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Full Length Research Paper

The effect of different treatments on improving seed germination characteristics in medicinal species of *Descurainia sophia* and *Plantago ovata*

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Creating optimal conditions for germination of medicinal plants seed is essential for their cultivation. Therefore, to evaluate the effect of different treatments on seed germination of two medicinal species, *Descurainia sophia* and *Plantago ovata* collected in 2009 from Tehran Province, an experiment with a factorial randomized complete block design in 6 treatments with 4 replications was conducted. Treatments included KNO_3 with concentrations of 0.1, 0.2 and 0.3%, acetylsalicylic acid with levels of 50 and 100 mg/l, prechilling (4°C for 10 days), thiourea with levels of 0.1 and 0.2%, boiling water for 5 and 10 min. To compare, distilled water was used as control. Results showed that the effect of different treatments on germination percentage of two medicinal species was significantly different ($p < 0.05$). It was shown that prechilling was the most effective treatment on seed germination of both species.

Key words: *Descurainia sophia, Plantago ovata*, germination, acetylsalicylic acid, prechilling, thiourea and KNO_3.

INTRODUCTION

In any seed production program, readiness of seed to germinate for further multiplication is much needed (Shanmugavalli et al., 2007). By definition, germination incorporates those events that commence with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis (Bewley and Black, 1994).

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations, as those described in ancient texts such as the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties (Hoareau and Dasilva, 1999).

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Abbreviations: ABA, Abscisic acid; **GR,** germination rate; **MGT,** mean germination time.

Descurainia sophia and *Plantago ovata* are two of the most economically and ecologically important species in Iran. *D. sophia* from Cruciferae family is an annual or biannual herb with a height of about one meter. This plant was used medicinally by the Navajo and Paiute tribes among others. A poultice of the plant has been used to ease the pain of toothache. The seed is considered to have agents that are a tonic for the heart, that are locally soothing and softening, urine-inducing, inducing the removal (coughing up) of mucous secretions from the lungs, fever-reducing, and a tonic. The seeds form a special remedy for sciatica. A poultice of the ground up seeds has been used on burns and sores. Semi-dried oil is obtained from the seed. The leaves are stored with corn to prevent it from spoiling (Zargari, 2008). *P. ovata* is a plant from Plantaginaceae, an annual herb that grows to a height of 12-18 in. The seeds are enclosed in capsules that open at maturity. Seeds are translucent and concavo-covex.Isagol (Zargari, 2008). Rezaeipoor et al. (2000) indicated that *P. ovata* can suppress the humeral immune responses, especially in the primary immune response. To obtain high percentage of germination, seed should be

Table 1. Variance effect analysis of treatments on measured characteristics of two medicinal species, *D. sophia* and *P. ovata.*

Source of changes	Degrees of freedom	Germination percentage	Mean germination time	Germination rate
Replication	3	90.182	0.820	0.003
Species	1	2795.64**	3.102**	0.001^{ns}
Treatment	10	8104**	27.773**	0.310**
Treatment * species	10	1011.64**	5.018**	0.060**
Test error	63	128.228	0.787	0.001

*, ** and ns, 0.1, 0.5% and not significant, respectively.

taken from the crop harvested at the end of the preceding crop season. Old seeds tend to lose viability under ordinary storage conditions. Germina-tion begins in four days after sowing. If delayed, it could be stimulated (Zargari, 2008). Seed of this plant and its products are used in pharmaceutical industries for the production of relaxant compounds. In addition, it is used in blood cholesterol reduction drugs (Dinda and Craker, 1998; Gupta et al., 1994; Trautwein et al., 1997).

In Iran, there is limited information concerning the potential seed germination problems of *D. sophia* and *P. ovata.* Lack of literatures on comprehensive study of improving seed germination characteristics is still lacking.

In order to domesticate and cultivate, information on seed germination and how to improve these plants are needed. Initial germination test indicated that germination percentage was low in both species. Therefore we decided to test different treatment effects on seed germi-nation of the mentioned species.

Association of official seed analysts (AOSA) and the international seed testing association (ISTA) have sug-gested several methods to break dormancy and stimulate seed germination. The most known of these methods are prechilling, scarification (mechanical and chemical), different solutions (including: abscisic acid (ABA), KNO_3, nitric acid, thiourea, polyethylene glycol, ethanol etc), light alternatives and temperature (Kaplnd, 1997). Researches show that many of the plant hormones including auxin, gibberelin, cytokinin, ethylene and ABA may control nucleic acids performance stimulation of seed germination and contribute to dormancy (Chiwocha et al. 2005). Shariati et al. (2003) examined the effect of different treatments on dormancy breaking of *Achillea millefollum*. They found that giberellic acid (GA_3) with concentration of 500 ppm, 0.2% KNO_3, light and alternative temperatures (8/16) were the best treatments for breaking dormancy.

Therefore, the objective of this study was to assess the effects of different treatments on seed germination and devise an effective method for improving seed germi-nation in medicinal species of *D. sophia* and *P. ovata.*

MATERIALS AND METHODS

This study was conducted in the seed laboratory of the Natural Resources Faculty, University of Tehran, Iran, in January, 2009 to determine the effective methods of improving germination charac-teristics in two medicinal species, *D. sophia* and *P. ovate*. The matured seeds of *D. sophia* and *P. ovata* were collected in June, 2008 from rangelands of Tehran province. Immature and damaged seeds were removed. The treatments included 1- pre-treatment with KNO3 (0.1, 0.2 and 0.3%) for 48 h, 2- acetylsalicylic acid (50 and 100 mg/l) 3-prechilling (4°C for 10 days) 4- pretreatment with thiourea (0.1 and 0.2%) for 72 h, 5- boiling water (for 5 and 10 min) and 6-control treatment (irrigation with distilled water). Treatments were arranged in a factorial randomized complete block design with four replications. Seeds were sterilized by carboxin tiram for two minutes and then washed twice with sterilized water before use. For germination test, 25 seeds were sown on one filter paper in sterilized Petri dishes with 10 cm diameter moistened with 6 mL of distilled water. Germination percentage was recorded daily during the study period. Final ger-mination was calculated when no further germination took place for several days (after 14 days). Measured traits included germination percentage, germination rate (GR) and mean germination time (MGT). MGT was calculated to assess the germination rate (Ellis and Roberts, 1981). MGT and GR were calculated as follows:

$$MGT = \frac{\sum D.N}{n}$$

$$GR = \frac{1}{MGT}$$

Where, N is the number of seeds which grow in D day, n the total number of seeds grown and D is the number of days from the date of germination. The data were statistically analyzed using a factorial randomized complete block design with four replications. ArcSin transformation was used for germination percentage before analysis (Khan et al., 2006). Experimental data was analyzed by MSTAT-C program (MSTAT-C, 1990). Treatments means were compared using Duncan's multiple comparison tests at 5% level of probability.

RESULTS

Results showed that the interaction of treatments by species significantly affected germination percentage (Table 1). As Table 2 shows, prechilling, 50 mg/l acetyl-salicylic acid, 0.1, 0.2 and 0.3% KNO_3 for 48 h increased germination percentage of *D. sophia*, but the effect of 50 mg/l acetylsalicylic acid did not show significant difference when compared to the other treatments. It was found that using 0.1 and 0.3% thiourea and 100 mg/l acetylsalicylic acid resulted in reduction of *D. Sophia* germination.

Table 2. Comparison of mean different treatments on the germination characteristics of two species of medicinal *D. sophia and P. ovata.*

Characteristics	Treatment		*D. sophia*		*P. ovata*	
Germination percentage	Boiling water	5 min	0	J	0	J
		10 min	0	J	0	J
	KNO_3	0.01	69%	BCD	73%	BCD
		0.02	76%	BC	69%	BCD
		0.03	59%	CDEF	98%	A
	Thiourea	0.01	35%	GH	77%	BC
		0.03	15%	IJ	4%	IJ
	Acetylsalicylic acid	50 mili	57%	DEF	69%	BCD
		100 mili	42%	FG	19%	HI
	Prechilling 10 days		63%	CDE	97%	A
	Control treatment		50%	EFG	84%	AB
Mean germination time (MGT)	Boiling water	5 min	0	J	0	J
		10 min	0	J	0	J
	KNO_3	0.01	4.385	BCDEF	4.543	BCDEF
		0.02	4.743	BCDE	4.63	BCDE
		0.03	5.242	B	3.72	CDEFG
	Thiourea	0.01	2.76	GH	4.795	BCD
		0.03	4.4	BCDEF	3.083	FGH
	Acetylsalicylic acid	50 mili	3.27	EFGH	5.132	BC
		100 mili	3.334	DEFGH	7.083	A
	Prechilling 10 days		1.944	HI	1.023	IJ
	Control treatment		3.546	DEFG	3.747	CDEFG
Germination rate	Boiling water	5 min	0	I	0	I
		10 min	0	I	0	I
	KNO_3	0.01	0.2298	EF	0.2206	F
		0.02	0.2111	F	0.2173	F
		0.03	0.1938	F	0.2703	DE
	Thiourea	0.01	0.364	C	0.2088	F
		0.03	0.2366	EF	0.08115	H
	Acetylsalicylic acid	50 mili	0.3072	D	0.1958	F
		100 mili	0.3113	D	0.1427	G
	Prechilling 10 days		0.5235	B	0.9792	A
	Control treatment		0.2865	D	0.2698	DE

Means sharing the same letters do not differ significantly according to Duncan's multiple range tests at $P \leq 0.05$.

In *P. ovate* seeds, prechilling and 0.3% KNO_3 showed positive and significant impact on impro-ving germination of seeds, while the effect of 0.1 and 0.2% KNO_3, 0.1 and 0.3% thiourea, and 50 and 100 mg/l acetylsalicylic acid, induced reduction of seed germi-nation. A significant decrease in germination percentage could be due to the effect of acetylsalicylic acid (100 mg/l) and thiourea (0.3%). Prechilling with KNO_3 0.3% had the highest effect on improvement of germination percentage in the two medicinal species (Figure 1). It was revealed that boiling water had no effect on germination percentage of *D. sophia* and *P. ovate* (Table 1).

MGT of *D. sophia* increased when prechilling, as well as when 50 and 100 mg/l acetylsalicylic acid and thiourea 0.1% were applied but only MGT of prechilling showed significant difference with the rest. No remarkable decrease or increase in MGT was observed when 0.3% thiourea and 0.1, 0.2 and 0.3% KNO_3 were used but this characteristic decreased significantly when 0.3% KNO_3 was applied. MGT of *P. ovate* decreased due to the use of acetylsalicylic acid (50 and 100 mg/l), thiourea (0.1%) and KNO_3 (0.1 and 0.2%) but MGT of 100 mg/l acetylsalicylic acid showed significant reduction when compared with the rest treatments. Although prechilling, thiourea (0.3%) and KNO_3 (0.3%) influenced MGT, this characteristic significantly increased only when prechilling was used. Prechilling associated with 0.3% KNO3 were the best treatment to decrease MGT of the two medicinal

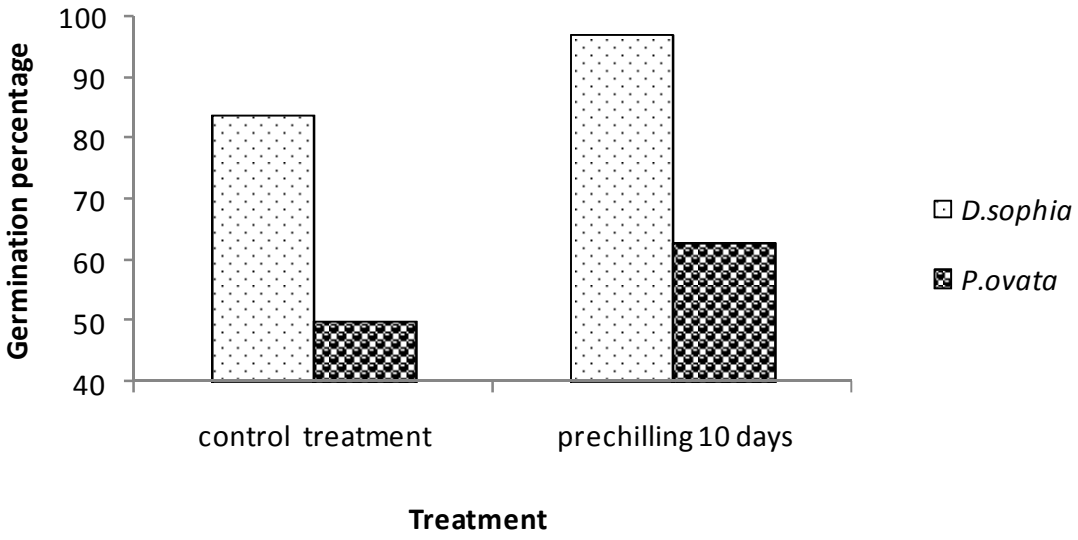

Figure 1. Comparison of control treatment with prechilling in germination stimulation of two medicinal species, *D. sophia* and *P. ovata*. Prechilling treatment with 0.3% KNO_3 was the best treatments to increase germination.

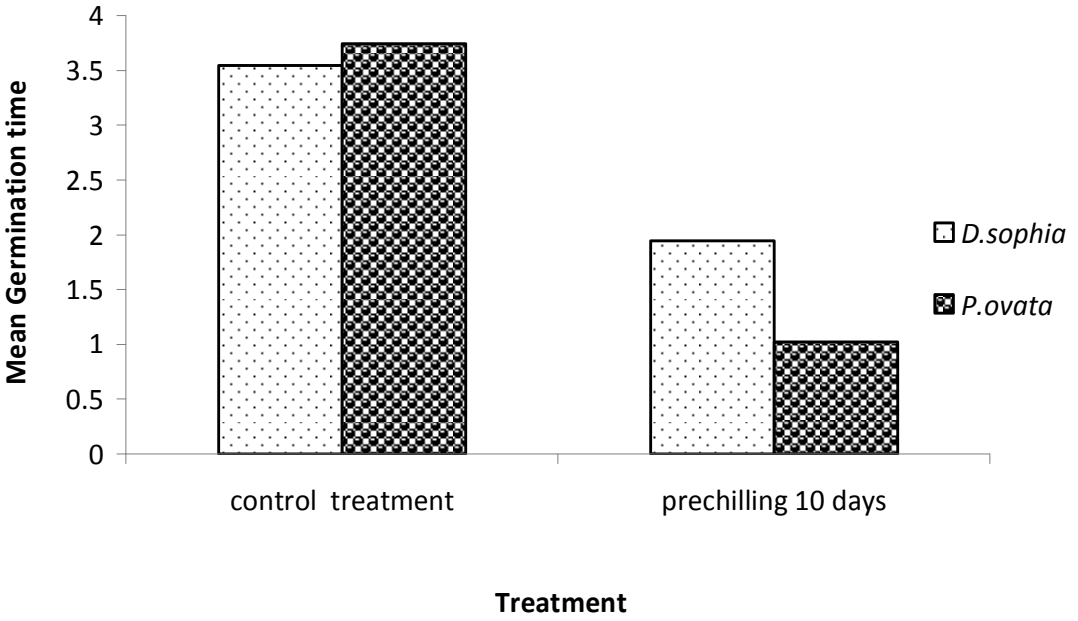

Figure 2. Comparison of control treatment with prechilling in the mean germination time of two medicinal species, *D. sophia* and *P. ovata*. Prechilling treatment with 0.3% KNO3 was the best treatments to decrease MGT.

species (Figure 2).

GR of *D. sophia* increased when prechilled, as well as when 50 and 100 mg/l acetylsalicylic acid were applied and only GR that resulted from prechilling showed a significant difference. The treatments, 0.1 and 0.3% thiourea and 0.1, 0.2 and 0.3% KNO_3 had significant positive effects on GR compared with the control treatment. Germination rate of *P. ovate* significantly increased when prechilled, and when 0.3% KNO_3 was applied. But germination rate of *P. ovate* decreased when acetyl-salicylic acid (50 and 100 mg/l), thiourea (0.1 and 0.2%) as well as KNO_3 (0.1 and 0.2%) were applied. Reduction of germination rate in *P. ovate* under application of these treatments showed a significant difference when compared with control. Figure 3 shows comparison of GR in two understudy species affected by prechilling and control.

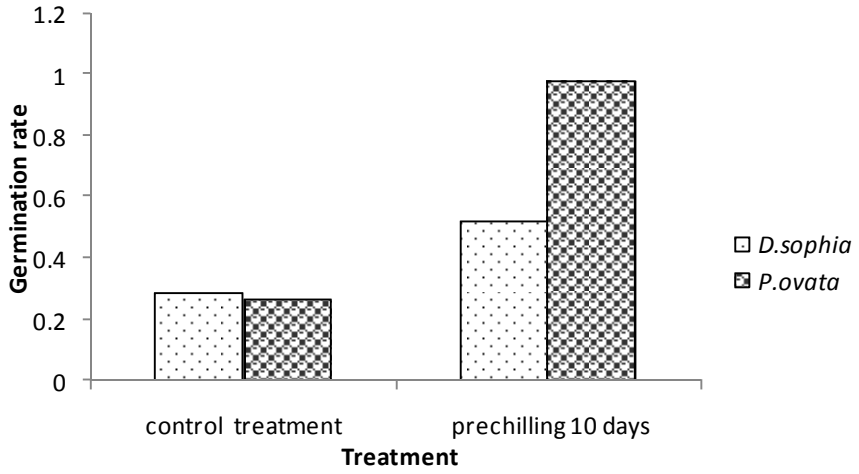

Figure 3. Comparison of control treatment with prechilling in germination rate of two medicinal species of *D. sophia and P. ovata*.

DISCUSSION

Seeds germination status depends on embryo growth potential or inhibitors (Koorneef et al., 2002). This potential depends on seed structure especially embryo structures (endosperm, pericarp and capsules) and affective factors on embryo growth. For example, hormones and environmental factors influence embryo growth (Benech et al., 1998; Mares, 2005). Based on current research results, treatments of prechilling, 0.1, 0.2 and 0.3% KNO_3 and 50 mg/l acetylsalicylic acid were effective in stimulating seed germination of *D. sophia*. Prechilling for 10 days was known to produce the best treatment. Bianco et al. (1984) is of the opinion that low temperatures may have influence on membrane permeability, due to the movement of gibberellin toward their activity places. El-Refaey and El-Dengawy, (2005) found that germination increase in seeds that were prechilled was related to increase of soluble proteins content. Sari et al. (1999) and Davazdah et al. (2005) tested the effect of prechilling on seed germination of some medicinal species like *Echinaceae angustifolia*, *Lallemantia royleana, P. ovata*, *Plantago psyllium, Silybum mariamum* and *Cuminum cyminum*. The results indicated that prechilling had a positive effect on germination of the mentioned species.

One reasons for the positive effect of chemical stimulators such as KNO_3 on seed germination of studied species is related to creating a balance between hormonal ratios in seed and reducing the growth preventable materials, like ABA.

Using 0.1 and 0.2% (KNO_3) did not have huge effect on germination of *P. ovata*. Derkan and Karssen (1993) explained that seed reaction against KNO_3 is related to sensitivity of seed. *D. sophia* had higher germination compared to *P. ovate* which may be due to its soft coat. Soft coat of *D. sophia* facilitate water absorption and the exchange of gas, thus, enhancing germination. Thiourea caused reduction of seed germination in both species. This is may be due to the negative effect of osmotic potential of thiourea in high concentrations which could affect the environment. This result agrees with Mirzaei et al. (2009).

The stimulation effect of acetylsalicylic acid or salicylic acid on plant growth was confirmed by El-Wahed et al. (2006) on yellow maize plants and Emongor (2007) on cowpea plants. The effect of acetylsalicylic acid on endogenous phytohormones levels has been mentioned by Tompsett and Schwabe (1974).

El-Shraiybe and Hegazi (2009) reported that plants treated with ABA showed high level of ABA in seeds at harvest. The high levels of ABA induced seeds dormancy and subsequently prevent seeds sprouting in pods. The present results indicated that 100 mg/l acetylsalicylic acid significantly reduced germination percentage in both species.

Totally, according to our results, although KNO_3 (0.1 and 0.2%) and KNO_3 (0.3%) affected *D. Sophia* and *P. ovate,* respectively and increased their germination, prechilling is the best effective stimulator in germination improvement of *D. sophia* and *P. ovate*.

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