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Short communication

Seed germination and dormancy breaking techniques for *Ferula gummosa* and *Teucrium polium*

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Abstract

Dormancy and germination requirements were investigated in seeds of *Ferula gummosa* Boiss (Apiaceae) and *Teucrium polium* L. (Labiatae). Seeds of both species were subjected to different treatments including various levels of GA_3, HNO_3, H_2SO_4, chilling and soaking with water at different temperatures. Germination of both species increased at higher concentrations of GA_3. In the case of *F. gummosa* highest germination percentage was obtained when the seeds were treated, soaked in water at 5 °C. For *T. polium* seeds, the highest germination percentage was found when they were exposed to GA_3. The highest germination rate and percentage of *T. polium* seeds were obtained at concentrations of 500–2500 ppm GA_3. Washing and chilling (5 °C) for a period of 14 days was most effective in breaking dormancy in *F. gummosa*. Acid scarification by H_2SO_4 (75% v/v), for 5 and 10 min with GA_3 (1500 ppm, 48 h), broke dormancy and induced, 31.9% and 34.1% germination of *T. polium*, respectively. In contrast to *F. gummosa* which showed no response to water soaking, *T. polium* germination

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was induced by up to 32.7%. For both species, germination rate was positively correlated with germination percentage.

Keywords: Medicinal plant; Dormancy; GA_3; Temperature; Seeds; *Teucrium polium*; *Ferula gummosa*

1. Introduction

One of the main problems that prevent sustainable use of medicinal plants, native to the arid lands is that they readily germinate within the native environment, but fail to show good germination under laboratory conditions (Gupta, 2003) or when cultivation is attempted. Galbanum *(Ferula gummosa,* Apiaceae), is one of the most important rangeland products of Iran, with a high export demand due to a large number of applications within both traditional medicine and industry (Islami-Manuchehri, 1994). Its main habitats are located within Pakistan, Turkmenistan, and a vast area in northeast Iran at an altitude of 2000–4000 m, with average annual precipitation of 250–400 mm (Batuli, 1994).

Teucrium polium (Labiatae) is a perennial herb, which has a wide distribution in Iran and is used in traditional medicine (Passmore and Eastwood, 1986). In contrast to *F. gummosa*, *T. polium* has a hard seedcoat. As a consequence of dormancy, the germination percentage of both *F. gummosa* and *T. polium* species is very low.

Gibberellic acid (GA_3) is one of the hormones proposed to control primary dormancy by inducing germination (Iglesias and Babiano, 1997). The effect of GA_3 as a germination promoter is hypothesized to increase with chilling treatment. Chilling also plays an important role in providing the stimulus required to overcome dormancy. Chilling has been reported to induce an increase in GA_3 concentration (Bretzloff and Pellett, 1979).

The aim of this research was to determine treatment(s) which are able to stimulate and enhance germination of the two important medicinal plants in Iran.

2. Materials and methods

Seed source: The mature seeds of *F. gummosa* and *T. polium* were collected from Northeast of Iran (Lat: 36° 27′ N; Lon: 59° 63′ E), in 2003. After collection, immature seeds and those damaged by insects were removed. The seeds were surface sterilized by soaking in 1% sodium hypochlorite (NaOCl) for 5 min and subsequently rinsed thoroughly with sterilized water prior to applying any treatment. All germination experiments were conducted using three replications of 25 seeds per each treatment. Seeds were placed on double layered Wathman No.1 filter paper moistened with 5 ml of distilled water in sterilized Petri dishes. The physico-chemical treatments varied as a consequence of differences in the seedcoat hardness for each as follows:

F. gummosa seeds were exposed to:

Chemical scarification: Seeds of *F. gummosa* and *T. polium* were soaked in HNO_3 (25% v/v), and H_2SO_4 (75% v/v), respectively, for 10 min and then washed thoroughly by distilled water, before transfer to the germination test process.

Cold stratification: Seeds after moisturized with distilled water were maintained at a temperature of 5 °C, for 7 days.

Ethanol (96% v/v)*:* Seeds were soaked in ethanol (96% v/v), for 24 h.

Soaking in cool water, GA_3 treatment and cold stratification: Seeds were soaked in distilled water for periods of 24, 48 and 72 h, and also soaked initially in distilled water for 24 h followed by GA_3 (250 ppm) for 48 h in one treatment and kept at 5 °C for 48 h in another treatment.

Effect of GA_3: Seeds were soaked in 100, 250, and 500 ppm for 48 h, and 1000, 1500, and 2500 ppm GA_3, for 72 h.

Washing and cooling: Seeds were washed every day thoroughly in running water and kept at 5 °C and −10 °C for periods of 7 and 14 days followed by washing at 20 °C for 5 days.

T. polium seeds were exposed to:

Soaking in cool water: Seeds were soaked in distilled water for a period of 72 h, before transfer to the germination test process.

Hot water treatment and chemical scarification: Seeds were immersed into hot water at 80 °C for 5 min then soaked in H_2SO_4 (75% v/v) for 5 min followed by a thorough wash within distilled water.

Chemical scarification and GA_3 treatment: Seeds were soaked in H_2SO_4 (75% v/v) for 5 min and then washed thoroughly by distilled water followed by soaking in 1500 ppm GA_3, for a period of 48 h.

Chemical scarification and cold stratification: Seeds were soaked in H_2SO_4 (75% v/v) for 5 min and then washed thoroughly within distilled water followed by contact within a moist substrate at −10 °C for 48 h.

Effect of GA_3: Seeds were soaked in 100, 250, and 500, 1000, 1500, and 2500 ppm GA_3 for 72 h.

After each treatment, seeds were transferred to germinators with continuous darkness, constant temperature of 20 °C and relative humidity between 70% and 75%. Germinated seeds were counted and removed every 24 h for 45 days. A seed was considered germinated when the tip of the radicle had grown free of the seedcoat (Wiese and Binning, 1987; Auld et al., 1988). The germination rate was calculated as follows (based on Wiese and Binning, 1987):

$$\text{germination rate} = \sum_{n=1}^{45} (\text{number germinating since } n-1)/n,$$

where, n is the days of incubation.

After Arcsin transformation, the percentage of germination was subjected to an analysis of variance. The data were analysed using a randomized complete design

with three replications and the LSD for all pairs comparison at $P<0.05$ was calculated using Turkey's *t*-test (Li, 1964).

3. Results and discussion

In our experiment, application of GA_3 stimulated the germination of both species. This response was dependent on the concentration of applied GA_3. At lower concentrations, germination of both species was lower. In *F. gummosa*, increasing the concentration of GA_3 above 500 ppm and increasing the duration of soaking from 48 to 72 h, improved both the germination percentage and rate significantly ($P<0.05$). The highest germination percentage and rate were obtained in the concentrations range of 1000–2500 ppm (Table 1). For *T. polium* highest germination percentage was obtained at 500 ppm GA_3 (Table 2), with a positive response across all applied GA_3 concentrations. Increasing GA_3 concentration increased both germination rate and percentage but there was no significant difference ($P>0.05$) among GA_3 concentrations. *F. gummosa* did not respond to GA_3 concentrations of less than 500 ppm. GA_3 is widely used to break dormancy of seeds of various plant species. Dormant seeds which require chilling, dry storage after ripening and light as a germination stimulator, are often treated with GA_3 to overcome their dormancy (Gupta, 2003).

Table 1
Seed germination and dormancy breaking and applied treatments for *Ferula gummosa*

Dormancy breaking treatments	Germination rate (seeds per day)	Germination (%)
HNO_3 (10 min)	0.26	4.5
HNO_3 (30 min)	0	0
Soaking cool water (72 h)	0.03	0
Soaking cool water(24 h) + GA3 (250 ppm, 48 h)	0	0
Soaking cool water (24 h) + 5 °C (48 h)	0	0
Cold stratification (5 °C, 7 days)	0.06	14.6
Ethanol 96% (24 h)	0.01	3.84
GA_3 100 ppm (48 h)	0	0
GA_3 250 ppm (48 h)	0	0
GA_3 500 ppm (48 h)	0.01	3.84
GA_3 1000 ppm (72 h)	0.1	23.1
GA_3 1500 ppm (72 h)	0.1	17.1
GA_3 2500 ppm (72 h)	0.1	22.3
Washing (7 days, 5 °C)	0.07	12.4
Washing (14 days, 5 °C)	0.45	26.1
Washing (7 days, −10 °C)	0.01	8.1
Washing (14 days, −10 °C)	0.09	6.7
Washing (5 days, 20°)	0.13	20.9
LSD 5%	0.18	10.9

Table 2
Seed germination and dormancy breaking and applied treatments for *Teucrium polium*

Dormancy breaking treatments	Germination rate (seeds per day)	Germination (%)
Soaking cool water (72 h)	0.48	32.7
Hot water (80 °C, 5 min) + H_2SO_4 75%, 5 min	0	0
H_2SO_4 75%, 10 min	0.5	31.9
H_2SO_4 75%, 5 min + GA_3 1500 ppm, 48 h	0.48	34.1
H_2SO_4 75%, 5 min + cold stratification (−10 °C, 48 h)	0.15	22.8
GA_3 100 ppm (48 h)	0.43	32.9
GA_3 250 ppm (48 h)	0.55	35
GA_3 500 ppm (48 h)	0.64	45.3
GA_3 1000 ppm (72 h)	0.64	42.3
GA_3 1500 ppm (72 h)	0.71	43.09
GA_3 2500 ppm (72 h)	0.6	39.2
LSD 5%	0.29	9.17

Washing and chilling are standard procedures which have been used to enhance the germination of dormant seeds (ISTA, 1996). Washing and chilling at 5 °C water for a period of 14 days resulted in the highest germination rate and percentage of *F. gummosa* (Table 1). Both germination rate and percentage of *F. gummosa* increased by increasing the number of exposure days at 5 °C. However, when the chilling temperature was decreased from 5 to −10 °C, a negative response was monitored for both germination rate and percentage. The response to the washing and chilling treatments is in accordance with results by Negatali et al. (2001), who found that, in the natural habitats of *F. gummosa*, higher seed germination percentage occurred in colder regions with higher precipitation.

For *T. polium* seeds, acid scarification with H_2SO_4 (75% v/v) for 10 min, and acid scarification with H_2SO_4 (75% v/v) for 5 min together with GA_3 (1500 ppm, 48 h), resulted in germination of 31.9% and 34.1%, respectively (Table 2). The response to acid scarification was stronger when GA_3 was combined, which suggests a synergistic response. The seedcoat is not the main inhibitor of germination of *F. gummosa* but for *T. polium* its hard seedcoat can be considered as an inhibitor trait. Ethanol treatment failed to stimulate the germination. In this investigation soaking seeds of *T. polium* in cool water for a time period of 72 h induced 32.7% germination (Table 2), however it did not show any effect on *F. gummosa* (Table 1).

Regardless of applied treatment to seeds of both species, germination rate was positively correlated with germination percentage ($r = 0.85, P < 0.05$) for both species. Therefore, fast germination was associated with high germination percentage. It can be concluded that for both species, GA_3 alone or in combination with washing stimulates seed germination and has a larger effect than the other treatments applied in this study.

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