potential (Ψ) of PEG solutions and seed treatment with PEG on shoot and root weight and root/shoot weight ratio

Ψ(MPa)	Root dry weight (g)				Shoot dry weight (g)				Root/Shoot Weight Ratio			
	0.5	1	1.5	Mean	0.5	1	1.5	Mean	0.5	1	1.5	Mean
0	4.39	4.11	5.00	4.50b	5.89	6.28	6.05	6.07a	0.82	0.66	1.00	0.83c
0.5	8.40	7.16	6.94	7.50a	4.83	4.77	4.22	4.61b	1.76	1.76	1.78	1.76b
1	4.85	3.51	4.87	4.41b	1.38	1.60	1.77	1.58c	3.79	2.48	2.88	3.05a
1.5	0.76	1.82	1.43	1.34c	0.00	0.00	0.00	0.00d	0.00	0.00	0.00	0.00d
Mean	4.60a	4.15a	4.56a		3.02a	3.16a	3.01a		1.59a	1.22b	1.41ab	
Significance Ψ		***				***				***		
Seed treatment		ns				ns				*		
Interaction		*				ns				*		

Values represent means, and different letters indicate significant difference p < 0.05 level.

n.s., not significant, * significant at P < 0.05 level, ** Significant at P < 0.01 level, *** significant at P < 0.001 level

The root/shoot ratio increased by increasing osmotic potential until -1 MPa and decreased at -1.5 MPa (table 5).

Comparison of different osmoprming levels (-0.5, -1 and -1.5 MPa) showed that there was significant difference in germination characteristics. Highest germination percentage (78.1%) obtained from -0.5 MPa osmopriming treatment, but osmopriming at -1 and -1.5 MPa osmotic potential didn't show significant effect on germination percentage (table 5).

Lowest MGT (3.29 day) was obtained at -0.5 MPa seed treatment and other two levels of osmopriming treatments didn't show significant difference. Osmopriming treatments had significantly effect on root length as by seed osmopriming at -0.5 MPa had highest (56.74 mm) and -1 Mpa had lowest (50.4mm) shoot length. Osmopriminig don't had significant effect on root length and root and shoot dry weight but length and weight root/shoot ratio was significant, as by increasing in osmotic potential on seed treatment, decreased length root/shoot ratio and lowest weight root/shoot ratio was at -1 MPa seed treatment (tables 5 and 6).

A two-way ANOVA indicated that total germination percentages, MGT, shoot length, root/shoot length ratio and root/shoot weight ratio were significantly affected by seed priming and osmotic potential, and their interaction also was significant, but the interaction of shoot dry weight and root length were not significant (table 5 and 6).

3.5. Effect of different times of osmopriming and drought stress

Result showed that between different times of osmopriming in germination percentage, MGT, root length and root/shoot length ratio there weren't significantly difference, but 6h seeds osmopriming was more effective and by increasing seed treatment time duration, means of these traits declined (table 7). Root and shoot dry weight significantly affected by osmopriming times as 6 hours seed treatment had highest root and shoot dry weight (table 8).

There was significantly interaction between osmopriming times and osmotic potential of PEG in germination percentage ($P < 0.001$), MGT ($P < 0.001$), shoot length ($P < 0.01$), root/shoot length ratio ($P < 0.05$), and root/ shoot weight ratio ($P < 0.05$) but weren't significantly interaction in root length and shoot dry weight (tables 7 and 8).

In this study, the seed germination and seedling growth of rye was investigated under drought stress after hydro and osmopriming treatments. Seedlings showed moderate tolerance to drought stress and grew well at -0.5 MPa osmotic

potential. Hydropriming without aeration (HP-O2), enhanced germination and shortened the delay in germination time under both stress and non-stress condition.

The best hydropriming time was 6 hours in both of the germination and seedling characteristics. Moreover, it could be used as a mean of selecting seeds with high germination potential by only using hydro primed seeds without O_2 after 6h of contact with water. These combined results could be applied to improve rye seedling production.

Priming allows some of the metabolic processes necessary for germination to occur without germination take place. In priming, seeds are soaked in different solutions with high osmotic potential. This prevents the seeds from absorbing in enough water for radicle protrusion, thus suspending the seeds in the lag phase [38].

Seed priming has been commonly used to reduce the time between seed sowing and seedling emergence and to synchronize emergence [29].

Hydropriming of wheat seeds may improve germination and emergence [4] and may promote vigorous root growth [12]. In maize (*Zea mays* L.) inbred lines, maximum invigoration was observed in seeds hydroprimed for 36h as indicated by higher germination rate and longer radical length.

Previous work suggested that the adverse and depressive effects of water stress on germination can be alleviated by various seed priming treatments [1, 2, 7, 34].

Under these stresses there is a decrease in water uptake during imbibitions and furthermore salt stress may cause excessive uptake of ions [28]. Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops, particularly seeds of vegetables and small seeded grasses [8].

Seed germination and early seedling growth are sensitive to drought stress in many crop species [5], and our results confirm those observations for rye. In PEG solution, the strongest decline in germination percentage and the slowest germination rate were observed at the highest PEG concentration. Least germination percentage occurred at osmotic potential of -1.5 MPa. High drought stress inhibited the growth of shoot and root.

Similar observations were made for canola and sweet sorghum when seeds were exposed to similar stress conditions (9, 31].

In this study, hydropriming without O_2 promoted germination and rapid seedling emergence of rye both in non-drought and drought stress, which are in line with findings for example in sunflower, triticale and potato seeds [20, 41, 14].

Table 7. Effects of osmotic potential (Ψ) of PEG solutions and seed treatment time (PEG) on germination percentage, mean germination time (MGT), shoot and root length of secale

Prime time (h)	Germination(%)				MGT(days)				Shoot length(mm)				Root length(mm)				Root/Shoot ratio			
	6	12	24	Mean	6	12	24	Mean	6	12	24	Mean	6	12	24	Mean	6	12	24	Mean
Ψ(MPa)																				
0	98.33	99.17	98.33	98.61a	2.82	2.59	2.70	2.70d	132.9	143.20	133.70	136.6a	147.6	142.8	142.1	144.2a	1.113	1.008	1.068	1.06c
0.5	98.33	95.83	93.33	95.83b	3.35	3.24	3.57	3.3c	67.50	66.83	58.97	64.43b	119.2	118.2	107.1	114.9b	1.785	1.777	1.87	1.81b
1	88.33	84.17	80.00	84.17c	4.61	4.43	4.29	4.44b	13.68	9.86	12.67	51.70c	56.22	49.33	49.55	51.70c	5.302	5.280	4.358	4.98a
1.5	24.00	28.33	31.67	28.00d	5.60	5.63	5.12	5.54a	0.00	0.00	0.00	7.79d	6.60	8.40	8.38	7.79d	0.000	0.000	1.00	0.00d
Mean significance	77.25a	76.88a	75.83a		3.92a	3.97ab	4.09a		54.97a	53.53ab	51.34b		82.41a	79.70a	76.80a		2.05a	2.01a	1.83a	
Ψ			***				***				***				***				***	
Seed treatment			ns				*				*				ns				ns	
Interaction			***				***				**				ns				*	

Values represent means, and different letters indicate significant difference p < 0.05 level.
n.s., not significant, * significant at P < 0.05 level, ** Significant at P < 0.01 level, *** significant at P < 0.001 level

Table 8. Effects of osmotic potential (Ψ) of PEG solutions and seed treatment time (PEG) on shoot and root weight and root/shoot ratio

Ψ(MPa)/Prime time (h)	Root dry weight (g)				Shoot dry weight (g)				Root/Shoot Weight Ration			
	6	12	24	Mean	6	12	24	Mean	6	12	24	Mean
0	4.39	4.11	5.00	4.50b	5.89	6.28	6.05	6.07a	3.83	6.06	5.12	5.00c
0.5	8.40	7.16	6.94	7.50a	4.83	4.77	4.22	4.61b	12.09	11.56	8.19	10.61b
1	4.85	3.51	4.87	4.41b	1.388	1.60	1.77	1.58d	15.52	22.77	16.65	18.31a
1.5	0.76	1.82	1.43	1.34c	0.00	0.00	0.00	0.00d	0.00	0.00	0.00	0.00d
Mean significance	4.85a	4.65a	3.81b		3.52a	2.86b	2.81b		7.86b	10.09a	7.49	
Ψ			***				***				***	
Seed treatment			**				*				**	
Interaction			**				ns				*	

Values represent means, and different letters indicate significant difference p < 0.05 level.
n.s., not significant, * significant at P < 0.05 level, ** Significant at P < 0.01 level, *** significant at P < 0.001 level

The beneficial effects of hydropriming have been attributed to the mobilization in embryonic tissues of enzyme activities required for rapid seed germination and of compounds such as free amino acids, proteins, and soluble sugars from storage organs [5]. However, the effects of hydropriming on seed germination was limited at −0.9 MPa and −1.2 MPa in PEG, other seed primed methods, such as halopriming, should be compared to select the optimal priming conditions in later studies. In PEG solutions, the total germination percentage at -1.5 MPa was only ¼ of that at --0.5 MPa. However, Mehra et al. (2003) showed that the PEG molecules did not enter the seeds, and, hence had no toxicity effect on seeds [8, 20]. This contrast may be due to the long-term drought stress conditions in PEG solutions, which led to the decline of seed vitality.

The results of this experiment indicate that osmopriming by PEG especially at lower concentration could improve some parameters of rye seed germination and seedling growth. Osmopriming at -5.0 MPa osmotic potential after 6h could be used to improve germination characteristics. Osmopriming is the most widely used type of seed priming in which seeds are soaked in low water potential solutions. Examples of such osmotica used include polyethylene glycol (PEG), NaCl, $MgSO_4$ and manitol. PEG and NaCl, however are the most commonly used osmoticum for rice priming [21]. Osmopriming contributes to significant improvement in seed germination and seedling growth in different plant species. Seeds of tomato and asparagus (*Asparagus offcinalis*) osmoconditioned in -0.8 MPa PEG-8000 showed increased germination under saline media [32]. Osmotic priming to improve seed germination performance may also enhance general crop performance. Osmoconditioning of Italian ryegrass (*Lolium multiflorum*) and sorghum (*Sorghum bicolor*) seeds with 20% PEG-8000 for 2 d at 10°C increased germination percentage, germination rate, seedling establishment and dry matter production under water stress, water logging, cold stress and saline conditions [18].

4. Conclusion

The results of this experiment indicate that drought stress causes seedling growth and germination reduction. The present results suggested that osmo- and hydro-priming method could enhance the ability of drought tolerance in rye seeds by improving seed germination under high drought stress condition. The osmopriming by PEG especially at lower concentration could improve some parameters of rye seed germination and

seedling growth. Hydropriming without aeration is a simple and useful technique for enhancing seedling growth and germination. However, suggested that optimum soaking time for rye need to further investigation. Hydropriming as an ideal priming method is cheaper than osmopriming and it can process a large number of seeds at a time, and this method seems to be possible for application in rye production in high drought stress in the future. Finally, for the next studies, other seed primed methods, such as halopriming, should be compared to select the optimal priming conditions.

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