

## The Study Effects of Stratification, Temperature and Potassium Nitrate on Seed Dormancy Breaking *Ferula assa-foetida*

<sup>1</sup>A. Raisi, <sup>2</sup>S.M. Nabavi Kalat and <sup>2</sup>A.R. Sohani Darban

<sup>1</sup>Seed Science and Technology, Faculty of Agriculture,  
Mashhad Branch, Islamic Azad University, Mashhad Iran

<sup>2</sup>Department of Agronomy and Plant Breeding, Faculty of Agriculture,  
Mashhad Branch, Islamic Azad University, Mashhad Iran

**Abstract:** Assafotida or Anghouzeh (in Persian) (*Ferula assa-foetida*), is one of the medicinal plant belong to *Apiaceae* family, which is a perennial and herbaceous plant, native to Iran and parts of Afghanistan. This plant is one of the most important species among the 30 species of *Ferula* genus. The seeds of many species of *Apiaceae*, have different degrees of dormancy, which is a serious problem for geowing these plants. So, in order to seed dormancy breaking a factorial experiment laid out in completely randomized design with 3 replications conducted in Seed Technology Laboratory, Faculty of Agriculture, Islamic Azad University, Mashhad Branch, in 2012. Factors including: stratification in 4 levels (control, 30, 60, 90 days), temperature in 2 levels (8 and 10°C) and potassium nitrate in 4 levels (0.1, 0.2, 0.3 and 0.4%). Analysis of variance showed that the potassium nitrate, stratification and temperature had significant effects on germination percentage and germination rate in 1% probability and highest germination percentage (76%) and germination rate (27.8 seedling/ day) obtained in interaction of potassium nitrate (0.2%), temperature (8°C) and stratification (60 days).

**Key word:** Assafotida • Percentage germination • Rate germination • Stratification

### INTRODUCTION

Assafotida or Anghouzeh (in Persian) (*Ferula assa-foetida*), is one of the medicinal plant belong to *Apiaceae* family, which is a perennial and herbaceous plant, native to Iran and parts of Afghanistan [1-2]. This plant is one of the most important species among 30 species of *Ferula* genus which have been distributed in Iran grow wild in warm, dry and calcareous grasslands. Assafotida is an oleo- gum resin obtained by cutting roots [3-5]. It has a strong stem and, at least during first five growth years, generates rosette leaves which are placed on the ground. A straight and cylindrical stem grows from among its leaves. Stem height reaches 2 to 2.5 m. Inflorescence is compound umbel. This plant is monocarpic, so that only once flowering during the growth period (depending on local climatic conditions, after 6 to 10 years). The fruit is Schizocarp (two achenes). Double achenes are separated from each other when they are ripe and each one turns into a seed and fall [6-8].

Assafotida has anticonvulsant, hypnotic and antiworm effects. It is used for neural-origin respiratory system disease, Throat spasm, asthma and constipation relief in the elderly. In terms of worm excretion, it affects some types of worms like pinworm and *Lubricus*. Asafetida has also wide application in veterinary [5, 9].

Seeds of most *Apiaceae* species have different degrees dormancy, which is a serious issue in cultivating these plants. They germinate in native environments and fail to germinate in laboratory or field conditions [10, 11].

According to ISTA, seed dormancy of many *Apiaceae* species, is internal primary dormancy which is of physiological type [12]. Depending on the type of plant, to break the seeds physiological dormancy, they should be stratified, exposed to alternating temperatures or treated potassium nitrate or gibberlic acid. Baskin *et al* [13] stated in several reports that various species of *Osmorhiza*, *Erythronium*, *Thaspium pinnatifidum* and *Ferula gummosa* of *Apiaceae* family had different degrees of physiological dormancy, which can be broken after

applying appropriate stratification [12-7]. Cavieves *et al.* [14] investigated dormancy break of *Phacelia seunda* seed and demonstrated that one period of stratification treatment could increase germination of the seed [15]. In many garden plants, potassium nitrate breaks seed dormancy and increases healthy seedlings. According to the studies by Bewley and Black [16], potassium nitrate increased the level of oxygen by decreasing the oxygen available for citric acid cycle [17].

Therefore the aim this research was to investigate the effect of stratification, potassium nitrate and temperature on breaking the dormancy of *Assafotida* seeds.

## MATERIAL AND METHODS

This research was conducted in seed technology laboratory, Faculty of Agriculture, Islamic Azad University, Mashhad Branch, in 2012. The experiment arranged in factorial laid out in completely randomized design with three replications. Factors including: stratification in 4 levels (control, 30, 60 and 90 days), temperature in 2 levels (8 and 10°C) and Potassium nitrate in 4 levels (0.1, 0.2, 0.3 and 0.4 Percentage). For stratification treatments, seeds maintained on a wet towel at 4 °C for the desired time periods. 25 seeds in each Petri dish were placed. Then 3 ml of potassium nitrate solution was added to each Petri dish. All Petri dishes were placed in germinator at 8 and 10°C. Germination was continued for 30 days and germinated seeds were counted on a daily. Seeds were considered germinated when their radical length was 2 mm. At the end experiment indexes such as: germination percentage and germination rate was measured. Statistical analysis was carried out using MSTAT-C. Mean comparison was performed with Duncan's multiple range test at the 5% level of significance.

## RESULTS AND DISCUSSION

Analysis of variance shown in Table 1. Evaluation of stratification effect on traits showed that the highest germination percentage (61%) and highest germination rate (20 seedling/ day) obtained in 60 day stratification, that indicated percentage and rate germination increased 57% and 20% respectively comparison with control (Table 2). This results are consistent with reports Rahnama *et al.* [15] and Nadjafi *et al.* [18] on *Ferula gummosa*. Razavi *et al.* [19] reported that 10 weeks of stratification at 5 °C resulted in embryo growth and increases the germination percentage in *Prangos ferulaceae*.

Table 1: Analysis of variance ( M.S)

S.O.V	D.F	Germination percentage	Germination rate
Potassium nitrate (A)	3	115/32 **	748 **
Temperature (B)	1	117 **	294 **
(A*B) interaction	3	55/27 **	42 <sup>ns</sup>
Stratification (C)	3	1827/28 **	14254/66 **
A*C interaction	9	69/89 **	88/29*
(B*C) interaction	3	56/35 **	126/44 *
(A*B*C) interaction	9	66/63 **	131/63 **
Error	64	3/74	42/66

ns not significant, \* and \*\* significant at 5% and 1% respectively.

Table 2: Stratification effect on seed germination percentage and germination rate

Stratification (day)	Germination percentage	Germination rate
0	4d	0.76d
30	36b	8.03b
60	61a	20.3a
90	18c	3c

Means with same letters in each column are not significantly different at 5% probability

Table 3: Potassium nitrate effect on seed germination percentage and germination rate

Potassium nitrate (%)	Germination percentage	Germination rate
0.1	31b	9.7a
0.2	37a	7.9b
0.3	27c	9.4a
0.4	24c	4.9c

Means with same letters in each column are not significantly different at 5% probability

Probably stratification causes an increase in gibberellic acid and reduces abscisic acid in seeds. Gibberellic acid is transferred to the Aleurone layer and cause to activate several enzymes. One of these enzymes is amylase. Amylase that break down sugars and starches and provides the energy needed for the embryo [20]. So can stimulate germination.

potassium nitrate effects on traits showed that the highest germination percentage (37%) obtained at potassium nitrate 0.2% and the highest germination rate (9.7 seedling/day) obtained in potassium nitrate 0.1% [Table 3]. Hormonal balance and reduced seed growth inhibitors such as Absciscic acid, is one of the reasons for the positive effects of the chemical stimulants such as potassium nitrate. Chemical stimulants cause physiological seed dormancy breaking [21].

Table 4: Temperature effect on seed germination percentage and germination rate

Temperature (°C)	Germination percentage	Germination rate
10	28b	6.6b
8	32a	9.3a

Means with same letters in each column are not significantly different at 5% probability

Table 5: Interaction Potassium nitrate \* germination temperature on germination rate

Temperature (°C)	Potassium nitrat(%)			
	0.1	0.2	0.3	0.4
10	9.7a	5.2c	9.3a	2.3d
8	9.6a	10.7a	9.5a	7.5b

Means with same letters in each column are not significantly different at 5% probability

Table 6: Interaction Potassium nitrate \* stratification on seed germination percentage and germination rate

Stratification (day)	Potassium nitrat(%)							
	Germination percentage				Germination rate			
	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4
0	6hi	10hg	1i	1i	1gh	1/5gh	0/28h	0/16h
30	34c	47b	34c	31cd	8/1d	8/8d	9/4cd	5/7e
60	63a	69a	62a	49b	26/5a	17/4b	25/5a	11/5c
90	24de	22ef	13gh	15fg	3fg	4ef	2/6f-h	2/3f-h

Means with same letters in each column are not significantly different at 5% probability

Table 7: Interaction temperature \* stratification on seed germination percentage and

Temperature (°C)	Stratification (day)			
	0	30	60	90
10	5e	31c	59a	18d
8	4e	41b	62a	19d

Means with same letters in each column are not significantly different at 5% probability

Table 8: Interaction temperature \* potassium nitrate \* stratification on seed germination percentage and

Stratification (day)	Temperature							
	10				8			
	Potasium nitrat (%)							
	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4
0	8i-m	8i-m	1lm	2k-m	4j-m	12h-m	1lm	0/0m
30	21f-h	52bc	26ef	26ef	46cd	42cd	41cd	36de
60	62b	62b	61b	52bc	64b	76a	64b	46cd
90	22f-h	20f-i	13g-l	16f-j	25e-g	25eg	13g-m	14f-k

Means with same letters in each column are not significantly different at 5% probability

Evaluation of temperature effect on percentage and rate of germination showed that the highest germination percentage (32%) and germination rate (9.3 seedling/ day) obtained in temperature (8°C) (Table 4).

The mean comparison of germination rate (interactions between potassium nitrate and temperature) showed that the lowest rate obtained of potassium nitrate (0.4%) and temperature (10°C) (Table 5).

Evaluation of interaction between stratification and potassium nitrate on percentage and rate of germination showed that the highest percentage germination (69%) obtained at stratification (60 days) and potassium nitrate (0.2%). However, had no significant difference with stratification (60 day) and potassium nitrate (0.1 and 0.3%) (Table 6).

The highest germination rate obtained at stratification (60 day) and potassium nitrate (0.1 and 0.3%) respectively 26 and 25.5 seedling/ day. The results showed that stratification was an effective factor in the breaking of seed dormancy of this plant and potassium nitrate had resonance effect. Because potassium nitrate concentrations alone and in control treatment (without stratification) had not positive effect. Reports of Centebass *et al.* [22] on cherry seeds, Stidham *et al.* [23] on 18 species of shrubs and Ruhi *et al.* [17] on *Tulipa kaufmanniana Regel* is consistent with our results.

The mean comparison of germination (interactions between temperature and stratification) showed that the highest percentage germination (62%) obtained in temperature (8°C) and stratification (60 days). However, had no significant difference with stratification (60days) and temperature (10°C) (Table 7). Evaluation of interaction between potassium nitrate, germination temperature and stratification showed that the highest germination percentage (76%) and germination rate (28 seedling/day) obtained in potassium nitrate (0.2%), temperature (8°C) and stratification (60 days) (Table 8).

Table 9: Interaction temperature\* potassium nitrate \* stratification on seed germination rate

Stratification (day)	Temperqtur							
	10				8			
	Potasium nitrat (%)							
	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4
0	1.3j-g	1.2g-j	0.19ij	0.32f-j	0.8j-h	1.6g-j	0.3h-j	0/0j
30	6.6f-d	9d	8.9d	3.2f-j	9.6d	8.5d	9.8d	8d
60	28.8a	7d-e	27ab	3.9e-i	24b	27.8a	24b	19c
90	2j-g	3.4f-j	1.2g-j	1.7g-j	3.9e-i	4.7e-g	4e-h	2.8g-j

Means with same letters in each column are not significantly different at 5% probability

## REFERENCES

1. Abd El-Razek, M.H., S. Ohta, A.A. Ahmed and T. Hirata, 2001. Sesquiterpene coumarins from the roots of *Ferula assa foetida*. *Phytochemistry*, 58: 1289-1295.
2. Ghassemi Dehkordi, N., S.A. Sajjadi, A.R. Gannadi, Y. Amnzadeh, M. Azadbakht, G.R. Asghari, G.R. Amin, A. Haji Akhondi and A.M. Taleb, 2003. Iranian Herbal Pharmacopoeia. *Medicine*, 6(3): 69-63. (In Persian).
3. Khosravi, H. and A.A. mhraby, 2005. Economic study of *Ferula* harvisting in Tabass rigion. *Natural Resources*, pp: 58-4. (In Persian).
4. Mellati, F., M. Parsa and B. Llah Gani, 2010. Evalution of germination treatment and sowing date on *Dorema*, *Asafotida* and *Galbanum*. *Journal of agricultural research*, pp: 4-8. (In Persian).
5. Hassani, B., A. Saboor, T. Radjabian and H. Fallah Hussini, 2008. Somatic Embryogenes is of *Ferula assa-foetida*. *Iran. JSUT*, 33(4): 15-23.
6. Omid Beygi, R., 2009. Production and processing medicinal plants. Vol 2. Publications Razav., 2(4). (In Persian).
7. Otroshy, M., A. Zamani, M. Khodambashi, M. Ebrahimi and P.C. Struik, 2009. Effect of exogenous hormones and chilling on dormancy breaking of seeds of *Asafoetida* (*Ferula assafoetida* L.). *Research journal of seed Science*, 2(1): 9-15.
8. Daniel, M., 2006. Medicinal plant: chemistry and properties. Science publishers, USA.
9. Zargari, A., 1996. Medicinal plants. Tehran University Press. Edition, 6: 592-595. (In Persian).
10. Zare, A.R., M. Solouki, M. Omid, N. Irvani, A. Oladad Abasabadi and N. Mahdi Neza, 2011. Effect of various theatments on seed germination and dormancy breaking in *Ferula assa foetida* L. (*Asafetida*), a threatened medicinal herb. *Trakia journal of sciences*. ity Press. Edition, 6: 592-595.
11. Gupta, V., 2003. Seed germination and dormancy breaking techniques for indigenous medicinal and aromatic plants. *Journal of Medicinal and Aromatic Plants Science*, 25: 402-407.
12. Amooaghaie, R., 2006. The effect of soaking, temperature and duration of prechilling on seed dormancy breaking of *Ferule ovina*. *Journal of Biology*, 18: 350-359. (In Persian).
13. Baskin, C.C. and J.M. Baskin, 1999. Seed ecology, dormancy and germination. *Amodern synthesis*. *American Journal of Botany*, 86: 903-905.
14. Cavieres, L.A and M.T.K. Arroyo, 2000. Seed germination response to cold stratification period and thermal regime in *phacelia secunda* (*Hydrophyllaceae*). *Plant Ecologi.*, 149: 1-8.
15. Rahnama-Ghahfarokhi, A. and R.T. Afshari, 2007. Methods for Dormancy Breaking and Germination of *Galbanum* Seeds(*Ferula gummosa*). *Asian Journal of Plant Sciences*, 6(4): 611-616.
16. Bewley, J.D. and M. Black, 1994. Seeds physiology of development and germination. Plenum press, New York.
17. Rouhi, H.R., K. Shakarami and R. Tavakkol Afshri, 2010. Seed treatments to overcome dormancy of waterlily tulip(*Tulipa kaufmanniana* Regel). *Australian Journal of Crop Science*, 4(9): 718-721.
18. Nadjafi, F., M. Bannayan, L. Tabrizi and M. Rastgoo, 2006. Seed Germination and Dormancy Breaking Techniques for *Ferula gummosa* and *Teucrium Polium*. *Journal of arid Environments*, 64: 542-547.

19. Razavi, S.M. and R. Hajiboland, 2009. Dormancy breaking and germination of *Prangos ferulaceae* seeds. *EurAsian Journal of Biosciences*, 3: 78-83.
20. Copeland, L.O. and M.B. McDonald. 1995. *Principals of seed science and technology*. Third Edition. Chapman and Hall, New York, pp: 236.
21. Farhadi, M., M. Sharifani, H. Heshmatallah and A. Kohrokhi, 2006. Effect-coat seed and Stratification on the germination of the seed *Accr velutinum Boiss*. *Journal of Gorgan University of Agricultural Sciences and Natural Resources*, 13(2): 49-44.
22. Cetinbas, M. and F. Koyuncu, 2006. Improving Germination of *Prunus avium* L. Seeds by Gibberellic acid, Potassium nitrate and Thiourea. *Hort. SCI. (Prague)*, 33(3): 119-123.
23. Stidham, N.D., R.M. Ahring, J. Powell and P.L. Claypool, 1980. Chemical scarification moist prechilling and thiourea effects on germination at 18 shrub species. *Journal of Range Management*, 33: 115-118.