ECOPERSIA

2017, 5 (4): 1969-1980

Effect of Pre-Sowing Seed Treatments on Germination Traits and Early Seedling Growth of Eldar Pine

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Received: 25 June 2017 / Accepted: 5 September 2017 / Published Online: 31 December 2017

Background: Seed energy and seed vigour are the most important qualitative attributes influencing plant's growth and establishment that can be improved by techniques generally known as seed priming, which enhances the percentage, speed and uniformity of germination. Effect of various priming techniques was conducted on seed germination and seedling's early growth of elder pine *(Pinus eldarica* Medw.) in Seed Technology Lab of Natural Resources Faculty, Tarbiat Modares University, Iran.

Materials and Methods: Seeds were treated through hydropriming with distilled water, halopriming with NaCl at -4 and -8 bar concentrations, osmopriming with polyethylene glycol 6000 (PEG 6000) at -4 and -8 bar concentrations and hormonopriming with salicylic acid (SA) at 1 and 2 mM solutions for 48 h. Un-primed dry seeds were taken as control. The seeds were kept in germinator at 20 ± 0.5 °C, 65% relative humidity and 16.8 h light/dark photoperiod for 42 days.

Results: The highest germination percentage (92%) and germination speed (5.13 seeds/day) were obtained with hydropriming. The best results to improve germination energy, time to 50% germination, seedling length, seedling dry weight and seedling vigour index were achieved with hydropriming and hormonalpriming 1 and 2 mM. Osmopriming and halopriming -8 bar compared to control in most mentioned traits showed poor performance.

Conclusions: Hydropriming and hormonalpriming can be suitable techniques to support nursery practices of elder pine seed in order to improve germination percentage, emergence and early seedling growth.

Keywords: Pinus eldarica, Priming, Seed germination, Vigour index

1. Background

Despite the genus *Pinus* is one of the most widely distributed around the world (1), eldar pine (*P. eldarica* Medw.) is found in a much smaller biogeographic region from 200 - 600 meter elevations in the semi-arid steppe region in Georgia (2). However, its distribution area has been extended, via plantations, to numerous countries in Europe, Middle East and Asia, as well as Central America, Africa and Australia (3). This tree is highly valued for establishment of windbreak, reclamation, reforestation, biomass and energy, landscape and soil conservation. It shows potential to provide chemical extractives, or for the manufacture of particle board and paper pulp, too (3, 4). In developing countries, it is valued principally for its benefits as a short-rotation wood and fuelwood crop, and for establishing windbreaks (5). It is also used as ornamental tree in the southwestern United States (6). In reality, these benefits make this species important for landscape planning and multipurpose forestry. As a consequence, the interests for landscape designers, forest managers, growers and researchers in the ecology of elder pine have increased. Germination is a critical stage in the life cycle of plants and often controls population dynamics (7). It must be explained that although potential of the seed for germination is high, it is often variable in speed and capacity. Therefore, improvements in germination and establishment would help growers meet local seedling demands.

To ensure successful seed germination and stand establishment, particularly under unfavourable environmental conditions, high seed and seedlings vigour are required as they are the most important qualitative attributes affecting the plant growth (8). The seed vigour can be improved by an easy, low-cost and low-risk technique called seed priming (9), which improves seed invigoration, germination rate and germination percentage, reduces germination time, suites germination uniformity and seedling growth under both normal (10, 11, 12) and stressful conditions (13, 14). During priming, seeds are partially hydrated so that pre-germinative metabolic activities start, but radicle emergence is prevented; then the seed is dried back to the original moisture level (15, 16).

Several seed priming methods have been examined, including halopriming, osmopriming, hydropriming, thermopriming, solid medium priming, hormonopriming and biopriming (13), each of which may have different effects, which is dependent on plant species, growing phase, concentration/dose of priming agent and incubation period. Mechanism of priming seed may be not completely understood, however it's physiological and biochemical advantages is expectable (17, 18).

Various works on seed priming and germination characteristics have been conducted on various pine trees (19, 20, 21, 22, 23, 24), but no reported work on eldar pine seed was available.

2. Objective

Because the osmopriming and halopriming are applied as effective strategies for increasing the growth and survival of seedlings in drought and saline conditions, therefore the present investigation for the first time was carried out to assess the effect of various priming treatments on germination and seedling early growth in eldar pine.

3. Materials and Methods

The study was conducted in Seed Technology Lab of Faculty of Natural Resources, Tarbiat Modares University, Iran. The elder seeds of equal size and weight were collected from the Caspian Seed Center of Amol, Mazandaran province, northern Iran. Some traits of the seeds are listed in Table 1.

3.1. Seed priming

Seed treatments included soaking in distilled water (hydropriming), soaking in -4 and -8 bar solutions of polyethylene glycol 6000 PEG (osmopriming), soaking in -4 and -8 bar solutions of NaCl (halopriming) and soaking in 1 and 2 mM solutions of salicylic acid SA (hormonopriming). Likewise, some un-primed dry seeds were taken as control. All priming treatments were conducted in room temperature (25 ± 1°C) for 48 h; this period was chosen for being the best treatment compared to other periods (24, 72 and 96 and 120 hours) in the pretest. After treating, the solutions were decanted and seeds rinsed for 2 min. with distilled water to remove the chemical traces, then dried at room temperature for 4 days at ambient room conditions (25 ± 1°C) in order to bring the seeds to their approximate original moisture content.

3.2. Germination test

Germination test was conducted using distilled water. Three replications of 25 seeds of primed and un-primed (control) seeds were kept in 9 cm petridishes at 20 ± 0.5°C, 65% relative humidity and 16/8 hours light/dark photoperiod for 42 days (25). The required moisture for the seeds was provided by adding 5 ml distilled water to each petridish. Seed germination was recorded daily after the commencement of the experiment. Seeds were considered germinated when root length reached 2 mm. Test germination was terminated when no further germination occurred. Then some traits were calculated according to listed equations in Table 2.

To determine the seedling shoot length and root length, 7 seedlings were selected randomly from each petridish, their lengths measured using 1 mm scale ruler. Seedling dry weight was measured using 0.0001 gr digital scale, after drying root and shoot at 103°C for 17 h in an air oven.

3.3. Data analysis

The statistical analysis was performed in a completely randomized design (CRD), with three replications (25 seeds per replicate) and 8 treatments: control (unprimed seeds), soaking in distilled water (hydropriming), soaking in -4 and -8 bar solutions of PEG 6000 (osmopriming), soaking in -4 and -8 bar solutions of NaCl (halopriming) and soaking in 1 and 2 mM solutions of SA (hormonopriming). Data were statistically analyzed using SPSS software program (Ver. 19 for Windows). Distribution was tested for normality by Kolmogorov-Smirnov test. Homogeneity of variances was tested by Levene's test. The data were analyzed through ANOVA and the mean comparisons was done using Duncan's Multiple Range and Dunnet' T3 tests. Excel software was used to draw figures.

Table 1Some quantitative traits of elder pine seeds

Seed provenance	Viability (%)	Purity (%)	Moisture (%)	Number (per Kg)	1000 seed weight (per gr)
Khorasan	93%	99.7%	9%	17000	67.93

Table 2 Germination indices calculation equations

Studied trait	Equation	Reference
Germination Percentage	$\mathrm{GP} = \frac{n}{N} * 100$	(25)
Germination Speed	$\mathrm{GS} = \sum (\frac{n_i}{t_i})$	(26)
The time to 50% germination (T50)	$G_{50} = \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{n_j - n_i}$	(27)
Germination Energy	$\mathrm{GE} = \frac{Mcgr}{N} * 100$	(28)
Seedling Vigour Index	$\mathrm{SVI} = GP * (\frac{SL+RL}{100})$	(29)

n = total number of germinated seeds during the germination test, N= number of seeds initiated, ni = number of germinated seeds on day ti, ti = number of days during the germination period (between 0 and 42 days), Mcgr=maximum of cumulative percentage germination, SL=shoot length, RL= root length

[DOR: 20.1001.1.23222700.2017.5.4.3.7]

4. Results

Significant differences ($p < 0.01$) were observed among priming treatments for all characters examined (Table 3).

Maximum germination percentage were obtained at treatments of hydropriming, osmopriming -4 bar, hormonopriming 1 mM and 2 mM; the lowest trait was observed in osmopriming -8 bar followed by control and halopriming at -8 bar (Figure 1). All priming treatments (except halopriming -8 bar) enhanced the germination energy significantly ($p < 0.01$) compared to the unprimed seeds. The best result of germination energy was obtained in 1 mM SA primed seeds (Figure 2).Except osmopriming and halopriming treatments with -8 bar concentrations, other treatments improved germination speed (Figure 3). In contrast, the highest average time to 50% germination was recorded in both listed treatments; however, there was no significant difference among the control and osmopriming -8 bar. The lowest G50 was observed with hydropriming and hormonopriming 1 and 2 mM (Figure 4).

Table 3 Analysis of variance of the seed germination traits and early seedling growth in elder pine in response to priming treatment

Source of variance		GP	GE	GS	G50	SL	SDW	SVI
Priming treatment	F	22.83	343.31	1585.86	105.35	120.49	93.51	97.9
	p	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
	Sum of Squares	2446.23	8252.48	72.96	270.36	10760.55	0.009	17324.46

** Significant at $p < 0.01$.

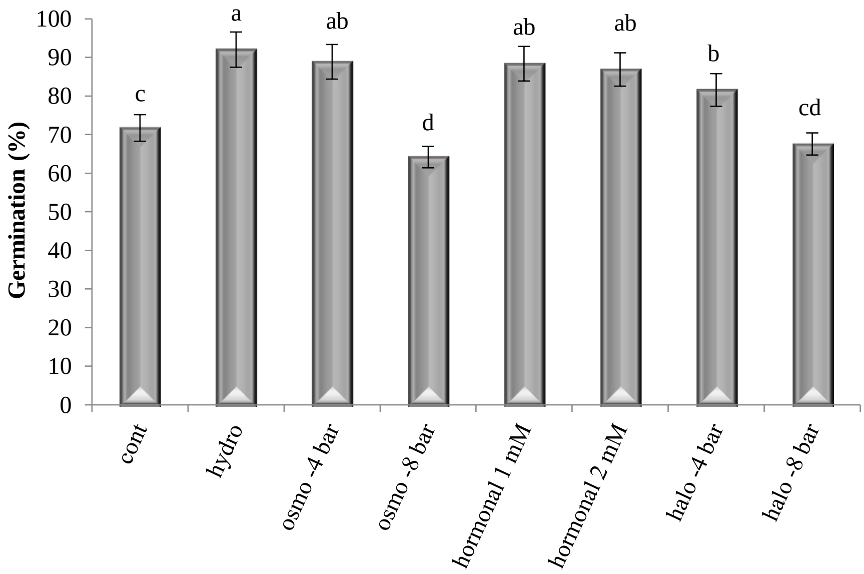

Figure 1 Effect of different treatments on the germination percentage of elder pine seeds
cont= control; hydro= hydropriming, osmo -4 bar= osmopriming -4 bar, osmo -8 bar= osmopriming -8 bar, hormonal 1 mM= hormonalpriming 1 mM, hormonal 2 mM= hormonalpriming 2 mM, halo -4 bar= halopriming -4 bar, halo -8 bar= halopriming -8 bar

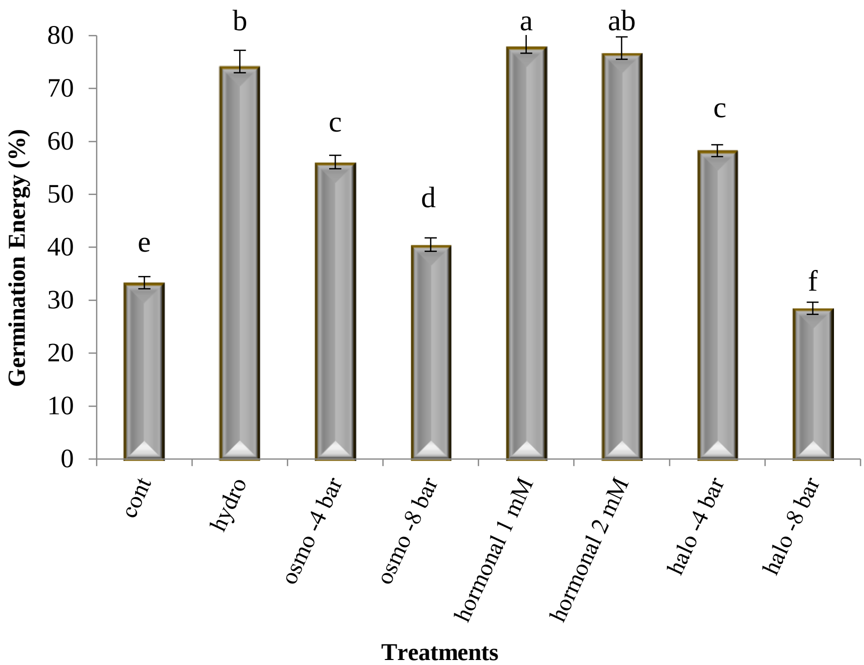

Figure 2 Effect of different treatments on the germination energy of elder pine seeds
cont= control; hydro= hydropriming, osmo -4 bar= osmopriming -4 bar, osmo -8 bar= osmopriming -8 bar, hormonal 1 mM= hormonalpriming 1 mM, hormonal 2 mM= hormonalpriming 2 mM, halo -4 bar= halopriming -4 bar, halo -8 bar= halopriming -8 bar

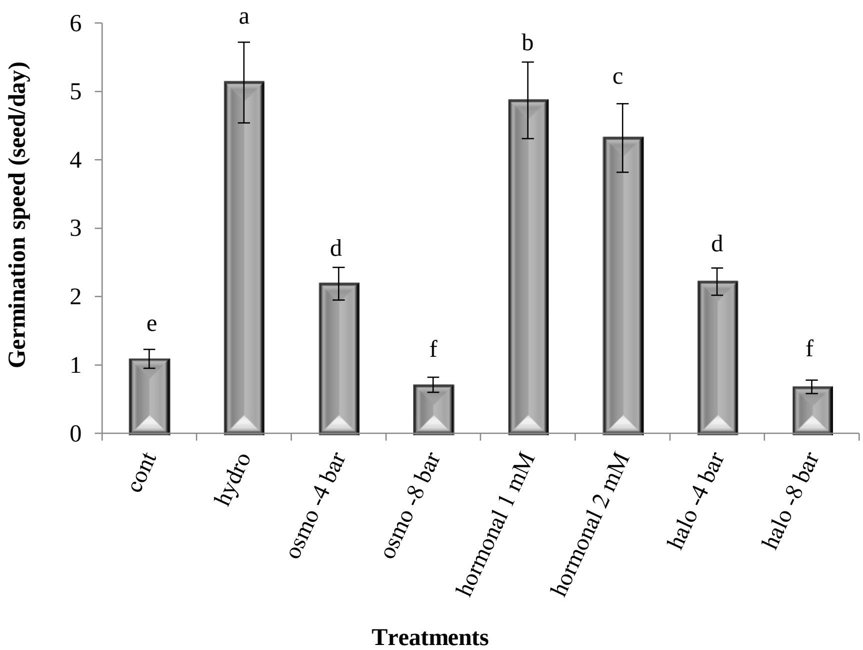

Figure 3 Effect of different treatments on the germination speed of elder pine seeds
cont= control; hydro= hydropriming, osmo -4 bar= osmopriming -4 bar, osmo -8 bar= osmopriming -8 bar, hormonal 1 mM= hormonalpriming 1 mM, hormonal 2 mM= hormonalpriming 2 mM, halo -4 bar= halopriming -4 bar, halo -8 bar= halopriming -8 bar

[DOR: 20.1001.1.23222700.2017.5.4.3.7]

The response of seedlings hydroprimed and hormonalprimed with 1 mM were statistically greater in seedling length compared to other treatments. Control and halopriming with -8 bar had the lowest seedling length (Figure 5).

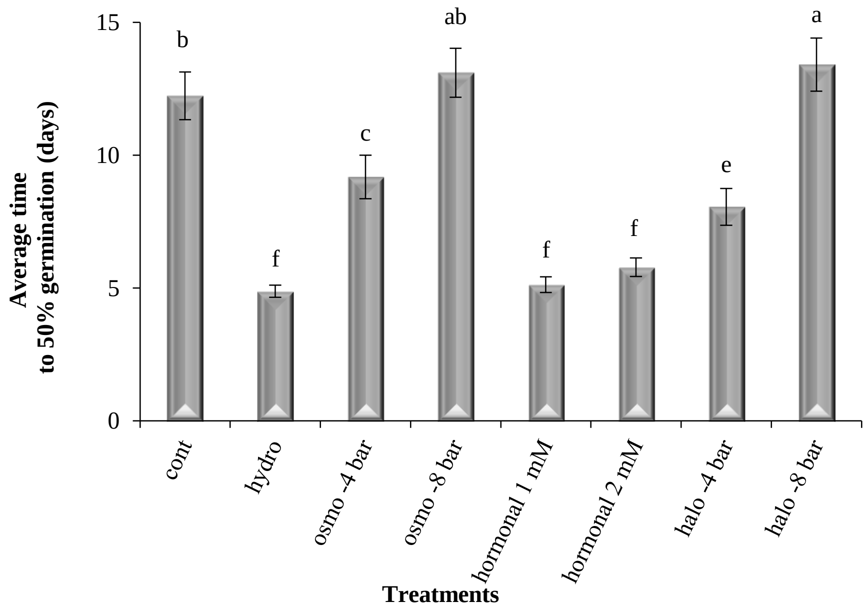

Figure 4 Effect of different treatments on the average time to 50% germination of elder pine seeds
cont= control; hydro= hydropriming, osmo -4 bar= osmopriming -4 bar, osmo -8 bar= osmopriming -8 bar, hormonal 1 mM= hormonalpriming 1 mM, hormonal 2 mM= hormonalpriming 2 mM, halo -4 bar= halopriming -4 bar, halo -8 bar= halopriming -8 bar

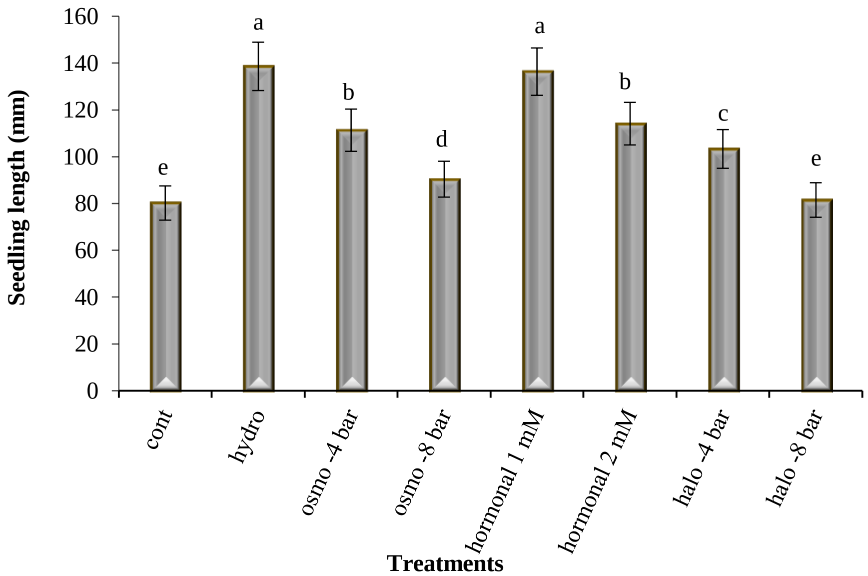

Figure 5 Effect of different treatments on seedling length of eldar pine seeds
cont= control; hydro= hydropriming, osmo -4 bar= osmopriming -4 bar, osmo -8 bar= osmopriming -8 bar, hormonal 1 mM= hormonalpriming 1 mM, hormonal 2 mM= hormonalpriming 2 mM, halo -4 bar= halopriming -4 bar, halo -8 bar= halopriming -8 bar

[DOR: 20.1001.1.23222700.2017.5.4.3.7]

All priming treatments (except halopriming -8 bar) significantly increased seedlings' dry weight, the maximum of which was obtained in hormonalpriming 1 and 2 mM, hydropriming and osmopriming -4 bar treatments (Figure 6).The highest seedling vigor index belonged to hydropriming and hormonalpriming 1 mM and the lowest values to halopriming -8 bar, control and osmopriming -8 bar (Figure 7).

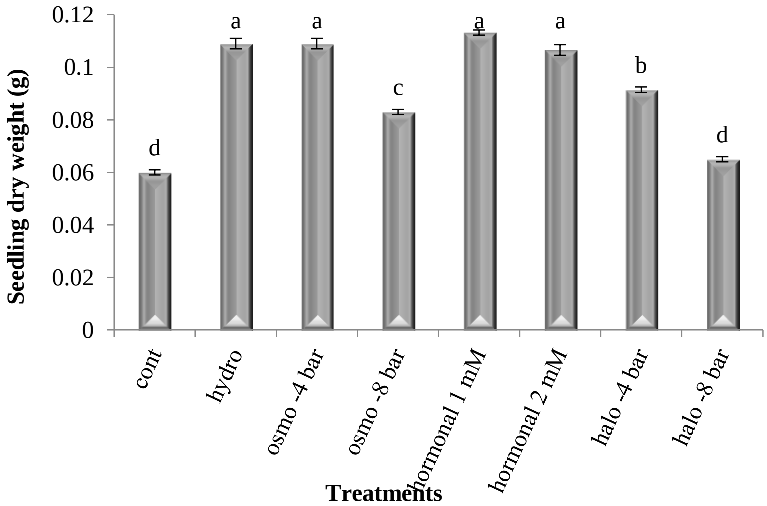

Figure 6 Effect of different treatments on seedling dry weight of elder pine seeds
cont= control; hydro= hydropriming, osmo -4 bar= osmopriming -4 bar, osmo -8 bar= osmopriming -8 bar, hormonal 1 mM= hormonalpriming 1 mM, hormonal 2 mM= hormonalpriming 2 mM, halo -4 bar= halopriming -4 bar, halo -8 bar= halopriming -8 bar

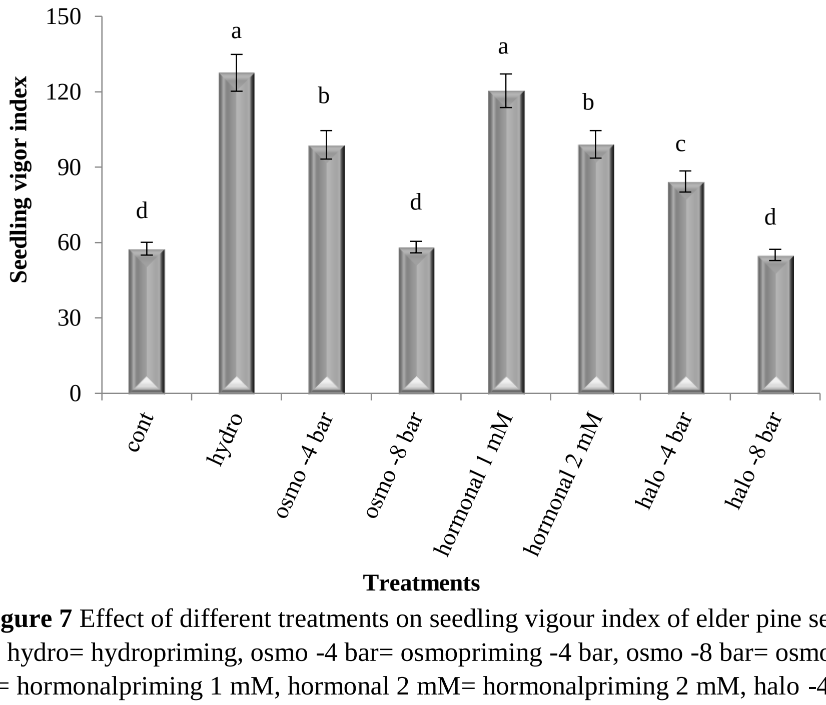

Figure 7 Effect of different treatments on seedling vigour index of elder pine seeds
cont= control; hydro= hydropriming, osmo -4 bar= osmopriming -4 bar, osmo -8 bar= osmopriming -8 bar, hormonal 1 mM= hormonalpriming 1 mM, hormonal 2 mM= hormonalpriming 2 mM, halo -4 bar= halopriming -4 bar, halo -8 bar= halopriming -8 bar

[DOR: 20.1001.1.23222700.2017.5.4.3.7]

5. Discussion

The present study revealed that germination percentage and energy significantly improved in most of the treatments as compared to the unprimed seeds, which was consistent with another finding (24) in which hydropriming and halopriming with -3, -6 and -12 bar KNO_3had improved the germination percentage and energy of white-barked pine seed. The positive effect of priming treatments on germination may be due to induction a range of biochemical changes in seed that are required to initiate the germination process, hydrolysis or metabolism of inhibitors, imbibition and enzymes activation (31). In the present study, osmopriming and halopriming in -8 bar concentration resulted in lower germination percentage, which might be related to the inhibitory effect of the solutions low osmotic potential and/or to specific ion toxicity (32).

Germination speed is among the essential parameters in determining the quality of seeds, and normally, has direct relation to plant growth and amount of products. Also, the germination time is an index of seed germination speed (33). In our study, hydropriming, hormonalpriming 1 and 2 mM, osmopriming and halopriming -4 bar treatments resulted in improvement of speed germination and lower mean time taken to 50% germination, compared to the control, which were in line with those obtained by others researchers on brutia pine(6) and white-barked pine(24). The significantly less time taken to 50% germination could be the result of shortening of the lag phase during priming (34).

According to Mcdonald (35) when seeds are in the primed state, important pre-germination steps such as DNA and RNA synthesis are accomplished in the seed; hence, the seeds are physiologically close to germination and have fewer steps to complete than un-primed seed in order to accomplish germination. Moreover, increased germination rate due to seed priming may be due to increased rate of cell division in the root tips of seedlings from primed seeds (28, 34). Early reserve breakdown and mobilization might be the cause of significant reduction in G50 (24).

Seedlings' length and dry weight were significantly influenced by seed priming treatments, which was in correspondence with the findings on brutia pine (6) and benguet pine (36). The higher performance of primed seedlings in comparison with un-primed seedlings can be attributed to earlier germination caused by priming (37) or to the increased cell division within the apical meristem of root that enhancesthe growth (38). Seedling vigor index as a function of seedling length and germination percentage is the power and ability of seed emergence in difficult conditions (39). In present study, the highest seedling vigor index obtained for hydropriming treatment that was not significantly different from hormonalpriming 1 mM treatment. The lowest values of this parameter belonged to halopriming -8 bar, control and osmopriming -8 bar. Halopriming levels with KNO_3 (1 and 3 %), SA levels (0.2 and 0.5 mM) and hydropriming improved seedling vigor index in the black cumin (33). It seems that higher water absorption during hydropriming leads to improved seedling vigor index as the result of mobilization of the reserved food material, activation and re-synthesis of some enzymes (40).

6. Conclusions

Osmopriming and halopriming in -8 bar concentration showed poor performance in most traits, but the best results in germination and seedling growth were achieved with hydropriming and hormonalpriming 1 and 2 mM. Therefore, treatments such as hydropriming and hormonalpriming on eldar pine seed can support nursery practices by improving the seed germination and early growth performance. In reality, these treatments are economical, environmentally friendly and easily applicable by nursery workers and can encourage direct seeding where applicable.

Conflict of Interest

The authors have no conflict of interest.

Acknowledgement

The authors do acknowledge Dr. Fatemeh Ahmadloo, Assistant Professor at Research Institute of Forests and Rangelands for the scientific cooperation.

Authors' Contributions

First author is M.Sc. student, who conducted the Laboratory works and provided the initial draft of manuscript. Second author is supervisor of thesis, who planned the methodology of research, contributed technical points and modified the draft.

Funding/Support

The research was conducted as a part of M.Sc. Thesis and financially supported by Faculty of Natural Resources, Tarbiat Modares University, Iran.

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[DOR: 20.1001.1.23222700.2017.5.4.3.7]

اثر پیش تیمارهای بذر بر صفات جوانه‌زنی و رشد اولیه کاج تهران

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تاریخ دریافت: ۴ تیر ۱۳۹۶/ تاریخ پذیرش: ۱۴ شهریور ۱۳۹۶/ تاریخ چاپ: ۱۰ دی ۱۳۹۶

مقدمه: قدرت و بنیه بذر صفات کیفی مهمی هستند که تاثیر مهمی روی رشد و استقرار گیاه دارند. بنیه بذر می‌تواند به‌واسطه تکنیک‌هایی موسوم به پرایمینگ بذر که درصد، سرعت و یکنواختی جوانه‌زنی را افزایش می‌دهند بهبود یابد. اثر تکنیک‌های مختلف پرایمینگ روی جوانه‌زنی و رشد اولیه گیاهچه کاج تهران (*Pinus eldarica* Medw.) در آزمایشگاه تکنولوژی بذر دانشکده منابع طبیعی، دانشگاه تربیت مدرس، ایران انجام شد.

مواد و روش‌ها: بذرها توسط هیدروپرایمینگ با آب مقطر، هالوپرایمینگ با کلرید سدیم در غلظت‌های ۴- و ۸- بار، اسموپرایمینگ با پلی اتیلن گلایکول ۶۰۰۰ در غلظت‌های ۴- و ۸- بار و پرایمینگ هورمونی با اسید سالیسیلیک در غلظت‌های ۱ و ۲ میلی‌مولار به مدت ۴۸ ساعت تیمار شدند. بذور خشک پرایم نشده به‌عنوان شاهد در نظر گرفته شدند. بذرها در ژرمیناتور با شرایط دمایی ۰/۵±۲۰ درجه‌سانتی‌گراد، رطوبت نسبی ۶۵ درصد و فتوپریود ۸ ساعت تاریکی و ۱۶ ساعت روشنایی به مدت ۴۲ روز نگه‌داری شدند.

نتایج: بیش‌ترین درصد (۹۲ درصد) و سرعت جوانه‌زنی (۵/۱۳ بذر در روز) در تیمار هیدروپرایمینگ به‌دست آمد. بهترین نتایج برای بهبود قدرت جوانه‌زنی، زمان رسیدن به ۵۰ درصد جوانه‌زنی، طول گیاهچه، وزن خشک گیاهچه و شاخص بنیه گیاهچه در تیمارهای هیدروپرایمینگ و پرایمینگ هورمونی ۱ و ۲ میلی‌مولار حاصل شد. اسموپرایمینگ و هالوپرایمینگ ۸- بار در مقایسه با شاهد در بیش‌تر صفات مورد بررسی عملکرد ضعیفی نشان دادند.

جمع‌بندی: در کل، می‌توان نتیجه‌گیری کرد که هیدروپرایمینگ و پرایمنیگ هورمونی می‌توانند تکنیک‌های مناسبی برای بهبود درصد جوانه‌زنی و رشد اولیه گیاهچه تهران در اقدامات نهالستانی باشند.

کلمات کلیدی: بنیه بذر، پرایمینگ، جوانه زنی بذر، کاج الدار

[DOR: 20.1001.1.23222700.2017.5.4.3.7]