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# Effect of Exogenous Hormones and Chilling on Dormancy Breaking of Seeds of Asafoetida (*Ferula assafoetida* L.)

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**Abstract:** Asafoetida (*Ferula assafoetida* L.) is a medicinal plant with a problematic seed germination. Seeds of this plant have a long dormancy. The present research was carried out to investigate whether exogenous application of the hormones Gibberellic Acid ($GA_3$), N6-furfurylaminopurine (kinetin) and 6-benzylaminopurine (BAP), alone or in combinations and chilling (for 0 or 28 days) could be used to alleviate the problem of dormancy. Germination was increased from 53.3% in a medium free of any hormones to 74.4% in a medium supplemented with 0.25 mg $L^{-1}$ BAP. This was the only single hormone treatment giving a statistically significant increase in germination. Chilling increased germination from 37.5% (0 days of chilling) to 82.9% (28 days of chilling). Combining exogenous application of 0.25 mg $L^{-1}$ BAP with 28 day chilling even gave more than 90% seed germination. Present findings suggest that seeds germination of Asafoetida can be greatly improved by combining chilling with BAP application.

**Key words:** Asafoetida, chilling, dormancy breaking, *Ferula assafoetida* L., exogenous application of hormones

## INTRODUCTION

Asafoetida (*Ferula assafoetida* L.), also known as devil's dung, is an important species of the Apiaceae family. It is a stout perennial, monocarpic herb (Singh Puri, 2003) reaching a height of 2-3 m with 2-4 pinnate pubescent leaves having cauline sheaths, yellow flowers in large terminal compound umbels and dorsally compressed cremocarps (Daniel, 2006). It is native to Iran.

This medicinal plant has important effects on the functioning of the digestive system and it is reported to be useful for breath, secretions, flatus and gastric eructation (Bown, 1995; Chevallier, 1996). The resin gum from the roots is vermifuge; it is antispasmodic, expectorant and appetizing (Chiej, 1984; Duke and Ayensu, 1985). The seeds of Asafoetida have a long dormancy. The dormant seeds do not germinate on the mother plant and they have enough time for dispersal (Baskin *et al*., 1995). This characteristic is very useful. Seeds of most cultivated and garden plants lose their dormancy before or shortly after they are separated from the mother plant, but in contrast most wild plants have a long seed dormancy (Bryant, 1996). Usually a change in environmental conditions caused by seasonality of the climate is a main factor determining the level of dormancy through their effects on the seed hormonal balance (Bewley and Black, 1994; Peng and Harberd, 2002; Thomas, 1990; Thomas and Sambrooks, 1985). Because seed dormancy is a nuisance in propagation programmes, removing or breaking dormancy has always been a challenge for plant physiologists (Baskin and Baskin, 1991).

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Primary seed dormancy can be divided into two types: external and internal. Morpho-physiological dormancy belongs to the internal type of primary dormancy. It depends on plant species and seeds must be exposed to cold, heat, gibberellic acid or chemical materials for dormancy breaking (Bewley and Black, 1994). Seeds of many species of the Apiaceae family have different types of morpho-physiological dormancy that c hilling can remove (Baskin and Baskin, 1991, 2004; Baskin *et al*., 1999; Phillips *et al*., 2003). In this family, different species of the genera Erythronium, Osmorhiza (Baskin and Baskin, 1984, 1989, 1991; Baskin *et al*., 1995) and species like *Ferula gummosa*, *Teucrium polium* (Nadjafi *et al*., 2006) and *Thaspium pinnatifidum* (Baskin *et al*., 1992) need different periods of chilling to break seed dormancy.

$GA_3$ is one of the most important hormones that have been suggested to break primary dormancy (Nadjafi *et al*., 2006). $GA_3$ can improve seed germination in Apiaceae which also requires light and cold (El-Dengawy, 2005; El-Nabawy *et al*., 1980; Finch-Savage and Leubner-Metzger, 2006; Peng and Harberd, 2002; Thomas, 1990; Thomas and Sambrooks, 1985).

Asafoetida's seeds have a long dormancy and the aim of this research was to find efficient ways to break it.To the best of our knowledge no earlier studies on seed dormancy and dormancy breaking methods for *F. assafoetida* have been reported. For the first time we established *in vitro* culture systems for dormancy breaking of seeds of this plant species.

## MATERIALS AND METHODS

The experiment was carried out at the tissue culture facility of the Agricultural Biotechnology Research Institute of Iran in October 2007.

Seeds of Asafoetida were obtained from the Medicinal Plants Collection of the Shahid Fozveh Research Center in July 2007. Seeds were washed under running tap water for 10 times to remove surface contamination. To eliminate probable pathogens, seeds were subsequently placed in a fungicide solution containing 2.5% (w/v) Benomyl and Carboxyn Tiram for 4 h. Seeds were rinsed again with sterile distilled water and then were soaked in ethanol 96% for 1 min. At the end of this sterilization process, seeds were placed in 25% chlorax supplemented with Tween 20 (1 drop) for 25 min. Seeds were rinsed again with sterile distilled water 3 times in a laminar flow cabinet. Fifteen seeds were put in a single Petri dish containing water agar and different concentrations of various hormones or hormone compositions.

In this study, 10 different hormone treatments and two chilling treatments (0 (control) and 28 days) were applied. Hormone treatments included (in addition to a control): 0.1 and 0.25 mg $L^{-1}$ BAP, 0.1 and 0.25 mg $L^{-1}$ kinetin, 5 mg $L^{-1}$ $GA_3$, plus the combinations of the BAP treatments or the kinetin treatments with the $GA_3$ treatment. Treatments are coded by the abbreviation of the substance followed by the concentration (without the unit).

For the chilling treatment Petri dishes containing seeds were placed in a dark refrigerator at 5°C temperature during 0 (control) or 28 days. After chilling these Petri dishes were transferred to a growth chamber at 24°C and with 18.5 µmol/ $m^2$/sec light intensity.

Germination rates were observed 2, 3 and 4 weeks after the transfer to the growth chamber. The experiment was conducted as a completely randomized factorial experiment with two factors and 3 replications. Data were statistically analyzed using the SAS package (Version 8 of the SAS System) (SAS, 1999) statistical computer program and Duncan's Multiple Range Tests were used to compare the Mean values.

## RESULTS

The analysis of data showed that both experimental factors, chilling and hormone application, significantly affected seed germination. Chilling at 5°C for 28 days increased germination. For 0 day chilling treatment, only 37.5% of seeds were germinated, while chilling resulted in 82.9% germination (Fig. 1).

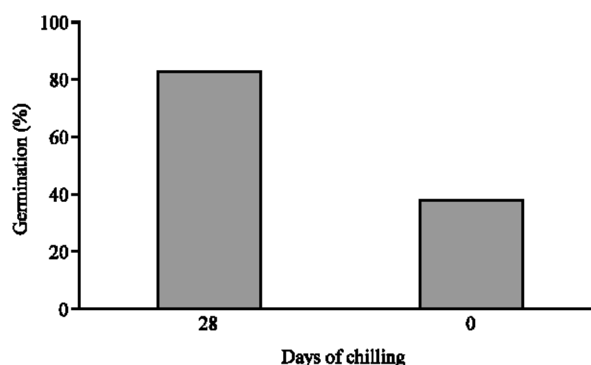


Fig. 1: The effect of cold on germination

Table 1: The effects of hormone application on germination

| Treatments (mg $L^{-1}$) | Germination (%) |
|---|---|
| BAP 0.1 | 61.1b |
| BAP 0.1+$GA_3$ 5 | 60.0b |
| BAP 0.25 | 74.4a |
| BAP 0.25+$GA_3$ 5 | 73.3a |
| Kin 0.1 | 50.0b |
| Kin 0.1+$GA_3$ 5 | 53.3b |
| Kin 0.25 | 54.4b |
| Kin 0.25+$GA_3$ 5 | 61.1b |

Mean values followed by the same letter(s) are not significantly different ($p<0.05$)

Table 2: Mean comparison of BAP concentrations

| Concentration of BAP (mg $L^{-1}$) | Mean of germinated seed (%) |
|---|---|
| 0.00 | 59.0b |
| 0.10 | 60.5b |
| 0.25 | 73.9a |

Mean values followed by the same letter(s) are not significantly different ($p<0.05$)

The maximum positive effect of hormone application was an increase in germination by 21.1% (Table 1). Treatment 0.1 mg $L^{-1}$ kinetin showed a (non-significant) negative effect on germination (Table 1).

In Asafoetida, kinetin had no effect on germination while other cytokinin (BAP) showed a large positive effect on seed germination at the higher concentration. These results showed that different cytokinins may have different influences on dormancy break. Treatment 0.25 mg $L^{-1}$ BAP was more effective than the treatment with $GA_3$ and increased germination significantly. Table 2 shows the BAP effects averaged over the single treatment and the combination with $GA_3$.

It was observed that seed germination in the treatment without chilling was almost fixed from the third week onwards and hardly any further germination was observed. For the treatment without chilling hormone applications had an effect on the dynamics of germination. The highest value after two weeks was observed for the treatment 0.25 mg $L^{-1}$ BAP + 5 mg $L^{-1}$ $GA_3$ (Table 3). During the third week $GA_3$ had the highest additional germination. All hormone applications showed little germination in the fourth week (Table 3).

For the treatment with 28 days chilling, germination increased over time and considerable additional germination took place in the last week (Table 4). This late germination contributed much to the effect of chilling on germination.

Table 3: The effects of hormones on percentage of additional germination at different intervals during germination without chilling

| | Germination (%) | | |
|---|---|---|---|
| Treatments (mg $L^{-1}$) | 2nd week | 3rd week | 4th week |
| BAP 0.1 | 40.0bc | 2.2hi | 6.7fghi |
| BAP 0.1 + $GA_3$ 5 | 26.7d | 4.4ghi | 0.0i |
| BAP 0.25 | 42.2b | 6.7fghi | 8.9fghi |
| BAP 0.25 + $GA_3$ 5 | 55.6a | 0.0i | 0.0i |
| Kin 0.1 | 13.3fgh | 6.7fghi | 0.0i |
| Kin 0.1 + $GA_3$ 5 | 17.8def | 6.7fghi | 2.2hi |
| Kin 0.25 | 37.8bc | 2.2hi | 0.0i |
| Kin 0.25 + $GA_3$ 5 | 26.7d | 8.9fghi | 0.0i |
| $GA_3$ 5 | 22.2de | 17.8defg | 0.0i |
| WA (control) | 15.6defg | 6.7fghi | 0.0i |

Mean values followed by the same letter(s) are not significantly different ($p<0.05$)

Table 4: The effects of hormones on percentage of additional germination at different intervals during germination with chilling

| | Germination (%) | | |
|---|---|---|---|
| Treatments (mg $L^{-1}$) | 2nd week | 3rd week | 4th week |
| BAP 0.1 | 13.3ij | 22.2h | 37.8d |
| BAP 0.1+ $GA_3$ 5 | 22.2h | 44.4c | 22.2h |
| BAP 0.25 | 13.3ij | 62.2a | 15.6i |
| BAP 0.25 + $GA_3$ 5 | 15.6i | 48.9b | 26.7g |
| Kin 0.1 | 2.2kl | 33.3e | 44.4c |
| Kin 0.1 + $GA_3$ 5 | 11.1j | 37.8d | 31.1ef |
| Kin 0.25 | 4.4kl | 37.8d | 26.7g |
| Kin 0.25 + $GA_3$ 5 | 15.6i | 42.2c | 28.9fg |
| $GA_3$ 5 | 11.1j | 37.8d | 33.3e |
| WA (control) | 6.7kl | 33.3e | 44.4c |

Mean values followed by the same letter(s) are not significantly different ($p<0.05$)

Table 5: Interaction between chilling and hormone treatment for germination

| | Germination (%) | |
|---|---|---|
| Treatments (mg $L^{-1}$) | 0 day chilling | 28 days chilling |
| BAP 0.1 | 48.9jkl | 73.3bcdefgh |
| BAP 0.1 + $GA_3$ 5 | 31.1mn | 88.9abc |
| BAP 0.25 | 57.8hij | 91.1ab |
| BAP 0.25 + $GA_3$ 5 | 53.3ijk | 93.3a |
| Kin 0.1 | 20.0mn | 80.0abcdefg |
| Kin 0.1 + $GA_3$ 5 | 26.7mn | 80.0abcdefg |
| Kin 0.25 | 40.0klm | 68.9defghi |
| Kin 0.25+ $GA_3$ 5 | 35.6klmn | 86.7abcd |
| $GA_3$ 5 | 40.0klm | 82.2abcdef |
| WA (control) | 22.2mn | 84.4abcde |

Mean values followed by the same letter(s) are not significantly different ($p<0.05$)

Germination showed different behaviours for treatments with chilling and without chilling. For 0 day chilling, most of the germination took place in the first two weeks, while after 28 days chilling most of the germination took place during the third week (Table 3, 4). Without chilling, all hormonal treatments, showed their maximum germination during the first two weeks and hardly any germination during the fourth week (Table 3). In contrast, after 28 days of chilling, all hormonal treatments showed most germination during the third or fourth week with relatively little germination during the first two weeks (Table 4).

The two-ways interaction of both factors (chilling and hormone application) was also significant regarding the final percentage of germinated seeds. Highest values for germination were obtained for 0.25 mg $L^{-1}$ BAP (91%) and 0.25 mg $L^{-1}$ BAP + 5 mg $L^{-1}$ $GA_3$ (93%) at 28 days of chilling while it was lowest with 0.1 mg $L^{-1}$ Kin without chilling (20%) (Table 5).

For chilling, highest and lowest percentage of germination was found with 0.25 mg $L^{-1}$ BAP + 0.5 mg $L^{-1}$ $GA_3$ and treatment 0.25 mg $L^{-1}$ kin, respectively. For non-chilling 0.25 mg $L^{-1}$ BAP + 5 mg $L^{-1}$ $GA_3$ resulted in the highest percentage. Of germination but 0.01 mg $L^{-1}$ kin showed lowest percentage of germination and there was no significant difference between this treatment and control.

It was interesting that on average 53.3% of final germination was obtained on a medium free of hormone (Table 1). When seeds were treated with chilling the control reached 84.4% but only 22.2% of germination was observed for the control without chilling (Table 5).

## DISCUSSION

The percentage of germination in Asafoetida can be increased with a chilling treatment. Walck and Hidayati (2004) reported that seed dormancy of *Osmorhiza depauperata* was broken by a 32 week incubation at 1-5°C and $GA_3$ was not a profitable substitute for cold. In *Thaspium pinnatifidum*, chilling was needed for breaking the physiological seed dormancy. It can stimulate immature embryos to grow and break their morphological dormancy (Baskin *et al*., 1992). The natural habitats of *F. gummosa*, higher seed germination percentage occurred in colder regions with higher precipitation. In *Chaerophyllum temulum* growth of embryo also happened on the long term chilling at 5°C (Vandelook *et al*., 2007). The main reason to explain the effect of chilling on breaking of seed dormancy is enhancing internal $GA_3$ accumulation (Phillips *et al*., 2003). $GA_3$ is suggested to break primary dormancy and stimulate germination (Eastmond and Jones, 2005).

Dormancy is one of the most important physiological properties that are controlled by ABA/$GA_3$ ratio. At high levels of ABA and low levels of $GA_3$, the embryo remains dormant and with a reversed ABA:$GA_3$ ratio, the intensity of the embryo's dormancy is reduced (Finch-Savage and Leubner-Metzger, 2006). If the chilling enhances the concentration of internal $GA_3$ in seed, so exogenous application of $GA_3$ should also cause more or faster germination, but in the case of Asafoetida, contrary to most species of the Apiaceae, application of this hormone had no effect on germination of seeds. Therefore, cold treatment may have other effects on dormancy breaking that still require elucidation. Tsukamoto (1972) applied cytokinins to break dormancy of *Gladiolus*. Tsukamoto (1972) reported that cytokinins can break dormancy of many species and also dormancy of buds in trees but he could not find any reason for that. Cytokinins are not necessary for germination, but they can affect ABA which inhibits germination and overcome thermo-dormancy (Litwack, 2005). It has been reported that cytokinins enhance the effect of $GA_3$ in the seed system of Celery and cause faster germination (Biddington and Thomas, 1978; Thomas and Van Staden, 1995). In Asafoetida, kinetin had no significant effect on germination but presence of BAP resulted in significant effects on germination, so it can be suggested that cytokinins may affect dormancy breaking in different ways or that different cytokinins have different activities.

Present result shows that the seeds of Asafoetida have a deep and complex morpho-physiological dormancy and like many species of Apiaceae, they can germinate without $GA_3$ but are enhanced by chilling (at 5°C temperatures). Application of BAP is also a simulative factor for dormancy breaking.

## CONCLUSION

From this study, it can be suggested that medium supplemented with BAP (0.25 mg $L^{-1}$) and without any nutrient can enhance dormancy breaking in Asafoetida seeds. $GA_3$ does not have sufficient effect on Asafoetida seed germination. Chilling can remove dormancy and enhances germination. With 28 days chilling and use of a medium containing 0.25 mg $L^{-1}$ BAP we could obtain more than 90% germination in seeds of Asafoetida. Longer chilling and higher concentrations of BAP may increase germination even further.

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