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Original Contribution

EFFECT OF VARIOUS TREATMENTS ON SEED GERMINATION AND DORMANCY BREAKING IN *FERULA ASSA FOETIDA* L.(ASAFETIDA), A THREATENED MEDICINAL HERB

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ABSTRACT

The aim of this study was to enhance the germination percentage and rate of *Ferula assa foetida*, a medicinally important vulnerable species, which have a low germination rate under normal laboratory conditions. The efficacy of different treatments including various levels of GA_3, chilling and soaking with running water for germination improvement was tested. Analysis of variance indicated that Cold stratification and GA_3 concentration had significant effects on seed germination percentage. Combined treatments chilling (4 ℃) for both periods of 30 and 60 days were most effective in breaking dormancy. Among the combined chilling treatments, Maximum germination was obtained at combination treatment cold stratification (60 days) with 2000 ppm of GA_3 solution and minimum germination was at socking in running water and control treatments. These results suggested that *Ferula assa foetida* seeds exhibit endogenous dormancy. A combination of cold stratifications and exogenous application of GA_3 alleviated seed dormancy in a relatively short period of time. Germination rate was positively correlated with germination percentage.

Key words: seed germination, dormancy breaking, cold stratification, *Ferula assa foetida* L., GA_3 treatment.

INTRODUCTION

The seeds of many medicinal plant species have dormancy; they readily germinate within the native environment, but fail to show good germination under laboratory conditions (1) or when cultivation is attempted.

Ferula assa-foetida (Apiaceae), commonly known as Asafetida is an herbaceous, perennial medicinal plant indigenous to Iran (2). Asafetida is a very effective medicinal herb that acts mainly on the digestive system, cleansing and strengthening the gastro-intestinal tract. The pungently flavored gum-resin that is obtained from the root by incisions is alterative, anthelmintic, antispasmodic, carminative, deobstruent, deodorant, expectorant, laxative, sedative and stomachic (3). The commercial and pharmacological need of Asafetida is achieved usually by exploiting the wild populations. Indiscriminate collection of this plant from the wild has posed a serious threat to its existence in the wild populations, especially when the plants are harvested well before seed set. The plant is conventionally propagated through seed but is hampered by the seed dormancy. Generally the germination capacity of Apiaceae family is very low due to seed dormancy (4, 5). Further, the methods of extraction employed are almost invariably crude and unsystematic (6). Consequently, Asafetida is recorded as a vulnerable species in the Red Data Book of Iran (7). Low seed germination has been a problem in stand establishment of *Ferula assa-foetida* on sites where its seeds are sown and in seedling production in nurseries.

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Unfortunately, there is limited information concerning the potential seed dormancy problems of Asafetida seeds. A significant barrier to germination is the need to develop methods, which release physiological dormancy in seeds. A period of chilling (Stratification) relieves primary dormancy of many northern hemisphere species (8). 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 (9). Gibberellic acid (GA_3) is one of the hormones proposed to control primary dormancy by inducing germination. The effect of GA_3 as a germination promoter is hypothesized to increase with chilling treatment (4). For the occasional species whose seed coats contain a readily water-soluble, germination-inhibiting chemical, this substance can be removed by soaking the seeds in tap water or by leaching the seeds in slowly running tap water for various lengths of time just prior to soaking (10).

To date, there is no report on seed germination of this plant. The objectives of this study were to determine the effect of chilling, exogenously applied GA_3 and running water on germination in finding effective methods for breaking the dormancy of *Ferula assa-foetida* seed as an initial step in its domestication.

MATERIAL AND METHODS

1. Seed source

The mature seeds of *F. assa foetida* were collected in July 2007 from a biological reserve in the nearby county of Nasr Abad, Yazd of Iran (Lat: 3147 N; Lon: 5352 E). The seeds were surface sterilized by soaking in 70 % alcohol for 1 min and afterwards soaking in 2.5 % sodium hypochlorite (NaOCl) for 15 min and subsequently rinsed thoroughly with double sterilized water prior to applying any treatment.

2. Implementation of the experiment

Soaking in running water: seeds were soaked in running water for 72 h and then transferred to the germination test process.

Cold stratification: the surface-sterilized seeds were maintained at moistened sand with water at temperature of 4 ℃, for 30 and 60 days and 0 ˚C for 7 and 14 days.

Effect of GA_3: seeds were soaked in 500, 1000, 1500 and 2000 ppm GA_3 for 72 h.

Soaking in running water and cold stratification: seeds were soaked in tap water for 72 h subsequently seeds were transferred to sealed plastic boxes containing moist sand. These boxes were placed in a refrigerator set at 0 ˚C for 7 and 14 days.

Cold stratification and GA_3 treatment: seeds were soaked in 500, 1000, 1500, and 2000 ppm GA_3, for 72 h and then maintained at moist sand at temperature of 4 ˚C, for 30 and 60 days.

3. Germination experiments

Germination experiments were tested using four replications of 25 seeds per each treatment. After any treatment, seeds were placed on 15 cm sterilized Petri dishes containing double layered Wathman No.1 filter papers moistened with 10 ml of double sterilized water and incubated at 23 ± 2 ℃ under 16 h photoperiod supplied by two Philips TL 40W fluorescent tubes.

Germinated seeds and rotted seeds were counted every other day and removed. The Germination test was recorded every other day for 60 days. A seed was considered germinated when the tip of the radicle had grown free of the seed coat (11). For each experiment, a batch of untreated seeds was used as control.

4. Statistical analysis

Germination percentage was calculated as [(the umber of germinated seeds)/the number of sampled seeds] ×100. The germination rate was calculated as follows (based on Yang *et al*., 2007):

$$\text{Germination rate} = \sum_{n=1}^{60} \frac{Gt}{Dt}$$

Where Gt is the number of germinated seeds after t days (Dt).

The results were arcsine transformed and statistically analyzed by a one-way analysis of variance (ANOVA) using SAS version 9.1. The data were analyzed using a randomized complete design and LSD test was used to determine if there were significant ($p < 0.05$) differences among treatment means.

RESULT

Seed germination and rate was significantly different among various treatments. Together with untreated controls, running water treatments resulted in the lowest germination percentage and rates **(Table 1)**. Chemicals that accumulate in the seed-coat during development and remain in the seed after

harvest can act as germination inhibitors. Some of the substances associated with inhibition are various phenols, coumarin and abscisic acid, which can be leached out by soaking in water (12). In the present study, seeds were soaked in tap water for 72 h. In a few minutes, the tap water turned dark in color indicating the presence of substances leached mainly from seeds. These results suggest that chemicals are not the main inhibitor of germination of Asafetida seeds. It is possible also that soaking seeds in running water for 72 h was not effective to removed substances from seeds.

Table 1. *Seed germination and dormancy breaking and applied treatments for Ferula assa foetida*

Dormancy breaking treatments	Germination (%)	Germination rate (seeds per day)
control	1.05^{h}	0.012^{j}
soaking in running water (72 h)	5.31^{h}	0.037^{ji}
chilling (4 ˚C for 30 d)	49.75^{ed}	0.647^{f}
chilling (4 ˚C for 60 d)	68^{c}	0.875^{e}
chilling (0 ˚C for 7 d)	23.75^{g}	0.237^{ghi}
chilling (0 ˚C for 14 d)	34.25^{gf}	0.337^{gh}
GA_3 treatment (500 ppm)	9.75^{h}	0.15^{hij}
GA_3 treatment (1000 ppm)	23.5^{g}	0.337^{gh}
GA_3 treatment (1500 ppm)	39^{ef}	0.43^{gf}
GA_3 treatment (2000 ppm)	34^{gf}	0.575^{f}
soaking + chilling (72 h + 0˚C for 7 d)	27.75^{gf}	0.262^{gh}
soaking + chilling (72 h + 0˚C for 147 d)	38.25^{f}	0.35^{gh}
chilling + GA_3 (4 ˚C for 30 d + 500 ppm)	53.66^{d}	1.1^{d}
chilling + GA_3 (4 ˚C for 30 d + 1000 ppm)	66.33^{c}	1.365^{c}
chilling + GA_3 (4 ˚C for 30 d + 1500 ppm)	69.66^{c}	1.3^{dc}
chilling + GA_3 (4 ˚C for 30 d + 2000 ppm)	76.33^{bc}	1.265^{dc}
chilling + GA_3 (4 ˚C for 60 d + 500 ppm)	85^{ab}	1.3^{dc}
chilling + GA_3 (4 ˚C for 60 d + 1000 ppm)	88.33^{a}	1.732^{ab}
chilling + GA_3 (4 ˚C for 60 d + 1500 ppm)	90.66^{a}	1.6^{b}
chilling + GA_3 (4 ˚C for 60 d + 2000 ppm)	91.66^{a}	1.9^{a}

Numbers with different letters (a–k) are significantly different by LSD test at $p < 0.05$.

Seed germination of *Ferula assa foetida* was significantly affected by cold stratification treatments. As a whole, cold stratification treatments obviously increased germination percentage and rate in different treatments. Germination percentage and rate varied with duration and temperatures of stratification. Increasing chilling times increased both germination rate and percentage. At 4 ˚C for 30 and 60 days, germination percentages were 49.75 and 68, respectively, and it was 23.75 and 34.25 at 0 ˚C for 7 and 14 days, respectively **(Table 1)**. These indicate and agree with that cold stratification at higher duration increase seed germination. These results agree with those reported by Yang *et al*. (2007), Nadjafi *et al*. (2006), Fang *et al*. (2006), in other plants. Compared with chilling treatments at 0 ˚C, combination treatments socking in running water with chilling at 0˚C for periods of 7 and 14 days did not significantly affect on germination percentage **(Table 1)**. In general, chilling for 60 days suffices to remove the embryo dormancy in many plants (13); the current study also indicate that stratification at 4 ˚C for 60 days yielded fastest germination of all stratification treatments in *Ferula assa foetida*. Since the method of cold stratification treatment is quite

simple and inexpensive compared with GA_3, this can be widely used in practice.

Gibberellins overcome seed dormancy and bud dormancy in many species, acting as a substitute for low temperatures, long days or red light (14). 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 (4). This response was dependent on the concentration of applied GA_3. At lower concentrations, germination was lower. Among all the GA_3 treatments, concentration 1500 ppm had the highest germination percentage and concentration 500 ppm induced germination significantly lower than other GA_3 treatments **(Table 1)**. Similar results were obtained from studies carried out on other species, such as *Ferula gummosa* (4), *Sesamum indicum* (15), *Rumex dentatus* (16).

The highest germination percentage and rate achieved was after a combined treatment of stratification for 60 days with the application of 2000 ppm GA_3 (91.66) **(Fig 1)**, although no significant differences were found among other combination treatments **(Table 1)**. In the cold stratification with 2000 ppm GA_3, germination rate increased from 1.26 after 30 days to 1.9 after 60 days. Considerable research on gibberellins as seed germination promoters shows that application of gibberellic acid to dormant seeds can eliminate their natural chilling requirement (17). Moist chilling has successfully alleviated endogenous dormancy for various dormant seeds (18). These results show that combination treatments cold stratification with exogenous GA_3 significantly increased germination.

Fig. 1. Seed germination of *Ferula assa foetida* after a combined treatment of stratification for 60 days with the application of 2000 ppm GA_3

Regardless of applied treatment to seeds of *Ferula assa foetida*, germination rate was positively correlated with germination percentage. Therefore, fast germination was associated with high germination percentage. The response to cold stratification was stronger when GA_3 was combined, which suggests a synergistic response. Thus, it is concluded that the seeds of these species exhibit endogenous dormancy which required some period of after ripening to breaking dormancy. It can be concluded that cold stratification alone or in combination with GA_3 stimulates seed germination and has a larger effect than the other treatments applied in this study.

In conclusion, the present work has established an effective strategy for breaking seed dormancy and enhancing seed germination of *Ferula assa foetida* through soaking in running water, application of gibberellin and cold stratification. Other possibilities are GA_3 treatment seeds in various concentrations and durations. These need further studies. To obtain optimum germination, both GA_3

treatments and cold stratifications or their combination are necessary for *Ferula assa foetida*. Much more work is needed to investigate the causes for the long seed dormancy of this plant. Particularly, further investigation to identify the responsible inhibition mechanisms and studies on the hormonal balance in the seeds such as that between ABA and GA are deemed important.

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