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EXPERIMENTAL PAPER

Effect of gibberellic acid, stratification and salinity on seed germination of *Echinacea purpurea* cv. Magnus

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Summary

This study was conducted in order to determine the appropriate treatment for breaking dormancy and the effect of salinity on seed germination of purple coneflower (*Echinacea purpurea* cv. Magnus), in two separate experiments. In the first experiment, five levels of gibberellic acid (GA_3) (0, 250, 500, 1000, and 1500 $mg \times L^{-1}$) with four levels of cold moist stratification period of seeds at 5°C (0, 5, 10 and 15 days) were launched. A factorial experiment was conducted in a completely randomized design with four replications. The statistical analysis showed that concentration of 250 $mg \times L^{-1}$ GA_3 with 10 days of cold moist chilling significantly increased the percentage of germination of normal seedlings and reduced the mean time of germination. In the second experiment, the seeds were chilled for 10 days at 5°C and half of them treated with 250 $mg \times L^{-1}$ GA_3 for 24 hours. The seeds treated with GA_3, and those non-treated were subjected to NaCl for salinity stress. The experiment was conducted using five salinity levels (0, 20, 40, 60 and 80 mM NaCl) in four replications in a completely randomized design. The results showed that purple coneflower is highly sensitive to salinity in the germination stage. The results also showed that by increasing salinity levels, the percentage of germination and normal seedlings significantly decreased and the mean time to germination increased, compared to the control treatment. But the seeds treated with GA_3 showed higher viability and better performance under salinity stress condition.

Key words: *purple coneflower, chilling, GA_3, NaCl*

INTRODUCTION

Echinacea genus is represented by 11 taxa found in the United States and in south central Canada. *Echinacea purpurea* (purple coneflower) is the most widespread species [1] and the most widely cultivated medicinal species of the genus [2]. *Echinacea* species have long been recognized as important medicinal plants used by Native Americans for the treatment of many diseases, including colds, toothaches, snake bites, rabies and wound infections [3]. Medicinally, purple coneflower is thought to activate the immune system by stimulating T-cell production, phagocytosis, lymphocytic activity, cellular respiration (anti-oxidation) activity against tumor cells [4, 5], and inhibiting hyaluronidase enzyme secretion [6].

The extracts of the roots and herbs of *Echinacea* species have a complex chemical composition. Unsaturated lipophilic compounds (alkamides and ketoalkenes/alkynes), glycoproteine, caffeic acid derivatives, and polysaccharides are believed to be responsible for the observed immunostimulatory and anti-inflammatory activities [3].

One of the major barriers in the optimal use of herbs, including purple coneflower outside the natural habitat, relates to the limitations in the rate of seed germination and long seed dormancy. Dormancy ensures that seeds do not germinate in temperate climates until conditions are optimal for seedling survival. During seed dormancy, even if suitable environmental conditions (e.g. humidity, temperature etc.) are also provided, germination does not occur.

Different methods such as stratification, scratch technique (mechanical, chemical), the use of different materials that can stimulate germination (gibberellins, ethephone, potassium nitrate, nitric acid, and ethanol), optical frequency and temperature for breaking seed dormancy of medicinal plants have been used [7]. Low seed germination percentage of *Echinacea* species was noted earlier by Shalaby *et al.* [8]. Bishnoi *et al.* [9] reported that seed dormancy is variable in commercially available purple coneflower seed lots. The germination in lots with high dormancy can start significantly earlier and can be enhanced by scarification and application of ethephon or priming seed with PEG 8000 [9]. In the purple coneflower also cold stratification, application of gibberellin, and potassium nitrate could improve seed germination [10]. Rajabian *et al.* [11] showed that the highest percentage of seed germination in medicinal plant *Ferula assa-foetida* was obtained as a result of chilling on wet filter paper for 8 to 9 weeks and showed the highly significant effect of gibberellic acid (GA_3) on germination. Some herbs, especially medicinal plants are sensitive to salinity in stage of germination. Given the importance of purple coneflower in pharmaceutical industry in most countries, this study was done to determine the appropriate method for breaking seed dormancy and the response of plant to salinity stress at germination stage.

MATERIAL AND METHODS

Selection and preparation of seeds

Seeds of purple coneflower (*E. purpurea* cv. Magnus) that had not been stratified, were obtained from Goldaro Company (a medical herb factory), Isfahan Iran. The seeds were dried for 2 days at ambient laboratory temperatures in darkness and the immature seeds or unwanted materials were removed. Then they were stored in a sealed plastic bag at 20°C until the test of germination. Two different experiments were conducted with seeds of purple coneflower.

Stratification and using GA_3

The first experiment was the two-factorial one (five concentrations of GA_3 and four levels of cold duration) in a complete randomized design with four replications and 50 seeds per replication. Seeds were surface-sterilized by submerging 10 s in 70% ethanol, rinsing three times with sterile water, and subsequently treating the seeds with a solution of 5% sodium hypochlorite for 10 min. Afterwards, seeds were rinsed three times with sterile water. The preliminary work showed that GA_3 was more effective than ethephon for stimulation of germination. Therefore, the germination was conducted with 0, 250, 500, 1000 and 1500 $mg \times L^{-1}$ GA_3, in combination of four chilling treatments (0 as a control, 5, 10 and 15 days). Eighty groups, 50 seeds each were placed on dry filter paper in separated Petri-dish. Different groups of seeds were soaked in GA_3 at the corresponding solutions for 12 h at ambient air temperatures (*ca.* 20°C). After GA_3 treatments, the seeds were chilled in a refrigerator at 5°C in darkness for periods of 0 (control), 5, 10 and 15 days. After the appropriate chilling period, Petri-dishes were removed from refrigerator and incubated in the growth cabinet at constant temperature of 20°C in darkness. The Petri-dishes containing seeds were opened every 12 h to count the germinated seeds, which were removed and discarded, and to add water where necessary to maintain an adequate moisture level. Additional samples of each treatment were used to visually estimate abnormal seedling development, according to ISTA [12] rules, within 10 days after initiating germination. Germinating was considered to have occurred when radical length of approximately 2 mm had been reached. Final germination percentage was determined after two consecutive days when no further seed germination was observed. Final germination percentage and mean time to final germination were calculated according to the equation of Ellis and Robert [13].

Effect of salinity on seed germination

From the results of the first experiment, all seeds were chilled for 10 days at 5°C and half of them were soaking in solution of 250 $mg \times L^{-1}$ GA_3 for 24 h. Seed

germination under salt stress was evaluated at 20°C by placing 50 seeds on filter paper in 9 cm Petri-dishes containing 10 ml distilled water (control) or selected salt solutions. Germination characteristic was determined as described above. The experiment was conducted in a completely randomized design with five salinity levels (0, 20, 40, 60 and 80 mM of NaCl) in four replications and 50 seeds per replication.

Data analysis

In the first experiment, data was analysed for significant differences using a two-way analysis of variance (ANOVA) (GA_3 concentration and level of cold duration as the main factors) and in the second experiment data was analysed with one-way analysis of ANOVA. All percentage data were transformed using the arcsine transformation before analysis. Statistical analysis was performed using the MSTATC Version 1.4 software programme (Michigan State University, East Lansing, MI, USA) and means were compared using the least significant differences (LSD) test at $p=0.05$.

RESULTS AND DISCUSSION

Experiment 1

Effect of GA_3 on seed germination

In general, GA_3 has significant effects on all aspects of germination in this experiment, compared to the control. The results showed that the highest germination percentage and the highest normal seedlings were observed in the seeds treated with 250 $mg \times L^{-1}$ of GA_3, which was approximately 2.5 and 1.7 times higher than controls, respectively (tab. 1, 2). The lowest germination was observed in the control non-treated seeds. On the other hand, the application of GA_3 significantly reduced the average mean time to final seed germination. GA_3 at a concentration of 250 $mg \times L^{-1}$ reduced the mean time to final germination by 2.5 times as compared with control (tab. 3). Our findings agree with those reported by Chuanren *et al.* [14], who found that GA_3 treatment, accompanying prechilling of *E. angustifolia* for two weeks, was the most effective treatment for enhancing germination. Also Nadjfy and Rastgoo [15] showed that treatment with GA_3 significantly increased germination percentage of *Ferula gummosa* and *Teucrium polium*. However, Macchia *et al.* [16] reported that GA_3 alone does not lead to high germination percentage. These in-consisting results may be due to genotype differences and environmental conditions during seed production. GA_3 stimulated seed germination via the synthesis of α-amylase enzyme, and other factors intensified the hydrolysis enzymes

which cause hydrolysis of storage materials, including starch, and convert them to simple sugars that move to growing points [11].

Table 1.

Effect of GA_3 and cold duration (5°C) on germination percentage of purple coneflower seeds

Cold duration (days)	GA_3 (mg×L^{-1})					
	0	250	500	1000	1500	Mean
0	19.17	55.36	48.22	28.60	22.60	34.80
5	28.22	64.22	49.22	38.20	28.42	42.70
10	29.22	65.22	62.42	55.22	33.42	49.10
15	20.22	59.72	61.22	46.35	23.22	39.15
Mean	24.20	61.10	55.20	42.10	26.90	

Cold duration: LSD ($p<0.05$)=5.1
GA_3: LSD ($p<0.05$)=12.1
Cold duration×GA_3: LSD ($p<0.05$)=3.4

Table 2.

Effect of GA_3 and cold duration (5°C) on normal seedling percentage of purple coneflower

Cold duration (days)	GA_3 (mg×L^{-1})					
	0	250	500	1000	1500	Mean
0	22.12	46.64	42.79	42.77	35.02	37.90
5	25.02	55.54	50.03	44.07	38.22	42.60
10	57.06	90.02	88.86	75.07	60.07	74.20
15	42.05	57.07	55.54	50.02	50.07	50.90
Mean	36.50	62.30	59.00	52.90	45.80	

Cold duration: LSD ($p<0.05$)=14
GA_3: LSD ($p<0.05$)=9.5
Cold duration×GA_3: LSD ($p<0.05$)=2.1

Table 3.

Effect of GA_3 and cold duration (5°C) on mean time (day) of purple coneflower seed germination

Cold duration (days)	GA_3 (mg×L^{-1})					
	0	250	500	1000	1500	Mean
0	11.22	9.22	9.52	9.52	10.18	9.90
5	10.00	8.02	8.37	8.54	9.00	8.80
10	8.02	5.92	6.05	6.53	7.42	5.80
15	8.34	6.70	6.77	7.05	7.55	7.30
Mean	13.80	5.40	7.60	9.70	11.80	

Cold duration: LSD ($p<0.05$)=1.2
GA_3: LSD ($p<0.05$)=2.3
Cold duration×GA_3: LSD ($p<0.05$)=1.01

Effect of stratification on seed germination

Duration of stratification increased the percentage of germination and normal seedlings. The highest germination percentage and the highest normal seedlings were obtained in the seeds stratified at 5°C for 10 days as compared with controls (tab. 1, 2). The mean time to final germination was affected by stratification. The stratification treatments decreased mean time to final germination. The lowest mean time to final germination was observed in the seeds stratified at 5°C for 10 days, as compared with controls (tab. 3). Our results confirm the dormancy breaking effect of cold stratification, observed in purple coneflower by Fariman *et al.* [10] who showed that 21 days cold stratification increased seed germination to 98.33%. Wartidiningsih *et al.* [17] and Baskin *et al.* [18] reported that prechilling is required in some of *Echinacea* species (*E. purpurea, E. pallida* and *E. angustifolia*) to overcome seed dormancy. The benefice of chilling in germination was reported as an increased gene expression of cold GA_3 ox1 (an active form of enzyme-producing gibberellic acid) in the aleurone [19, 20]. In addition, cold treatments stimulation of endogenous gibberellin synthesis, and other active drivers increased seed germination. It seems that the cold treatment reduced the level of inhibitor hormones (i.e. abscisic acid) and increased the promoter substances which resulted in increases of the germination potential [21]. Therefore, the maximum germination of purple coneflower seeds was obtained when those treated with GA_3 and chilled prior to germination.

Interactions between GA_3 and stratification

The interactions between GA_3 treatment and duration of stratification showed that the highest increase occurred in both germination percentage and normal seedlings when 250 $mg \times L^{-1}$ GA_3 along with 10 days of stratification were applied (tab. 1, 2). The lowest average germination time was also obtained on seeds treated with 250 $mg \times L^{-1}$ GA_3 and 10 days of stratification (tab. 3). Generally, the seeds not chilled or not treated with GA_3 showed poor germination performance.

Experiment 2

Effect of salinity on seed germination

The effect of salinity on germination showed that with increasing salinity, the percentage of germination and normal seedlings significantly decreased (fig. 1, 2). According to the results, the highest percentage of germination and normal seedlings were found in the control treatments. Also, the results showed that the percentage of germination in the lowest salt concentration (20 mM NaCl) significantly decreased, compared to the control non-treated with GA_3. The reduction of germination percentage at the concentration of 60 mM was about 2.6 times less than the control. The seed treated with 60 mM NaCl, also significantly decreased the percentage of

the normal seedlings. No germination was observed on application of 80 mM NaCl. The mean time to final germination with increasing salinity levels also increased significantly (fig. 3). However, when purple coneflower seeds were treated with GA_3 and then with salt stress, better performance was observed (fig. 1-3). The research showed that the negative effects of salinity on germination may be due to the reduction of water absorption by seeds and enzyme activity for starch degradation [22]. The salinity effect on germination also could occur due to the accumulation of toxic ions that cause changes in enzymatic activity or specific hormones, such as GA_3 [19]. Apparently, the physicochemical effect of salt stress on the seed could slow down the germination rate [23]. El-Darier and Yossef [22] reported that increasing salinity from 100 to 300 mM could reduce germination percentage in *Lepidium sativum*.

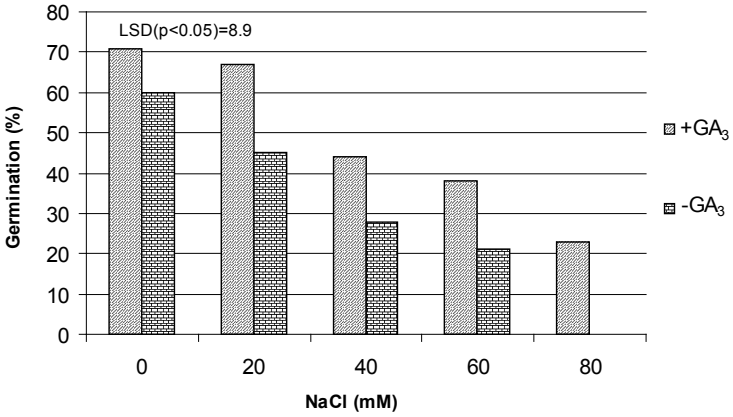

Figure 1.

Effect of NaCl and GA_3 (250 mg×L^{-1}) on germination percentage of purple coneflower seeds

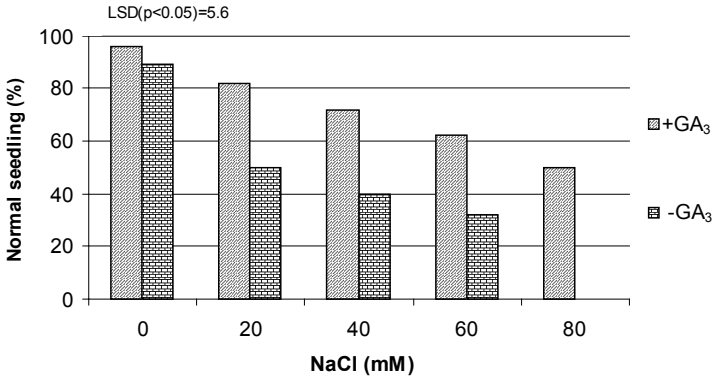

Figure 2.

Effect of NaCl and GA_3 (250 mg×L^{-1}) on normal seedling percentage of purple coneflower

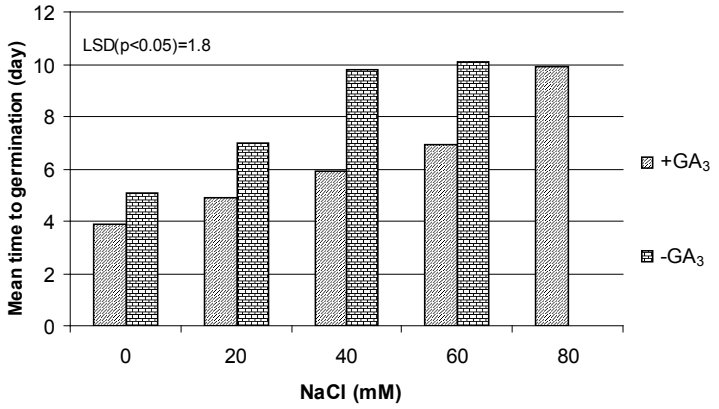

Figure 3.

Effect of NaCl and GA_3 (250 mg×L^{-1}) on mean time (day) of purple coneflower seed germination

CONCLUSION

The results showed that the GA_3 could break seed dormancy in purple coneflower cv. Magnus. It was found that the GA_3 concentration at 250 mg×L^{-1} with 10 days of cold treatment at 5°C was the most effective in increasing the percentage of germination and the normal seedlings. The responses of purple coneflower seeds to the GA_3 treatments suggested that the application of GA_3 could increase the percentage of germination and the normal seedlings and decrease the mean time to final germination under the salinity stress.

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WPŁYW KWASU GIBERELINOWEGO, STRATYFIKACJI ORAZ ZASOLENIA NA KIEŁKOWANIE NASION *ECHINACEA PURPUREA* CV. MAGNUS

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S t r e s z c z e n i e

Prezentowane badania obejmowały dwa odrębne doświadczenia, mające na celu określenie warunków przełamywania spoczynku nasion jeżówki purpurowej (*Echinacea purpurea* cv. Magnus) oraz wpływu zasolenia na ich kiełkowanie. W pierwszym z eksperymentów zastosowano pięć poziomów stężeń kwasu giberelinowego GA_3 (0, 250, 500, 1000 i 1500 $mg \times L^{-1}$) w połączeniu z chłodną stratyfikacją nasion trwającą odpowiednio: 0, 5, 10 i 15 dni, i przebiegającą w wilgotnym podłożu w temperaturze 5°C. Doświadczenie czynnikowe przeprowadzono w układzie całkowicie losowym, w czterech powtórzeniach. Analizy statystyczne wykazały, że zastosowanie dziesięciodniowej chłodnej stratyfikacji w połączeniu z GA_3 o stężeniu 250 $mg \times L^1$ istotnie zwiększa odsetek kiełkujących nasion i skraca średni czas kiełkowania. W drugim eksperymencie nasiona jeżówki były przechowywane przez 10 dni w temperaturze 5°C i połowę z nich poddano przez 24 godziny działaniu roztworu GA_3 o stężeniu 250 $mg \times L^{-1}$. Dla obu grup nasion badano wpływ stresu solnego (działania NaCl). Powyższe doświadczenie prowadzono w układzie całkowicie losowym, w czterech powtórzeniach, stosując pięć poziomów zasolenia (0, 20, 40, 60 i 80 mM NaCl). Otrzymane wyniki wskazują, że jeżówka purpurowa, na etapie kiełkowania nasion, jest bardzo wrażliwa na zasolenie. Zwiększanie jego poziomu powodowało spadek ilości kiełkujących nasion i dobrze wykształconych siewek oraz wzrost średniego czasu kiełkowania. Większą odporność w warunkach stresu solnego wykazywały nasiona poddane działaniu GA_3.

Słowa kluczowe: *jeżówka purpurowa, chłodna stratyfikacja, GA_3, NaCl*