DESERT

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Online at http://jdesert.ut.ac.ir

DESERT 13 (2008) 45-51

Seed dormancy-breaking and germination requirements of *Ferula ovina* and *Ferula gummosa*

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Received 1 June 2007; Received in revised form 22 December 2007; Accepted 26 April 2008

Abstract

Dormancy breaking and germination requirements were investigated in seeds of *Ferula ovina* and *Ferula gummosa*. Seeds of rather species were collected from north and eastern-north rangelands of Iran. Seeds of each of the species were subjected to different treatments including various levels of GA3, chilling and combination of GA3 and chilling (GA-chilling). In contrast to treated seeds, non-treated seeds did not germinate. Germination of both species increased at higher concentration of GA3. In the case of *Ferula ovina* the highest germination percentage was obtained when the seeds were treated with 1000 ppm GA-chilling with 76% of germination. For *Ferula gummosa* seeds, the highest germination percentage was found when exposed to 1500 ppm GA-chilling with 53% germination. Both species well responded to different periods of cold stratification (30/60 days), especially *F. ovina* with 36% and 55% germination, respectively. The results suggest that *F. gummosa* has deeper dormancy. For both species, germination rate was positively correlated with germination percentage.

Keywords: Chilling; Dormancy; *Ferula ovina*; *Ferula gummosa*; GA3; Germination

1. Introduction

Genus *Ferula* which belongs to tribe Peucedaneae, subfamily of Apioideae, Umbelliferae family has 133 species distributed throughout the Mediterranean area and central Asia (Mozaffarian, 1983; Heywood, 1985). More than 30 species of the genus *Ferula* (Apiaceae) grow in Iran (Mozaffarian, 1983, 1996). Most of them produce resins with distinct phytochemical properties. The area of distribution on extends through the mountainous and savanna-like regions with sub-desert climate (Safaian and Shokri, 1993).

Ferula ovina, is one of the most important rangeland species of Iran (Amooaghaie, 2006), with a high export demand due to its large number of applications within traditional medicine (Chen et al, 2000). Its main habitats are located within Pakistan, Turkmenistan, Afghanistan and a vast area in the east and central parts of Iran at an altitude of 2000-4000 m, with an average annual precipitation of 350-700 mm (Safaian and Shokri, 1993).

Ferula gummosa is a perennial plant native to Central Asia, growing in the northern parts of Iran. It is a resistant plant, native in semi arid regions. Its distribution could mostly be observed at an altitude of 2000-4000 m above sea level, with average annual precipitation of 250-400 mm (parsa, 1984; Ghahreman, 1986; Batuli, 1994).

The populations are closely linked to the winter rain and to snow falls, especially in lower altitudes. In the past years, the decrease in

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rainfall had an adverse impact on the density of *Ferula* populations. In certain habitats, in low altitudes of especially dry mountain ranges, the plant has already disappeared. With increasing altitude, the plant is encountered more frequently. Still, the lack of snowfall during the past years is expressed in an absence of young plants. The breaking of the seed dormancy depends on snowfall and germination upon the water released and provided by the melting snow.

Germination is a critical stage in the life cycle of weeds and crop plants, and often controls population dynamics, with major practical implications (Keller and Kollmann, 1999). Seeds of some taxa may have been nondormant at maturity, whereas those of others may have been dormant. In species with dormant seeds, dormancy break (after ripening) could have occurred as seeds were stored prior to sowing (Baskin *et al*, 2001). One of the main problems that prevent sustainable use of medicinal plants, native to the arid and semi arid lands, is that they readily germinate within the native environment, but fail to show good germination under laboratory conditions (Gupta, 2003).

Seed germination studies are key tools in conservation programs because they can be used for management programs and species reintroduction (Ortega-Base *et al*, 2005). Over the past 30 years, dormancy has been widely studied but the regulatory principles behind changes in several types of dormancies remain unclear. Abscisic acid (ABA) and gibberellins (GAs) are the hormones proposed to control primary dormancy (the form of dormancy that is acquired during seed development), ABA by inhibiting and GAs by inducing germination (Hilhorst and Karssen, 1992; Iglesias and Babiano, 1997). GAs is a naturally occurring plant hormone that triggers germination of seeds by helping them sprout (Jimenez, 2006). Moist chilling is often practiced to enhance the germination of dormant seeds (Bello *et al*, 1998). It is believed that cold treatment alters the inhibitor-promoter balance (Rehman and Park, 2000). The effect of GAs as a germination promoter is hypothesized to increase with chilling treatment. Chilling has been reported to induce an increase in GAs concentration (Bretzloff and Pellet, 1979; Frankland and Wareign, 1962).

Plant hormones such as gibberellins (GAs) are found to play an important role in the germination process (Ritchie and Gilroy, 1998). Gibberellins are generally synthesized by seeds (Bewley and Black, 1982) and their role in germination is thought to be hydrolysis of storage nutrient in seeds and a direct effect on the growth of the embryo (Karssen *et al*., 1989). External application of gibberellins to seeds can break seed dormancy and aid seedling establishment (Metzger, 1983; Pitel and Wang, 1988; Karssen *et al*., 1989; Dunand, 1992; Lecat *et al*, 1992). However, the stimulating effects of GAs on seed germination are not universal (Bell *et al*, 1995).

The objectives of this study were to determine the effect of exogenously applied GA and moist chilling on seed germination and to devise an effective method for breaking seed dormancy of both *Ferula ovina* and *F.gummosa*.

2. Materials and methods

Seeds of *Ferula ovina* were collected in August 2005 from Khorasan Shomaly province, around 37° 25′ N and 59° 39′ E, while seeds of *Ferula gummosa* were collected in July 2005 from Mazandaran province, around 36° 26′ N and 59° 63′ E. Seeds were separated from the undesired materials and unripe seeds on arrival at the laboratory and dry stored in a sealed plastic box at 5°C. The seeds were surface sterilized by soaking in 5% sodium hypochlorite (NaOCl), solution for 5 min and subsequently rinsed thoroughly with sterilized water prior to applying any treatment.

Germination experiments were conducted using three replications of 20 seeds per each treatment. Seeds were placed on double layered Whatman No.1 filter paper moistened with 5 ml of distilled water in sterilized Petri dishes. Treatments were as follows:

Cold stratification: Seeds were kept at 2-4°C in wet sand for 30 and 60 days before the germination test.

GA3 treatments: To determine the effect of gibberellins at seven concentrations (0, 100, 250, 500, 1000, 1500, 2000 PPM) for 72 h.

GA3 treatment and cold stratification: First, seeds were cooled at 2-4°C in wet sand for 30 days, then soaked in different concentrations of GA3 for 72 h.

After each treatment, seeds were transferred to germinator with alternative light/darkness and temperatures of 8°C N, 20°C D, and relative humidity of 70 to 75%. Germinated seeds were counted and removed every 24 h for 60 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).

Mean germination time was calculated as follows, according to Labouriau (1983):

Gr= Σ (number germinating since n-1)/ n
Where:
Gr= germination rate
n= the days of incubation

2.1. Statistical analysis

At first, the raw data were tested in SAS software for normality test and Root square transformation method was employed for data transformation. Then the data were analyzed through analysis of variance (ANOVA) and the Duncan test (P<0.05).

3. Results

3.1. GA3 stratification

The results of both species percent and rate of germination (variance analysis) are shown in tables 2 and 3. The result showed that the treatments effects were significant for both of above mentioned traits (P<0.01). Duncan Test was carried out for mean comparisons (P<0.01) (Table 4 and 5).

The effect of GA3 treatments on *F.ovina* and *F.gummosa* seeds germination are shown in Fig 1. With no GA3 (non-stratification), the seeds did not germinate in either one of the species. As a whole, GA3 treatments conspicuously increased germination rate and percent in different concentrations. At lower concentrations of GA3, none of the seeds germinated until after 60 days. In such a manner that, *F.ovina* did not respond to 100 ppm GA3 and *F.gummosa* did not respond to GA3 concentration of less than 500 ppm.

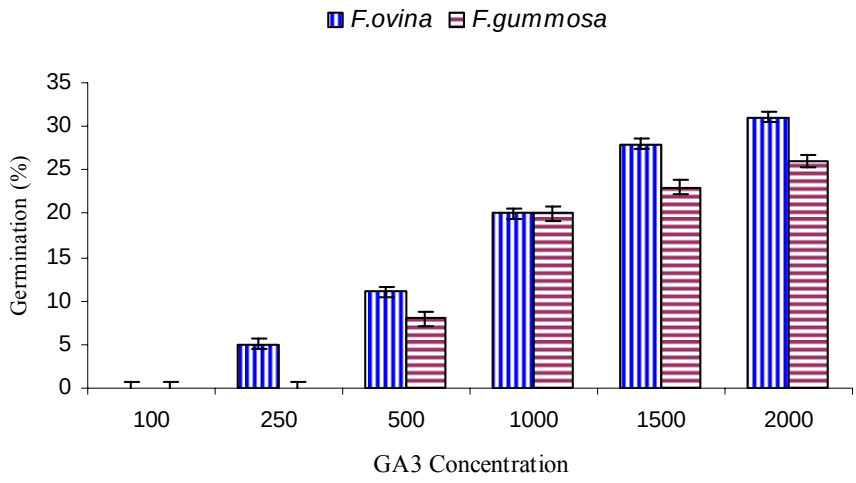

Fig. 1. Effect of GA3 concentrations on *F.ovina* and *F.gummosa* seeds germination

3.2. Cold stratification (chilling)

Seed germination was significantly different among difference treatments (P<0.01). Germination with and without stratification was compared. None of the fresh (non-stratification) seeds of either species germinated after 60 days of incubation. Both species well responded to different periods of cold stratification (30/60 days), especially *F.ovina* with 36% and 55% germination, respectively. The results indicated that an increase in stratification time (from 30 to 60 days) increased germination rate as well as germination percent. The results achieved are presented in Fig 2.

3.3. GA3 treatment with chilling

Seed germination was significantly different among difference treatments (P<0.01). In either of the species, GA3 treatments increased germination at different concentrations, but the difference was greatest when GA3 was accompanied by cold stratification. The results showed that germination rate was faster when chilled seeds were soaked in different concentrations of GA3. In none of the species increase in germination rate and percent was a linear increase. For example, in *F.ovina* specie, the highest germination was achieved by soaking seeds in 1000 ppm GA3 solution while in *F.gummosa* it was observed when soaking seeds in 1500 ppm GA3 solution. The results are shown in Fig 3.

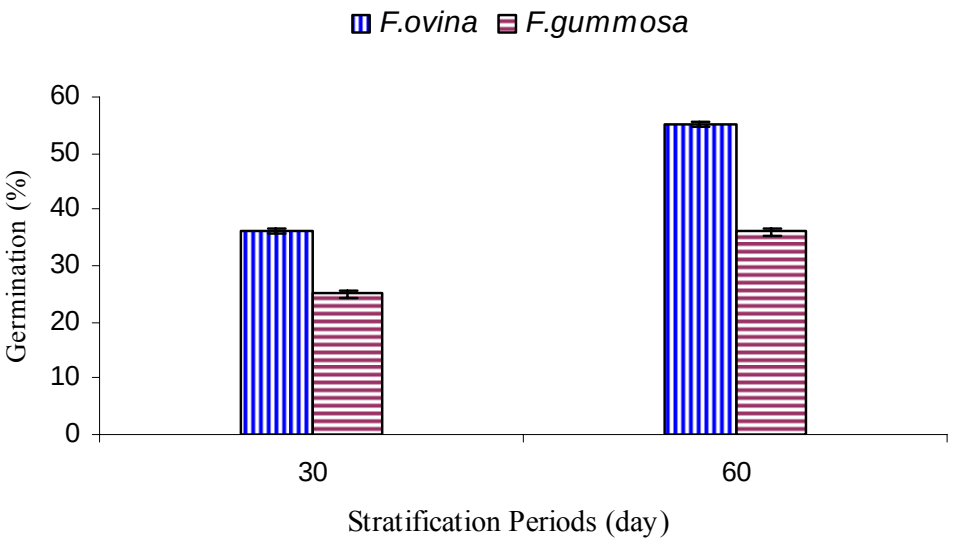

Fig. 2. Effects of stratification different periods on *F.ovina* and *F.gummosa* seeds germination

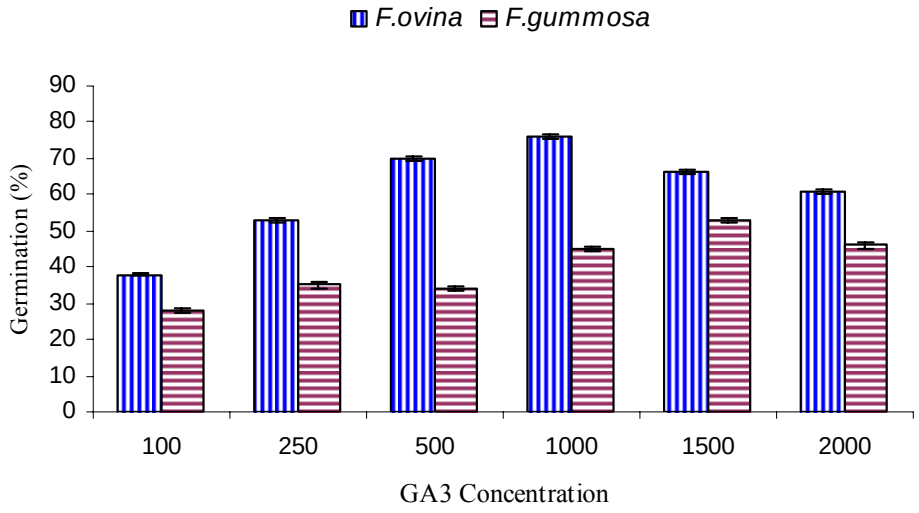

Fig. 3. Effect of GA3 concentrations on *F.ovina* and *F.gummosa* seeds germination after 30 days chilling

Table 1. Simple correlations between germination percentage and germination rate for two species

	F.ovina		*F.gummosa*	
	Gr	Gp	Gr	Gp
Gp	0.96^{**}	1	0.93^{**}	1

Gp= Germination percentage, Gr= Germination rate.

Table 2. Analysis of variance for treatments in *F.ovina* (** = significant for α =0.01)

S.O.V	chilling treatments			GA3 treatments			chilling+GA3 treatments		
	df	Ms		df	Ms		df	Ms	
		Gp	Gr		Gp	Gr		Gp	Gr
Treatment	2	0.23**	0.69**	6	0.2**	0.24**	6	0.2**	0.43**
Error	6	0.005	0.003	14	0.004	0.004	14	0.004	0.004

Table 3: Analysis of variance for treatments in *F.gummosa* (** = significant for α =0.01).

S.O.V	chilling treatments			GA3 treatments			chilling+GA3 treatments		
	df	Ms		df	Ms		df	Ms	
		Gp	Gr		Gp	Gr		Gp	Gr
Treatment	2	0.11**	0.47**	6	0.04**	0.26**	6	0.09**	0.25**
Error	6	0.002	0.005	14	0.001	0.001	14	0.003	0.003

Table 4. 4a: Mean comparison for different traits in chilling treatments, 4b: mean comparison for different traits in GA treatments, 4c: mean comparison for different traits in chilling+GA treatments in *F.ovina*

Treatments	Means	
	Gp	Gr
30 Days	36±0/02 b	0.75±0/04 a
60 Days	55±0/02 a	0.89±0/04 b
Control	0 c	0 c

4a

Treatments	Means	
	Gp	Gr
100 ppm (GA)	0 d	0 e
250 ppm (GA)	5±0/02 d	0.18±0/04 d
500 ppm (GA)	11±0/02 c	0.33±0/04 c
1000 ppm (GA)	20±0/02 b	0.49±0/04 b
1500 ppm (GA)	31±0/02 a	0.65±0/04 a
2000 ppm (GA)	28±0/02 a	0.67±0/04 a
Control	0 d	0 e

4b

Treatments	Means	
	Gp	Gr
100 ppm (GA) + chilling	38±0/02 d	0.7±0/04 c
250 ppm (GA) + chilling	53±0/02 c	0.86±0/04 b
500 ppm (GA) + chilling	70±0/02 ab	0.96±0/04 ab
1000 ppm (GA) + chilling	76±0/02 a	1.07±0/04 a
1500 ppm (GA) + chilling	66±0/02 ab	1.04±0/04 a
2000 ppm (GA) + chilling	61±0/02 bc	1.03±0/04 a
Control	0 e	0 d

4c

Table 5. 5a: Mean comparison for different traits in chilling treatments, 5b: mean comparison for different traits in GA treatments, 5c: mean comparison for different traits in chilling+GA treatments in *F.gummosa*.

Treatments	Means	
	Gp	Gr
30 Days	25±0/03 b	0.66±0/04 a
60 Days	36±0/03 a	0.70±0/04 a
Control	0 c	0 b

5a

Treatments	Means	
	Gp	Gr
100 ppm (GA)	0 d	0 d
250 ppm (GA)	0 d	0 d
500 ppm (GA)	8±0/03 c	0.31±0/04 c
1000 ppm (GA)	20±0/03 b	0.53±0/04 b
1500 ppm (GA)	23±0/03 ab	0.60±0/04ab
2000 ppm (GA)	26±0/03 a	0.61±0/04 a
Control	0 d	0 d

5b

Treatments	Means	
	Gp	Gr
100 ppm (GA) + chilling	28±0/03 c	0.68±0/04 b
250 ppm (GA) + chilling	35±0/03 bc	0.72±0/04 b
500 ppm (GA) + chilling	34±0/03 c	0.70±0/04 b
1000 ppm (GA) + chilling	45±0/03 ab	0.78±0/04ab
1500 ppm (GA) + chilling	53±0/03 a	0.83±0/04 a
2000 ppm (GA) + chilling	46±0/03 a	0.75±0/04ab
Control	0 d	0 c

5c

4. Discussion

It is suggested that the onset of embryo dormancy is associated with accumulation of growth inhibitors while breaking of dormancy with a shift in the balance of growth promoters that overcome the effect of inhibitors (Khan, 1971). Baskin *et al*., (1995), and Walck *et al*. (2002) reported that *Erythorium* and *Osmorhiza* species from Umbelliferae family possess a degree of physiological dormancy that can be broken with application of suitable stratification periods. They believe that this requirement for stratification is related to ecological distribution of seeds. Seeds of *F.ovina* and *F.gummosa*, because of belonging to cold climate, and finding their growth in these areas, may be stated to develop a kind of physiological dormancy in the form of ecological adaptation that we can break by use of chilling treatments.

Germination with and without stratification was compared. The results showed that stratification had a significant effect on seed dormancy, so that the germination percent increased with increasing stratification periods. Seeds with no stratification treatment did not germinate. These results suggested that *F.gummosa* has a deeper dormancy.

Cold stratification is a standard procedure used to enhance the germination of dormant seeds. It has been used for various dormant seeds and has been reported to successfully alleviate endogenous dormancy. Sharifi and Pouresmael (2006) found that stratification at 4°C in breaking seed dormancy of *Bunium persicum* was very helpful, such that increasing the duration of stratification resulted in an increase in germination percent. Eisvand *et al.* (2006) also reported that stratification of imbibed seeds of *Astragalus siliguosus* improve

germination percent as well as rate of germination.

Endogenous GA3 are widely studied in relation to the breaking of seed dormancy in various species. GA3 has been exogenously applied as a substitute for stratification. It has increased germination in many plant species. They have been exogenously applied as a substitute for stratification and have increased germination in many plant species, *Leucospermum* (Brits *et al*., 1995), *Fagus sylvatica* (Nicolas *et al*., 1996) and *Helianthus* (Sieler, 1998) being cited as same examples. In a previous study, it has also been reported that germination of *Echinacea angustifolia* seeds was improved by GA3 and it was suggested that GA3 affect physiological as well as metabolic activities of seeds, resulting in early germination (Chuanren *et al*., 2004).

Prasad *et al.* (1996) found that the use of GA3 at 100 $mg.L^{-1}$ increased the germination rate of lychee seeds in all the varieties studied, suggesting that gibberellic acid played an important promoting role in the germination process of these seeds. According to Salisbury & Ross (1992), gibberellic acid is the growth regulator that truly acts on seed germination, exerting a favorable effect on the break of dormancy.

The seeds in any of the species did not show any favorable reaction to low concentrations of GA3, because of their high rate of dormancy but the germination increased with increase in concentration of GA3 ($250 \leq X$). One of the objectives of the study was to determine the role of GA3 as a treatment instead of cold stratification treatments. The results indicated that we can not replace GA3 for cold stratification, even in high concentrations of this hormone.

With regard to germination percent and germination rate in the two different cold stratification periods (30 and 60 days) to be significant a combined treatment (30 days cold stratifications with different concentration of GA3) was employed for a reduction in cold stratification periods. The results indicated that the application of this complex treatment not only reduced the stratification period but also increased percent and rate of germination. These results show that although GA3 and cold stratification significantly increase germination, these treatments were not as effective as the treatment GA3 accompanied by chilling.

The response to chilling was more conspicuous when combined with GA3. 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 GA3 was significantly increased. Germination of seeds in 100, 200 and 300 ppm GA3 (after 30 days of chilling) was reported as 60, 51 and 45%, respectively. On the other hand, GA3-chilling treatments were reported as more effective than rather of exogenous GA3 or chilling alone.

In conclusion, these results suggest that GAs and moist chilling enhanced germination probably due to the inhibitor-promoter balance. However, the action of GAs or moist chilling alone may not be sufficient to bring inhibitor-promoter balance and therefore, failed to increase germination to its maximum level. The combination of GAs and chilling was perhaps more effective in bringing a hormonal shift that not only enhanced germination but also speeded it up. Therefore, GA-chilling may be recommended for breaking *F.ovina* and *F.gummosa* seeds dormancy in a relatively short time.

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