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Echinacea purpurea L. Seed Pretreatment with GA_3, Stratification and Light to Improve Germination

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ABSTRACT

Echinacea purpurea L. is a valuable medicinal plant with many seed germination problems. This objective of this study was to determine the factors required to break dormancy of *E. purpurea* seed. Treatments included: soaking in gibberellic acid (GA_3; 250 or 500 mg/l), stratification (4 or 7 weeks at 4°C), constant light exposure (24 h for 4 weeks) and a combination treatment (250 mg/l GA_3 + 4 or 7 weeks stratification). There were significant differences ($P < 0.01$) between all methods. Highest seedling fresh weight and seed germination percentage and lowest mean germination time occurred in the combination treatment.

Keywords: medicinal plants, seed dormancy, seed priming, seedling property
Abbreviations: GA_3, gibberellic acid; **MGT**, mean germination time

INTRODUCTION

High seed quality and seedling establishment are the cornerstones of profitable, efficient and sustainable crop production (Finch-Savage 1995). Dormancy is an important component of physiological seed quality and so plants with a long history of domestication and plant breeding generally have lower seed dormancy than wild or more recently domesticated species (Copeland and McDonald 2001). Seed dormancy is defined as the failure of an intact viable seed to complete germination under favorable conditions and is controlled by several environmental factors such as light, temperature and the duration of seed storage (Macchia *et al.* 2001). Dormancy can be broken by artificial scarification, low temperature treatment, the application of gibberellic acid (GA_3) and the removal of fruit (Copeland and McDonald 2001).

Echinacea purpurea is a valuable medicinal plant, originating from North America, but which has been extensively cultivated in Iran with good yield. There are several problems associated with seed germination and due to dormancy the germination percentage of *E. purpurea* commercial seeds of is generally low (authors' observation). Stratification (moist-prechilling or a period of cold-moist treatment) is a method traditionally used to break seed dormancy (Zhou *et al.* 2003; García-Gusano *et al.* 2004). *Echinacea angustifolia* propagation by achenes is limited by embryo dormancy, usually requiring light and scarification (physical abrasion) to increase germination (Feghahati and Reese 1994). GA_3 is an important endogenous growth regulator that has profound and diverse effects on plant growth and development. Specific roles of GA_3 include the induction of seed germination, the promotion of hypocotyls and stem elongation, and the regulation of pollen development and flower initiation (Peng and Harberd 2002). Tzortzakis (2009) suggested that KNO_3 and secondly GA_3 treatments may improve rapid and uniform seedling emergence and plant development of endive and chicory. Application of GA_3 or stratification of seeds increased emergence percentage of strawberry tree (Demirsoy *et al.* 2010). Gibberellins (GAs) are generally synthesized by seeds and their role in germination is thought to be hydrolysis of storage nutrients in seeds and a direct effect on the growth of the embryo (Lecat *et al.* 1992). External application of GAs to seeds and prechilling could break seed dormancy and enhance seedling establishment of *E. angustifolia* (Macchia *et al.* 2001) and *Leymus arenarius* (Greipsson 2001).

Since there are no reports on how improve *E. purpurea* seed germination in Iran, in this study, germination of seed under prolonged stratification and GA_3 treatment under controlled laboratory conditions was examined.

MATERIALS AND METHODS

Four replicates of 50 seeds each were placed in plastic Petri dishes. Seed dormancy treatments included: light exposure, GA_3 soaking (250 and 500 mg/l), scarification for 4 and 7 weeks at 4°C, or combination treatments (250 mg/l GA_3 + 4 weeks' scarification at 4°C (C1); 250 mg/l GA_3 + 7 weeks' scarification at 4°C (C2)). All chemicals were obtained from Sigma Co., St. Louis, USA. For the scarification treatment, seeds were allowed to imbibe deionized water at room temperature for 24 h and they were subsequently transferred to moist sand at 4°C in a cold-room for 4 or 7 weeks. For the GA_3 treatment, seeds soaked for 12 h in GA_3 solution were then transferred to Petri dishes. For the GA_3 and scarification combination treatment, after soaking in 250 mg/l GA_3 solution for 12 h, seeds were transferred to moist sand in a 4°C cold-room for 4 or 7 weeks. For the light exposure treatment, seeds were exposed to light for 24 h for a period of 4 weeks. Untreated seeds were used as control (16-h photoperiod). After treatment, all seed were evaluated under light and dark conditions (at 24 ± 2°C, 16-h photoperiod, 700 µmol s^{-1} m^{-2} photon in light period) using 15 × 100 mm Petri dishes on top of a single sheet of moistened filter paper that was kept moist with sterile water under a 12-h photoperiod with a 16/24°C night/day temperature regime. Water was added in 4 ml increments during the germination period when necessary to maintain seed hydration during the germination period. Germination was recorded daily over 40 days. When the emerging radicle could be observed by the naked eye, the seed was considered to have germinated. Measured traits were: germination

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Research Note

percentage, seedling fresh weight (determined by using a digital balance with sensitivity of ± 0.01 g), seedling shoot and root length (measured by using a digital caliper with sensitivity of ± 0.01 mm), mean germination time, the first observation of seedling emergence (E1st), and day of 50% emergence (E50) (Farhoudi *et al.* 2007).

Germination percentage was calculated using the following formula:

$$\text{Germination percentage (GP)} = 100 \times \frac{\text{Number of germinated seeds}}{\text{Total of number seeds}}$$

Mean germination time (MGT) was calculated to assess the velocity of germination and was calculated as follows (Schelin *et al.* 2003; Shim *et al.* 2008):

$$MGT = \frac{\sum f_i n_i}{N}$$

where *fi* = number of days during the germination period, *ni* = number of germinated seeds per day and N = sum of germinated seeds ($N = \sum n_i$).

Data analysis

Four replicates of 50 seeds each were placed in plastic Petri dishes. The experimental design was a completely randomized design (CRD) with four replications. Percentage data were arcsine transformed prior to ANOVA. Data was analyzed using MSTATC software. Mean comparison was performed with Duncan's multiple range test at $P < 0.01$ and graphs were drawn using Excel 2003 software.

RESULTS

Germination percentage

GP was significantly influenced by the treatment used to break seed dormancy (**Table 1**). Among all the treatments, the highest GP was observed after C2 treatment, independent of whether 250 or 500 mg/l GA_3 was used, although the result was not significantly different to C1. The lowest GP was observed in seeds treated with constant light and untreated controls (**Fig. 1**).

Mean germination time, E1st, E50

MGT, E1st and E50 were significantly influenced by the treatment used to break seed dormancy (**Table 1**). Among all treatments, lowest MGT was observed in C1 and C2. The highest MGT was found in seeds treated with 250 mg/l GA_3 (**Table 2**). Lowest E1st and E50 were observed in C1 (**Table 2**).

Seedling fresh weight

Results showed seedling fresh weight was significantly influenced by treatment (**Table 1**). Highest seedling fresh weight occurred in C1 and C2 (**Fig. 2**).

Seedling shoot and root length

The length of seedling shoots and roots was significantly influenced by seed dormancy breaking treatments (**Table 1**). Longest seedling roots were observed in seeds scarified for 7 weeks while longest shoots were observed in C1 (**Table 2**).

DISCUSSION

This study shows that combined GA_3 and scarification treatment are significantly more effective in improving seed germination and seedling establishment of *E. purpurea* compared to other treatments (**Fig. 1**, **Table 2**). Lowest seed germination of *E. purpurea* seeds was observed in the control treatment compared to the use of GA_3 or scarification alone proving the physiological dormancy of *E. purpurea* seeds. This result suggests that the onset of embryo dormancy is associated with the accumulation of growth inhibitors and that breaking dormancy involves a shift in the balance of growth regulators towards growth promoters that overcome the effect of inhibitors (Khan 1971; Fang *et al.* 2006; Farhoudi *et al.* 2006). Demirsoy *et al.* (2010) showed that the seed germination rate of *Arbutus unedo* L. increased as GA_3 concentration increase from 300 to 1200 mg/l and stratification duration from 5 to 15 weeks. Halopriming (KNO_3) or the use of a growth regulator (GA_3) improved the rate of germination of endive and chicory and reduced the mean germination time required (Tzortzakis 2009). There are various methods used to break seed dormancy: hormonal, temperature and/or light treatments. Moist chilling has successfully alleviated endogenous dormancy for various dormant seeds. Baskin *et al.* (1992) found prechilling increased seed germination of some Asteraceae family members. Prolonged stratification period improved seed germination of several plants (Bratcher *et al.* 1993; Macchia *et al.* 2001) but our study showed that seed germination and seedling fresh weight were lower in scarified seeds than those exposed to a combination of scarification and GA_3 (**Fig. 1, Fig. 2**). In some studies GA_3 was exogenously applied as a substitute for scarification, increasing the germination percentage of, *inter alia*, *Fagus sylvatica* (Nicolas *et al.* 1996) and *Echinacea* spp. (Macchia *et al.* 2001). Our study indicates that exogenously applied GA_3 is useful, when applied with chilling because the combination of GA_3 and scarification most likely increased endogenous seed GA_3 and helped dormant seeds to germinate. Gulzar *et*

Table 1 Analysis of variance of the effect of seed dormancy breaking treatment on *Echinacea purpurea* seed germination.

	GP	MGT	E1st	E50	Shoot length	Root length	Seedling fresh weight
Seed dormancy breaking treatment	1086.8*	457.9*	407.1*	588.5*	87.1*	517.1*	7.56*
Error	109.2	34.6	43.0	69.1	13.7	9.30	0.09
Cv (%)	13.1	10.2	12.1	11.2	8.2	13.1	8.1

*: Significant at P = 0.01

Table 2 Effect of seed dormancy breaking treatment on *Echinacea purpurea* seed germination and seedling growth.

Seed treatment	MGT (days)	E1st (days)	E50 (days)	Root length (mm)	Shoot length (mm)
7 weeks scarification + 250 mg/l GA_3	11.7 d	10.9 d	24.1 b	47 a	34 a
4 weeks scarification + 250 mg/l GA_3	12.3 d	11.3 d	24.9 b	41 b	32 a
7 weeks scarification	19.7 b	20.1 b	29.0 ab	45 a	28 b
4 weeks scarification	18.4 b	19.0 b	33.0 a	34 b	23 bc
500 mg/l GA_3	15.4 c	14.4 c	35.7 a	28 c	18 c
250 mg/l GA_3	23.4 a	24.6 ab	----	23 b	14 d
Light	30.1 a	30.8 a	----	16 d	11d
Control	28.3 a	29.0 a	----	18 d	12 d

Different letters within a column indicate significant differences at $P < 0.01$ according to DMRT.

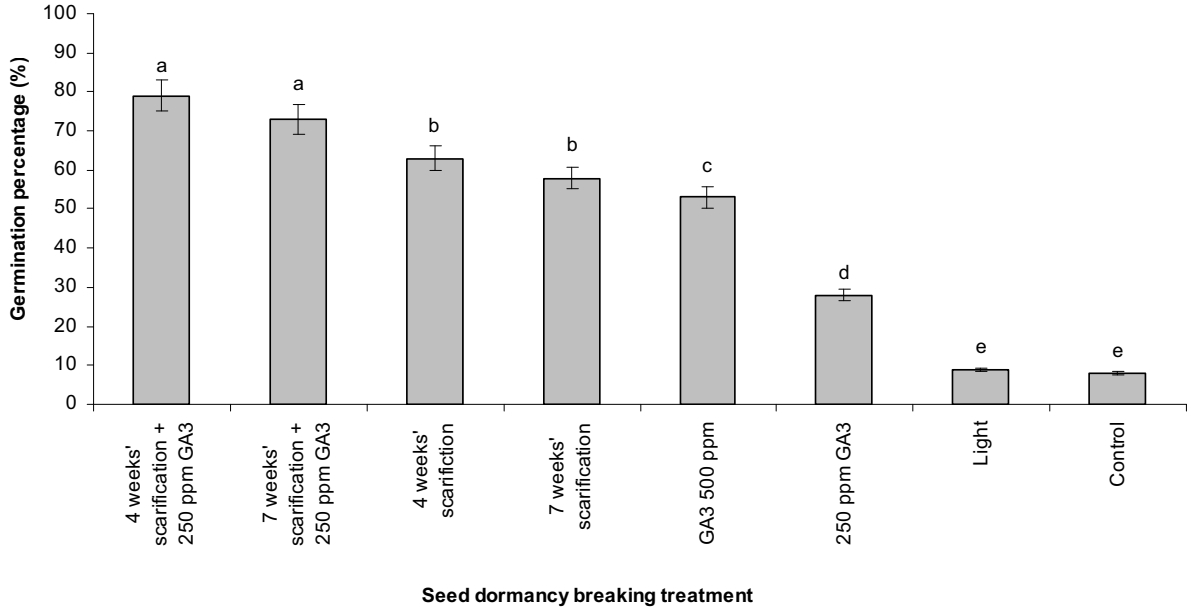

Fig. 1 Effect of seed dormancy breaking treatment on *Echinacea purpurea* seed germination percentage.

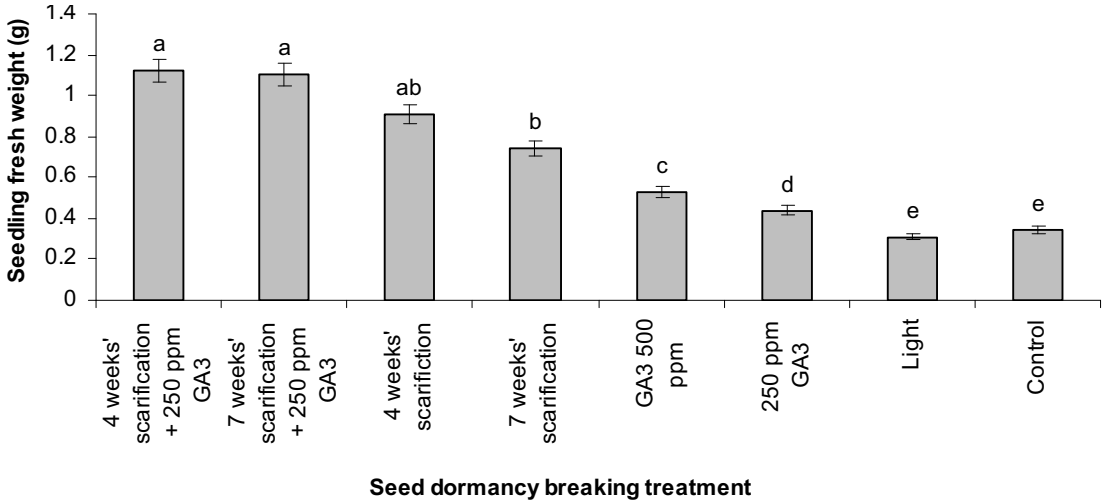

Fig. 2 Effect of seed dormancy breaking treatment on *Echinacea purpurea* seedling fresh weight.

al. (2001) suggested that GA_3 treatment involves the disappearance of ABA, mobilizes stored reserves and weakens the mechanical resistance of the endosperm cells around the radicle tip. Application of GA_3 to dormant seeds can eliminate their natural chilling requirement (Fang *et al.* 2006) but in this study we found that the use of GA_3 alone could not improve seed germination and seedling growth of *E. purpurea* compared to scarification alone. Greipsson (2001) found that the use of GA_3 and scarification increase seed germination of *Leymus arenarius*. Puppala and Fowler (2003) reported that when *Lesquerella fendleri* seeds were soaked only in GA_3 solution seed germination improved.

In conclusion, the marked improvement in *E. purpurea* germination following the combination of GA_3 and scarification indicated that these were suitable treatments to remove dormancy compared the use of GA_3 or scarification alone.

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