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Methods for Dormancy Breaking and Germination of Galbanum Seeds (*Ferula gummosa*)

Afrasyab Rahnama-Ghahfarokhi and Reza Tavakkol-Afshari
Department of Agronomy, Faculty of Agriculture, University of Tehran, Karaj, Iran

Abstract: The aim of this study was to examine whether *Ferula gummosa* seeds possess physical, physiological or combined dormancy. Seeds collected from Shahrekord zone in the center of Iran were subjected to different treatments. Dormancy breaking treatments were included: prechilling for 20, 30 and 40 days, exogenous application of GA_3 (250, 500 and 1000 ppm), prechilling for 40 days combined with various levels of GA_3 (250, 500, 1000 ppm), H_2SO_4 (80%) for 5 and 10 min, hot water at different temperatures (70 and 90°C) for 10 min, thiourea (3%) and KNO_3 (0.3%) for 72 h. Germination of *Ferula gummosa* significantly increased at higher concentrations of GA_3 (1000 ppm) and a longer period of prechilling treatments (for 40 days) and reached 41 and 69%, respectively. Consequently, these treatments were highly effective in dormancy breaking in comparison with other prechilling and GA_3 treatments. The highest germination was observed when seeds were exposed to prechilling (for 40 days) combined with GA_3 (1000 ppm) and induced up to 75% germination and suggested a synergistic response to GA_3 and prechilling. Radicle and plumule length, vigor index and Mean Germination Time (MGT) were also affected through treatments. Thiourea (3%), KNO_3 (0.3%) and 90°C hot water for 10 min had no effect on germination and other traits. Germination rate was positively correlated with germination percentage. These results showed that *Ferula gummosa* seeds exhibit both exogenous and endogenous dormancy.

Key words: Seed dormancy, GA_3, germination, prechilling, *Ferula gummosa*

INTRODUCTION

Galbanum (*Ferula gummosa*, Umbelliferae), is a wild plant indigenous to Iran with a high export demand due to a large number of applications within both traditional medicine and industry. In Iranian folk medicine, this plant has been used for stomach ache, epilepsy and as a wound healing remedy and there are some reports regarding the anticonvulsant, antispasmodic, expectorant and wound-healing activities of this plant (Sayyah and Mandegary, 2003; Zargari, 1989). The fruit essential oil of *F. gummosa* has been used as an antiepileptic remedy (Sayyah *et al*., 2001). Aerial parts of this plant have been demonstrated to be antinociceptive (Fazly *et al*., 1997). However, the main product which is used as a traditional medicine, is a gum obtained from the stem and the root (Sayyah and Mandegary, 2003).

Galbanum is mostly propagated from seeds and because of dormancy, the germination percentage of commercial seeds is generally very low. Seed dormancy is a great problem for cultivating galbanum and relatively little information is available on the seed dormancy-breaking and germination requirements of this plant as well as lack of literatures on suitable methodology for seed dormancy breaking.

Moist chilling can remove dormancy partially and is often practiced to enhance the germination of dormant seeds (Bello *et al*., 1998). It is believed that cold treatment and chilling alters the inhibitor-promoter balance in some plant species (Rehman and Park, 2000).

Furthermore, there are conflicting results on the required length of period of prechilling treatment, which varied from 2 to 15 weeks (Baskin *et al*., 1992; Parmenter *et al*., 1996; Smith-jochum and Albrecht, 1987). The chemicals which are used commercially in various places are including: potassium nitrate, thiourea, sulfuric acid, ethanol and cyanamid. All of these chemicals are inexpensive and can be used easily to break the true dormancy of seeds effectively (Chang and Sung, 2000).

To accelerate breaking seed dormancy, hormones have been applied in several studies (Zigas and Coombe, 1977; Mehanna *et al*., 1985; Chang and Sung, 2000). Gibberellic acid (GA_3) is one of the hormones proposed to control primary dormancy by inducing germination

Corresponding Author: Afrasyab Rahnama-Ghahfarokhi, Department of Agronomy, Faculty of Agriculture, University of Tehran, Karaj, Iran

(Iglesias and Babiano, 1997). Cavieres and Arroyo (2000) from a dormancy-breaking experiment with *Phacelia secunda* seeds showed that a long period of prechilling treatment resulted in increased germination level. Prechilling treatment increased the level of germination but the percentage of abnormal seedlings increased with longer treatment time (Rehman *et al.*, 1999).

Aliero (2004) reported that the use of hot water, sulfuric acid and sand paper scarification affected *Parkia biglobosa* seeds dormancy. El-Siddig *et al.* (2001) found that scarification of *Tamarindus indica* with hot water and sulfuric acid decreased mean emergence time and days to 50% emergence compared to control.

In Iran, there is limited information concerning the potential seed dormancy problems of *F. gummosa*. Lack of literatures on comprehensive study of galbanum seed dormancy breaking is still obvious. Therefore, the objectives of this study were to assess the effects of different seed dormancy breaking treatments on seed germination and seedling quality parameters and devise an effective method for breaking seed dormancy of *F. gummosa*.

MATERIALS AND METHODS

This research was carried out in Faculty of Agriculture, University of Tehran, Iran, in January, 2006 to determine the effective methods for galbanum dormancy-breaking. The matured seeds of *F. gummosa* were collected in June, 2005 from Shahrekord in the center of Iran, when seeds had desiccated to about 13% moisture on a dry weight basis. Immature and damaged seeds were removed and dry seeds were stored for 6 month at 18°C until germination testing carried out. The seeds were surface sterilized by soaking in 5% sodium hypochlorite (NaOCl) solution for 5 min and subsequently rinsed thoroughly with sterilized water for about 5 min prior to applying any treatment.

Untreated seeds were used as the control. For cold stratification, moisturized seeds with distilled water were placed in a sealed plastic box in a refrigerator at temperature of 5°C, for 20, 30 and 40 days under constant light conditions. GA_3 (Sigma, USA), were applied in three concentrations (250, 500 and 1000 ppm) and seeds were soaked in GA_3 for 72 h.

For cold stratification and GA_3 treatment, moisturized seeds with distilled water were placed in a sealed plastic box in a refrigerator at temperature of 5°C, for 40 days and then were treated with GA_3 (250, 500 and 1000 ppm) for 72 h.

For mechanical scarification treatment, seeds were treated in 70 and 90°C hot water bath, for 10 min. Then seeds were left in the water overnight (for 12 h) while it gradually cooled down to room temperature.

For chemical scarification, a set of seeds were soaked in H_2SO_4 (80%), for 5 and 10 min and then rinsed thoroughly by distilled water for 10 min, before transferring to the germination test process. Next set of seeds were treated with KNO_3 (Sigma, USA, 99.8%) at dosage of 0.3% for 72 h and the last set of seeds treated with thiourea were soaked in $CS(NH_2)_2$ solution (Sigma, USA, 99.9%) at dosage of 3% for 72 h at room temperature.

Treatments were arranged in a Completely Randomized Design (CRD) with three replications. For germination test 25 seeds were sown on one filter paper in sterilized Petri dishes with 15 cm diameter moistened with 10 mL of distilled water. All dishes were sealed with a strip of parafilm to reduce evaporation and water loss and no additional water was required during the test. Darkness was maintained by wrapping the dishes with two layers of aluminum foil. After the prechilling period, all of the treatments were transferred to germination chamber with 16 h light and 8 h dark. Constant temperature of 25±2°C and relative humidity of 70-75% were used. The first and the last germination count were carried out on 2nd and 45th days, respectively. Seeds were considered germinated when the tip of the radicle had grown free of the seed coat (Wiese and Binning, 1987; Auld *et al.*, 1988). Final germination was calculated when no further germination took place for several days.

Measured traits were including: germination percentage, germination rate, radicle and plumule length, seed vigor index and Mean Germination Time (MGT).

$$\text{Germination rate} = \sum_{n=2}^{45} \text{(Number germinating since n-1)/n}$$

The germination rate was calculated as follows (Wiese and Binning, 1987):

Where n is the day of incubation.

The percentage of germination was subjected to an analysis of variance.

Mean Germination Time (MGT) was calculated by following equation (Schelin *et al.*, 2003):

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

Where f_i is day during germination period (between 0 and 45 day), n_i is number of germinated seeds per day and N is the total number of germinated seeds in the treatment.

The seed vigor index was calculated as follows (Abdul-baki and Anderson, 1973):

$$\text{Vi} = \frac{\text{Ls} \times \text{Pg}}{100}$$

Where v_i is vigor index, Ls is the mean of seedling length and Pg is germination percentage.

The data were statistically analyzed using a Completely Randomized Design (CRD) with three replications. Data were subjected to analysis of variance using the SAS statistical software package (SAS Institute, 1988). Mean comparison was performed with Duncan's test at the $p<0.01$ level of significance.

RESULTS AND DISCUSSION

In this experiment, the results showed significant differences among the methods used for stimulating galbanum seed germination ($p<0.01$). Untreated seeds (control) did not germinate (Table 1). These results suggest that *F. gummosa* has deep exogenous and endogenous dormancy. Beginning of the embryo dormancy is associated with accumulation of growth inhibitors such as ABA and breaking of dormancy with a shift in the balance of growth regulators towards growth promoters such as GA_3 that overcome the effect of growth inhibitors (Rehman and Park, 2000).

GA_3 treatment stimulated the germination of *F. gummosa* (Table 1). Endogenous GA_3 is widely studied in relation to the breaking of seed dormancy in various species. GA_3 has been exogenously applied as a substitute for stratification and has increased the germination of many plant species. In fact, GA_3 is used to break seed dormancy of various plant species. In a previous study, it was also reported that germination of *Echinacea angustifolia* seeds was improved by GA_3 and was suggested that GA_3 affect physiological and metabolic activities of seeds resulting in early germination (Chuanren *et al*., 2004). The response to GA_3 was dependent on the concentration of GA_3 and a significant difference in germination was observed among seeds treated with various concentrations of GA_3. At lower concentration (250 ppm), germination was low and increasing the concentration of GA_3 above 250 ppm significantly improved germination percentage (Table 1). Some of studies also showed that the results of exogenous application of GA_3 on the breaking of seed dormancy and seed germination can be differed widely among species and within species (Tigabu *et al*., 2001). Other traits such as radicle and plumule length, MGT, germination rate and vigor index were also affected by GA_3 and the traits of MGT, germination rate and vigor index showed a significant difference in various concentrations of GA_3 (Table 1). Rehman and Park (2000) was also found a significant number of *K. paniculata* germinated seeds after treatment with GA_3, but no significant differences in germination was observed among seeds treated with 100, 200 and 300 ppm GA_3.

Washing and moist chilling are standard techniques which have been used for dormant seeds of many species to enhance the germination of dormant seeds and reduce endogenous dormancy successfully (ISTA, 1996).

Seeds showed a broad range of prechilling requirement for germination and prechilling at 5°C water for various durations significantly increased germination percentage of *F. gummosa*. Prechilling for 20, 30 and 40 days gave germination percentage of 8, 36 and 69%, respectively (Table 1). Sharifi and Pouresmael (2006) found that stratification at 4°C in breaking seed dormancy of *Bunium persicum* was very useful and increasing the duration of stratification resulted in an increase in germination percentage. In dormant seeds growth inhibitors have a balance with growth promoters. The balance between inhibitors and promoters is altered by exposing seed to moist chilling (Rehman and Park, 2000). Eisvand *et al*. (2006) also reported that stratification of imbibed Seeds of *Astragalus siliquosus* improve germination percentage and rate of germination. In prechilling treatments, higher seed germination percentage occurred in longer periods. Our results were consistent with results by Najdafi *et al*. (2006). Similar to our results Gupta (2003) reported that dormant seeds which require chilling are often treated with GA_3 for

Table 1: Effects of prechilling, GA_3 and prechilling-GA_3 treatments on germination percentage, root length, plumule length, Mean Germination Time (MGT), germination rate and vigor index in *F. gummosa* seeds

Treatments	Germination percentage	Radicle length (mm)	Plumule length (mm)	MGT (seed/day)	Germination rate	Vigor index
Prechilling for						
20 days	8cd	3.2bc	2.9bc	6.32e	0.158a	0.48e
30 days	36b	8.6b	10.5a	6.24e	0.162a	6.73c
40 days	69a	15.1a	12.1a	14.2b	0.07b	9.3b
GA_3 250 ppm, 72 h	11c	3.8bc	3.9bc	7.11d	0.14a	0.85f
GA_3 500 ppm, 72 h	37b	4.5 bc	5.3b	7.9d	0.126a	3.62d
GA_3 1000 ppm, 72 h	41b	4.6 bc	5.4b	13.9c	0.071b	4.1d
Prechilling for 40 days +						
GA_3 250 ppm, 72 h	69a	15.0a	12.0a	14.2b	0.07b	18.63b
GA_3 500 ppm, 72 h	71a	15.4a	13.1a	14.9a	0.067b	20.23ab
GA_3 1000 ppm, 72 h	75a	15.6a	13.9a	14.9a	0.067b	22.12a
Control	0d	0c	0c	0f	0c	0e

In each column values with the same letter(s) are not significantly different ($p<0.01$)

Table 2: Effects of hot water and chemical treatments on germination percentage, root length, plumule length, Mean Germination Time (MGT), germination rate and vigor index in *F. gummosa* seeds

Treatments	Germination percentage	Radicle length (mm)	Plumule length (mm)	MGT (seed/day)	Germination rate	Vigor index
Hot water 70°C for 10 min	2b	0.8a	0.9a	4.4c	0.224a	0.102b
Hot water 90°C for 10 min	0b	0a	0a	0d	0c	0b
H_2SO_4 (80%), 5 min	7b	3.6a	2.9a	6.13a	0.163b	4.55a
H_2SO_4 (80%), 10 min	25a	2.5a	3a	5.6b	0.160b	1.37b
Thiourea (3%), 72 h	0b	0a	0a	0d	0c	0b
KNO_3 (0.3%), 72 h	0b	0a	0a	0d	0c	0b
Control	0b	0a	0a	0d	0c	0b

In each column values with the same letter(s) are not significantly different ($p<0.01$)

breaking their dormancy. Among seed dormancy breaking treatments, the highest germination percentage was observed for 1000 ppm of GA_3 treatment and prechilling for 40 days. The response to prechilling was stronger when it was combined with GA_3. These results suggested a synergistic response to GA_3 and prechilling (Table 1). Rehman and Park (2000) reported that chilling increased germination of *Koelreuteria paniculata Laxm* up to 44 and 45% after 60 and 90 days of chilling, respectively. Moreover, after 15 days of chilling the germination of chilled seeds in GA_3 was significantly increased and germination of seeds in 100, 200 and 300 ppm GA_3 after 30 days of chilling was 60, 51 and 54%, respectively. On the other hand, GA_3-chilling treatments were more effective than exogenous GA_3 and chilling alone. Other traits were also affected by prechilling treatments and there was a significant difference among prechilling duration (Table 1). All of the traits except germination rate were increased with increased prechilling duration and this shows that prechilling is a proper method for breaking seed dormancy of *F. gummosa*. In some species, seeds need a broad range of stratification requirements for germination and also GA_3 can stimulate seed germination. It can be concluded that, prechilling can induce increasing in GA_3 concentration (Bretzloff and Pellett, 1979) and when GA_3 treatment accompanied with prechilling, they may show a synergistic effect on higher germination. These results also suggested that prechilling affects metabolic and physiological activities including changes in hormones, i.e., disappearance of ABA and activation of GA_3 and consequently, initiation of germination (Rehman and Park, 2000). As a result, application of GA_3 and prechilling together affect physiological and metabolic activities of *F. gummosa* resulting in early germination.

The response to acid scarification was low and application of H_2SO_4 (80%) for 5 and 10 min gave 7 and 25% germination, respectively (Table 2). It seems that H_2SO_4 (80%) was able to scarify seed coats through decreasing the inhibitory effect of seed coat and softening of seed coat by H_2SO_4 accelerated water uptake and resulted in earlier and faster germination (El-Siddig *et al*., 2001). The response to H_2SO_4 (80%) as a method for breaking seed dormancy was consistent with other studies (Hermansen *et al*., 2000; Najdafi *et al*., 2006). Other traits were also affected by H_2SO_4 (80%). Moreover, a reduction in seed vigor index, germination rate and MGT by increasing immersion time in H_2SO_4 (80%) was detected (Table 2). Similar results were reported by Rahman *et al*. (1999), Mohammad and Amusa (2003) and Najdafi *et al*. (2006). Although H_2SO_4 (80%) stimulates germination, however, seed coat can not be the only factor in seed dormancy of *F. gummosa*.

Soaking seeds in different hot water treatments showed no significant differences in seed germination. Soaking seeds in 70°C hot water for 10 min resulted in a small but insignificant increase in germination percentage and stimulated it only up to 2% (Table 2). In general, germination increased with hot water up to 80°C in some species (Mackay *et al*., 2001; Tigabu and Oden, 2001), but longer periods of hot water treatment decreased germination (Rinkon-Rosales *et al*., 2003). This result also suggested that seed coat can not be considered as the only dormancy factor in *F. gummosa*.

Thiourea and KNO_3 treatments had no significant differences in seed germination and failed to stimulate the germination of *F. gummosa*. These treatments did not also affect other traits (Table 2). Similar to these results nitrogenous compounds such as thiourea and KNO_3 were unable to alleviate seed dormancy in *Bunium persicum* (Sharifi and Pouresmael, 2006).

There was a significant positive correlation between germination percentage and germination rate ($r = 0.71$, $p<0.01$) and therefore, it can be concluded that fast germination was associated with high germination percentage (Najdafi *et al*., 2006; Rehman and Park, 2000).

CONCLUSIONS

In conclusion, these results suggest that since GA_3 and H_2SO_4 both induced seed germination of *F. gummosa*, therefore the ability of seed germination in this plant is a complicated process that is controlled by both external and internal regulating factors. It may be considered that seed coat can not be the main constraint in germination of

F. gummosa seeds. Since application of GA_3 or prechilling alone is not probably able to enhance germination to its maximum level and also is not sufficient to induce a balance between dormancy inhibitors and promoters, therefore, applying of GA_3 and prechilling together can be more effective and the highest germination percentage can be obtained when GA_3 treatment in higher concentrations accompanied with prechilling in longer periods. In this situation a hormonal balance will be created and prechilling may stimulate the synthesis or release of growth promoters necessary for germination such as GA_3 that consequently will enhance seed germination and other traits. Accordingly, it is recommended that for breaking *F. gummosa* seed dormancy and increasing seed germination percentages in a short time GA_3 treatments and prechilling should be applied together.

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