Journal of Medicinal Plants and By-products (2022) 1: 9-16

Original Article

Germination Responses of *Ferula assa-foetida* and *Ferula gummosa* Boiss. Seeds to Continuous Cold Stratification

Parvin Salehi Shanjani* **and Leila Falah Hoseini**

Natural Resources Gene Bank of Iran, Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization, Tehran, Iran

Article History: Received: 24 January 2021/Accepted in revised form: 23 February 2021

Abstract

Asafetida (*Ferula assa-foetida* L.) and galbanum (*Ferula gummosa* Boiss.), Apiaceae, are important endemic and endangered forage and medicinal plants of Iran. Survival of the species is threatened by climate change, overexploiting (as the source of oleo-gum resin and forage), and lack of cultivation management. Cultivation of these valuable plants is restricted by insufficient domestication knowledge. Germination characteristics of different accessions of *Ferula* taxa were studied to describe and compare their responses to continuous cold stratification conditions. Germination cues for the species were complex, with dormancy mechanisms present to restrict germination until cold stratification is fulfilled. Results indicated that a period of 4 weeks of stratification is sufficient for germination of asafetida, but optimal germination of galbanum requires stratification for periods of 8 weeks. Both species were able to germinate at very low temperatures (4 °C). These results indicated that *Ferula* seeds need to have a winter period of cold moist temperatures to break dormancy. Thus, it is concluded that fall and winter are the best times to sow the seeds. Within-taxon differences in dormancy breaking and seedling emergence may interpret as local adaptations. Pronounced differences occurred within both asafetida and galbanum, even though some studied sites in each taxon were adjacent. Variation within a taxon may depend on genetic differences, local weather during the growth of mother plants and seeds maturation, seed position on the mother plant, soil quality, or other naturally occurring factors.

Keywords: Asafetida, Forage, Galbanum, Medicinal plant

Introduction

Asafetida (*Ferula assa-foetida*) and galbanum (*F. gummosa*), Apiaceae, are important endemic and endangered medicinal plants. The taxa are monocarpic, herbaceous and perennials spread at altitudes of 1500-2500 m, with an average annual precipitation of 350-700 mm of Iran [1,2]. Recently, the survival of the species threatened by climate change, overexploiting (as a source of oleo-gum resin and forage), and lack of cultivation management. Cultivation of these valuable medicinal plants is restricted by edaphic and climatic factors, the low percentage of seed set and seasonal dormancy, and insufficient domestication knowledge [3].

Different dormancy breaking and germination stimulating treatments have been tried with seeds of many species of Apiaceae [4-7]. Results of different treatments including various levels of gibberellic acid, HNO3, chilling, and soaking with water at different temperatures showed that moist-chilling and gibberellic acid treatments seem the most promising methods for breaking dormancy of *Ferula* species. According to report of Nowruzian *et al*. [6] the best treatments for asafetida was moist-chilling for 4 weeks at 5 ± 1 °C or for 2 weeks of moist-chilling (at 5 ± 1 °C) followed by soaking GA3 (10 mg L-1) solution for 24 h. In a similar way treatment of moist-chilling for 6 weeks or 4 weeks followed by 500 ppm gibberellic acid is recommended for *F. ovina* [7]. Washing and chilling (5±1C) for 14 days was most effective in breaking dormancy in galbanum [5].

According to field observations of authors, cold stratification causes embryos to complete growth and germinate in the middle of winter in cold soil or covered with heavy snow. Shoots grow and emerge above the soil

*Corresponding author: Natural Resources Gene Bank of Iran, Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization, P.O. Box 13185-116, Tehran, Iran
Email Address: psalehi1@gmail.com

surface following increasing in temperature in early spring. Therefore, the first objective of the present work is the stimulation of the only natural treatment, cold stratification, and study of dormancy termination duration and seedling growth in asafetida, galbanum. Differences of the present work with earlier are in unlimited cold stratification duration for dormancy breaking and exposing moist chilling condition for seedling growth. Moreover, the study of differences and similarities among closely related taxa to increase understanding of adaptations and changes in seed dormancy and germination preferences. One difficulty when comparing seed dormancy and germination between taxa is the intra-taxon variation. Variation within a taxon may depend on genetic differences, local weather during the growth of mother plants and maturation of seeds, seed position on the mother plant, soil quality, or other naturally occurring factors. To be able to conclude a general level, for example for modeling or predicting changes in emergence patterns following climate change, knowledge about a taxon, including its variation, is needed. Therefore, for investigation of the impact of the habitat variability, germination characteristics among different accessions of asafetida and galbanum were studied under continuous moist chilling conditions. Information about germination can also improve the success rate of using the seed for rehabilitation, which is critical to the restoration of the high-altitude rangelands.

Material and Methods

Seed Materials

Seed material of 23 accessions of the asafetida (*F. assa-foetida*) and galbanum (*F. gummosa*) from all over Iran was provided from Natural Resources Gene Bank, Iran (Table 1).

Germination Methods

For each accession 150 seeds were sterilized with 70% ethyl alcohol for five minutes, and then washed with distilled water. Three replicates (50 seeds per replicate) of sterilized seed were placed in Petri dishes on double Whitman papers (TP). For protection against molds, the water used to moisten the seed samples and substrata contained 0.002% Binomial fungicide. The samples were immediately transferred into a germinator at 4±1 °C and 12/12 h light (600 lux)/dark for 60 days. Following germination, the percent and speed of germination were recorded every two days until the end of the experiment (two months). The length of roots and shoots of 10 randomly-selected seedlings from each replicate was measured in 60 day seedlings.

Table 1 Some details of the studied wild *F.* accessions

Type	Population	Abbreviation code	Accession code	Latitude (decimal)	Longitude (decimals)	Altitude (from sea level)	Mean annual precipitation (mm)
Asafetida	Bandar Abbas[1]	FaBandarA1	32209	28.17	56.83	2200	178
	Bandar Abbas[1]	FaBandarA2	31538	27.88	50.22	1845	179
	Haji Abad[1]	FaHajiAbad	31557	28.94	56.46	1900	179
	Boshroye[2]	FaBoshroye	38950	33.96	57.17	893	94
	Kashan[3]	FaKashan	35140	33.75	51.48	1800	137
	Kerman[4]	FaKerman	18333	30.09	57.76	2300	133
	Zarand[4]	FaZarand	18322	30.88	56.88	2300	47
	Lar[5]	FaLar	31038	27.46	54.39	2000	200
	Mehriz[6]	FaMehriz	33993	33.36	57.34	1565	84
	Tabas[6]	FaTabas1	33936	33.39	57.26	1536	56
	Tabas[6]	FaTabas2	33998	31.52	54.32	2090	56
	Taft[6]	FaTaft	39329	31.66	54.18	2122	60
	Elam[8]	FgEelam	21319	33.63	46.41	1000	575
	Haji Abad[1]	FgHajiAbad	34463	28.12	56.84	2200	178
	Lordegan[9]	FgLordegan	39659	31.42	51.26	2683	555
	Shahrod[10]	FgShahrod	30624	35.87	56.65	950	139
	Tabas[6]	FgTabas	33980	33.36	57.34	1565	84
	Taft[6]	FgTaft	39330	31.56	54.16	2439	60
	Dena[7]	FgDena	29823	30.50	51.72	2560	760
	Yasuj[7]	FgYasuj1	29819	30.48	51.79	2300	855
	Yasuj[7]	FgYasuj2	31218	31.94	51.44	1950	855
	Yasuj[7]	FgYasuj3	29822	30.45	51.65	2420	855
	Zarand[4]	FgZarand	18359	30.88	56.87	2400	47

[1] Hormozgan, [2] Khorasan, [3] Esfahan, [4] Kerman, [5] Fars, [6] Yazd, [7] Kohkeluye and Boyerahman, [8] Elam, [9] Charmahal Bakhtiali, [10] Semnan.

After measuring shoot and root lengths, the fresh seedling weight of each replicate was recorded. The seedlings were then placed in an oven at 80 °C for 24 hours, after which the dry weight of each replicate was recorded as a percentage of the fresh weight. The vigor indices and the germination percentage were estimated according to Abdul Baki and Anderson [8].

Data Analysis

Analyses of variance (ANOVA) were conducted for seed germination traits including dormancy termination duration (days), germination period (days), germination%, germination rate, germination index, seed vigor index, radicle length (mm), shoot length (mm), seedling length (mm), radicle/shoot length ratio, seedling fresh weight (mg), seedling dry weight (mg) and seedling dry matter%; and seed morphology traits including seed weight (g), seed length (mm), seed width (mm) and 1000 seeds weight (g) using the SAS9 software [9]. To assess the relationships among the 13 different traits Pearson's correlation coefficient was analyzed using statistical analysis system software [9]. The standardized morphological data were employed to calculate the Euclidean distances among the 23 genotypes of two *Ferula* species by NTSYS-pc version 2.1 [10]. Moreover, unweighted pair group methods of arithmetic mean (UPGMA) algorithm and SAHN clustering were also utilized to get the genetic relationships. The principal component analysis (PCA) of 23 Ferula genotypes was determined by Minitab software (version 15).

Results

Both asafetida and galbanum seeds failed to germinate without prior stratification. However, cold stratification stimulated the germination of both species. ANOVA suggested significant differences among wild populations of *Ferula* species for all the seed germination traits. A relatively high CV was obtained for the germination period, germination rate, seed vigor index, seedling fresh weight, and seedling dry weight; moderate to low values of CV were obtained for the remaining traits (Table 2, 3). Comparison of means verified that the duration of dormancy termination was significantly longer in galbanum (ranged from 31-51 days, with an average of 42 days) than asafetida (ranged from 12-28 days, with an average of 19 days) (Table 2, 3). Different accessions of asafetida had a significantly higher germination period, germination%, germination rate, germination index, seed vigor index, and radicle length values.

In the asafetida, the highest germination characteristics (germination percentage, rate, and index) were obtained in the population Tabas1 and the highest seedling parameters (radicle and shoot length, and seedling fresh weight) were obtained in the accessions Taft and Zarand; however, the seedling dry matter percentage for the Taft and Zarand showed the lowest values (Table 2).

In the galbanum, the highest germination characteristics (germination percent, rate, and index) were obtained in the accessions Lordegan and Taft, and the highest seedling parameters (radicle and shoot length, and seedling fresh weight and seedling dry matter percent) were obtained in the population Yasuj2 (Table 3). Accessions Yasuj1, 2, 3 of the galbanum, with closely similar habitats and geographical ranges, were found to have markedly different dormancy and germination characters (Table 3). Variation within a taxon may depend on genetic differences, local weather during the growth of mother plants and maturation of seeds, seed position on the mother plant, soil quality, or other naturally occurring factors.

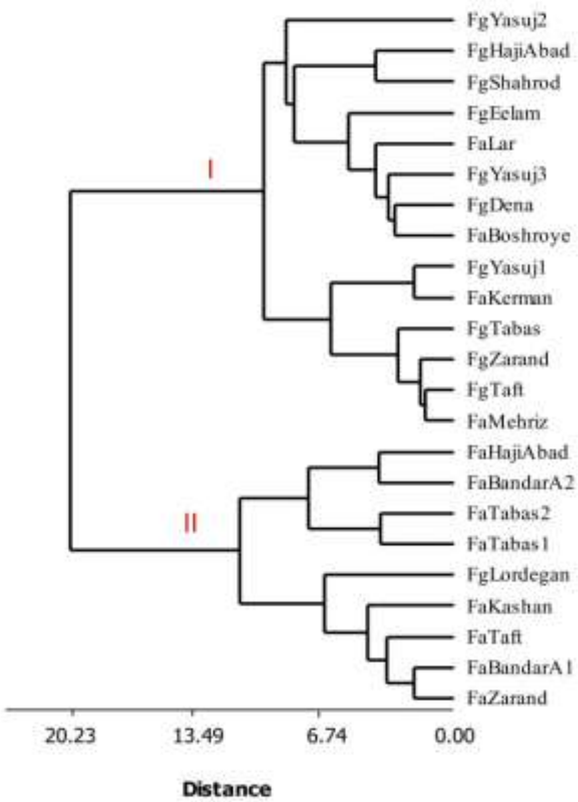

Fig. 1 Dendrograms of the 23 accessions of asafetida (with prefix Fa) and galbanum (with prefix Fg) under continuous cold stratification based on studied traits

Pearson's correlation indicated that dormancy termination duration, as the most important trait, was negatively and significantly correlated with important germination characters including germination percentage ($r=-0.49$, $p = 0.05$), germination rate ($r =-0.83$, $p=0.01$), germination index ($r=-0.79$, $p=0.01$) and seedling vigor index ($r=-0.45$, $p=0.05$). Germination percentage exhibited a positive and significant correlation with germination rate ($r = 0.69$, $p = 0.01$), germination index ($r=0.89$, $p=0.01$), and seedling vigor index ($r=0.78$, $p = 0.01$).

The correlation analysis indicated that the annual precipitation had a positive correlation with dormancy termination duration (r=0.61, p=0.01); but showed a negative correlation with the germination percentage (r = -0.47, p=0.05), germination rate (r=-0.60, p=0.01), and germination index (r=-0.60, p=0.01). The seedling fresh weight had a positive correlation with altitude (r = 0.59, p = 0.01). The above correlations implied that the annual precipitation plays an important role in influencing the dormancy and germination traits of *Ferul*a taxa.

The Euclidean distances matrix was subjected to agglomerative hierarchical clustering utilizing the UPGMA method to construct a dendrogram (Fig. 1). 23 accessions of *Ferula* taxa were classified into two main groups. Cluster I consisted of 10 accessions of galbanum and 4 accessions of asafetida; cluster II included eight accessions of asafetida and only one population of galbanum (Fig. 1). Comparison of means of two clusters indicated that accessions in cluster I have significantly higher dormancy termination duration, germination period, and seed weight, however, accessions cluster II showed higher germination percentage, germination rate, germination index, seed vigor index, and seedling length (Table 4). UPGMA trees of germination morphological characters partially separated the two species, a behavior also supported by the PCA plot (Fig. 2). However, almost within each species cluster, the accessions differed somewhat from each other and were joined together with different distances. Therefore, there was no obvious relationship between phenotypic traits and the origin of these *Ferula* accessions.

Table 2 Mean comparisons of seed germination characteristics of 12 accessions of asafetida under continuous cold stratification

Population	Dormancy termination duration (days)	Germination Period (days)	Germination %	Germination rate	Germination Index	Seed Vigor index	Radicle Length (mm)	Shoot Length (mm)	Seedling dry matter %
Bandar Abbas	14.33 d	10 a	50.67 cd	8.427 bc	478.2 cd	42.9 abc	28.13 a	46.45 abc	6.72 d
Bandar Abbas	16.33 cd	16.67 a	70.67 ab	6.3 cd	472.3 cd	32.4 a-d	16.33 abc	29.73 cd	5.7 d
Boshroye	23 ab	10.67 a	26.67 de	2.797 ed	220.4 de	14.94 dc	18.52 abc	37.95 bcd	11.84 b
Haji Abad	13.67 d	9.333 a	46.67 cd	7.337 bc	433.6 cd	17.28 bcd	11.17 c	29.49 cd	5.8 d
Kashan	21.67 bc	20.67 a	66.67 ab	7.95 bc	562.7 bc	46.78 ba	13.2 bc	55.03 ab	9.49 c
Kerman	28.33 a	20.67 a	58.67 ab	4.273 cd	390.6 cd	33.57 a-d	23.43 abc	34.07 bcd	8.4 c
Lar	28.33 a	20.67 a	20 e	1.343 e	122.9 e	6.51 d	11.92 c	19.36 d	16.57 a
Mehriz	26.33 ab	16 a	62.67 ab	5.86 cd	481.8 cd	26.45 a-d	14.2 bc	28.23 cd	6.2 d
Tabas	13 d	10 a	86.67 a	15.19 a	824.5 a	39.8 abc	16.9 abc	27.57 cd	9.83 c
Tabas	12.33 d	6.667 b	80 ab	14.43 a	771.6 ab	42.93 abc	21.1 abc	31 cd	8.15 c
Taft	12.33 d	8 b	62.67 ab	11.63 ab	607.1 ab	53.4 a	22.4 abc	60.97 a	6.49 d
Zarand	17 cd	13.33 a	53.33 bc	7.817 bc	486.7 cd	46.41 abc	25.73 ab	61.03 a	6.07 d
Mean	18.89	13.56	57.11	7.78	487.71	33.61	18.59	38.41	15.35
Cv	16.93	45.19	26.36	36.0	29.1	48.27	37.6	30.2	14.36

Different letters indicate significant differences among different accessions for the same species. P <0.05.

Table 3 Mean comparisons of seed germination characteristics of 11 accessions of galbanum under continuous cold stratification

Population	Dormancy termination duration (days)	Germination Period (days)	Germination %	Germination rate	Germination Index	Seed Vigor index	Radicle Length (mm)	Shoot Length (mm)	Seedling dry matter %
Dena	45 bcd	7.333 cb	20 c	0.763 e	91.77 d	13.28 ef	22.99 cb	42.69 b	11.53 bc
Elam	45 bcd	8.667 cb	30.67 c	1.133 de	138.5 d	12.98 ef	8.817 d	36.44 c	9.33 cd
Haji Abad	48.33 bc	4 c	69.33 ab	2.347 cd	295.8 c	29.25 cd	13.87 cd	28.4 c	4.35 f
Lordegan	38.33 de	11.33 cb	78.67 a	3.94 ab	439.1 ab	69.25 a	23.13 cb	66.33 a	11.11 cd
Shahrod	51.67 ab	4.667 c	29.33 c	0.823 e	106.7 d	13.03 ef	14.2 cd	28.59 c	11.97 cb
Tabas	35 e	9.333 cb	65.33 ab	4.11 ab	417.2 ab	33.72 cb	15.8 bc	36.03 c	7.257 e
Taft	32.33 e	14 b	72 ab	5.08 a	489.6 a	25.48 cd	11.6 d	24.13 c	7.51 e
Yasuj	33 e	22.67 a	70.67 ab	2.767 bc	322.4 bc	43.42 b	25.2 b	36.27 c	8.977 de
Yasuj	56.33 a	2.667 c	16 c	0.253 e	36.04 d	16.93 de	42.1 a	62.19 a	31.03 a
Yasuj	43 cd	10 cb	18.67 c	0.8 e	93.37 d	6.267 f	9.667 d	35.81 c	14.2 b
Zarand	31.67 e	14.67 b	56 b	3.577 bc	359.6 ab	26.12 cde	13.8 cd	32.43 cb	7.893 e
Mean	41.79	9.94	47.88	2.33	253.63	26.34	18.29	39.03	11.38
Cv	8.72	45.04	24.69	32.93	28.13	28.83	30.16	17.17	13.34

Different letters indicate significant differences among different accessions for the same species. P <0.05.

Table 4 Mean comparisons of seed germination characteristics of accessions that separated in two clusters of Fig. 1

Group	I	II
Dormancy termination duration (days)	36.85 a	17.28 b
Germination period (days)	12.40 a	11.67 a
Germination%	46.53 b	66.70 a
Germination rate	2.78 b	9.38 a
Germination Index	274.18 b	570.14 a
Seed vigor index	22.80 b	42.43 a
Radicle length (mm)	17.55 a	19.45 a
Shoot length (mm)	34.14 b	43.71 a
Seedling length (mm)	51.69 b	63.15 a
Radicle/shoot length ratio	0.52 a	0.48 a
Seedling fresh weight (mg)	23.18 b	29.41 a
Seedling dry weight (mg)	2.38 a	3.23 a
Seedling dry matter%	10.78 b	18.04 a

Different letters indicate significant differences among different accessions for the same species. P <0.05.

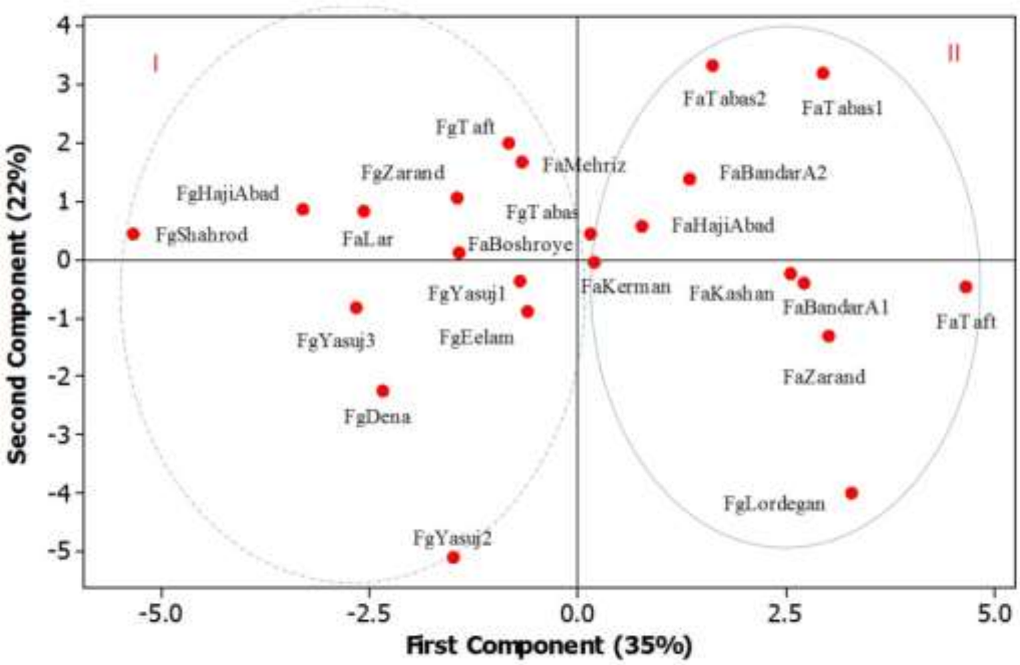

Fig. 2 Scatter diagram of the 23 accessions of asafetida (with prefix Fa) and galbanum (with prefix Fg) under continuous cold stratification based on studied traits.

PCA analysis of seed germination and morphological data revealed that the first four components comprise about 77% of the total variance. The first component accounted for 34.4% of the total variation in the data set while the second and third principal components contributed 21.2% and 14.4%, respectively. Together, these three components could explain 68% of the total variation in the characterized *Ferula* accessions. Analysis of the factor loadings of the characters in the retained PCs indicated that any of seed germination and morphological traits showed positive loadings in PC 1-3.

Discussion

Germination cues for asafetida and galbanum were complex, with dormancy mechanisms present to restrict germination until cold stratification or other requirements are fulfilled [5-7]. The existence of morpho-physiological dormancy (MPD) is very frequent in the Apiaceae [4, 11]. The cold stratification temperature used in this experiment (4 °C) provides an adequate moist chilling treatment. The temperature is also within the range of soil temperatures likely to be encountered in the field in high altitude Iran [12-15]. This cold stratification temperature has been reported as successful in breaking dormancy in studies of alpine and high altitude species [16].

Results indicated that the duration of dormancy termination was significantly longer in galbanum than asafetida. A period of 4 weeks of stratification is sufficient for germination of asafetida, but galbanum requires cold stratification for periods of 8 weeks for optimal germination. The final germination percentage of Ferula taxa at the present study was higher than the previous experiences [5-7], in which Ferula seeds transferred to standard germination condition following limited cold stratification treatment. Sommerville *et al.* [17] by studying several species of Australian Alps suggested species requiring stratification for periods of 8 weeks or more for optimal germination may be particularly sensitive to climate change. High altitude ecosystems are considered to be among the most sensitive to climate changes [18], and recent declines in average snow depth have been observed in alpine and high altitude areas in both the Northern and Southern Hemispheres [19-21]. For species in apiaceous depend on cold moist conditions (moist stratification) to break dormancy; reduced snow cover during winter may threaten the survival of these species, even if subsequent temperatures are suitable for germination [22]. Although the seed of some species may be able to tolerate winter temperatures in the absence of snow, a reduction in snow cover may also mean a reduction in the amount of available water (in total precipitation in winter and spring). As the level of seed hydration plays a role in breaking seed dormancy [16,23,24], relative drought during winter and spring may prove to be more important in limiting the germination of these species than the lack of snow cover per se [22]. Results of this research also indicated a significant correlation between precipitation and germination traits.

Both species were able to germinate at very low temperatures (4 °C). The ability to germinate at very low temperatures has been observed in several high altitude species [17]. The ability of germination at low temperatures may provide an advantage during a short growing season by allowing germination to begin under snowbanks [25]. *Aciphylla glacialis* (Apiaceae) germinated optimally at low temperatures, similar to the Asian and North American *Osmorhiza* species [25, 26] in the same family (Apiaceae). Cold stratification response having similar effects to high altitude and alpine species: improving final germination, widening the range of temperatures for germination, decreasing germination time, and synchronizing germination by reducing variability in time to germination [27].

The study species were highly variable in their dormancy and germination response to the moist chilling treatment. Variation of the dormancy termination duration parameter was significant among different accessions of each species; ranging from 31 to 51 days in the galbanum, and 12 to 28 days in the asafetida. Seed dormancy is a highly complex trait and is largely influenced by genetic and environmental factors. Dormancy intensity depends on age, nutritive conditions, and water supply of the plant, as well as the weather conditions during seed ripening [28]. Ecological factors, such as temperature, humidity, oxygen, and light, greatly influence the seed's dormancy period interruption, and there is a significant distinction of causes of dormancy discontinuance among species [29-33]. In concordance with the researches, significant correlations were found between germination characteristics (including dormancy termination duration) and precipitation.

The germination responses of asafetida and galbanum seeds were significantly affected by the origin of accessions. Several studies have been published attempts to explain the interrelation of the germination responses of populations of a particular species collected in different parts of their habitat. Haasis and Thrupp (34) and Skordilis and Thanos (35) working with coniferous species, and McNaughton (36) with *Typha* species all reported variations in the germination of different ecotypes. Lauer (37), on the other hand, failed to distinguish notable differences between populations of *Agrostemma githago* and *Datura stramoniam* collected in various locations in Europe. The variety of observed responses to germination is expected, as high altitude environments exhibit significant Spatio-temporal variability [27,38,39]. Even within a particular habitat, germination responses are unlikely to be consistent. For each species, germination is likely to vary between altitudes and populations. Variability in germination is an important strategy to ensure species survival in unpredictable environments, reducing the risk of exposing the seedlings to poor growing conditions [40-42]. For example, in the genus *Penstemon*, Meyer (43) reported that response to chilling was related to the probable chilling duration at the collection site. Populations from habitats with severe winters produced seeds with long chilling requirements, while those from habitats with mild winters produced seeds with short chilling requirements.

Conclusions

Cold stratification is the main prerequisite for breaking deep complex dormancy in asafetida and galbanum. A period of 4 weeks of stratification is sufficient for germination of asafetida, but galbanum requires stratification for periods of 8 weeks for optimal germination. Both species were able to germinate at very low temperatures (4 °C). These results indicated that *Ferula* seeds need to have a winter period of cold moist temperatures to break dormancy. Thus, it is concluded that fall and winter are the best times to sow the seeds. The characteristics of deep morpho-physiological dormancy in the taxa are part of the plant's adaptation to its environment. Highly significant intraspecific population differences in the germination parameters of the taxa might reflect local adaptation to a particular environment. Pronounced differences occurred within both asafetida and galbanum, even though some studied sites in each taxon were adjacent. Variation within a taxon may depend on genetic differences, local weather during the growth of mother plants and seeds maturation, seed position on the mother plant, soil quality, or other naturally occurring factors. To be able to conclude a general level, for example for modeling or predicting changes in emergence patterns following climate change, knowledge about a taxon, including its variation, is needed. Therefore, studies of germination behavior should include several populations from the same species.

Acknowledgments

This work was supported by the Agricultural Research, Education and Extension Organization, and Research Institute of Forests and Rangelands (RIFR), Iran; Project no. 14-09-09-9354-93198.

Authors' Contributions

PSS planned and directed the study, analysed the data and interpreted of results, and drafted the manuscript. LFH was responsible for the production of data. All authors read and approved the final manuscript.

References

1. Mozaffarian V. A dictionary of Iranian plant names: Latin, English, Persian: Farhang Mo'aser. 1996;505.
2. Amiri MS, Joharchi MR. Ethnobotanical knowledge of Apiaceae family in Iran: A review. Avicenna J Phytomed. 2016;6:621-635.
3. Golmohammadi F, Ghoreyshi SE, Parvaneh H. *Ferula assa-foetida* as a main medical plant in east of Iran (harvesting, main characteristics and economical importance). Inter J Farming Allied Sci. 2016;5-6:453-475.
4. Baskin CC, Milberg P, Andersson L, Baskin JM. Deep complex morphophysiological dormancy in seeds of *Anthriscus sylvestris* (Apiaceae). Flora. 2000;195:245-251.
5. Nadjafi F, Bannayan M, Tabrizi L, Rastgoo M. Seed germination and dormancy breaking techniques for *Ferula gummosa* and *Teucrium polium*. J Arid Environ. 2006;64:542-547.
6. Nowruzian A, Masoumian M, Ebrahimi M, Bakhshi Khaniki G. Effect of Breaking Dormancy Treatments on Germination of *Ferula assafoetida* L. Iran. J Seed Res. 2016;3:155-169.
7. Fasih M, Tavakkol Afshari R. The morphophysiological dormancy of *Ferula ovina* seeds is alleviated by low temperature and hydrogen peroxide. Seed Sci Res. 2018;28:52-62.
8. Abdul Baki AA, and Anderson JD. Vigor determinations in soybean seed multiple criteria. Crop Sci. 1973;13:630-633.
9. SAS Institute. SAS InstituteSAS⁄Stat user's guide, Version 9.1 SAS Institute, Cary, NC, USA. 2001.
10. Rohlf F. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System (2.1 Ed.), Department of Ecology and Evolution, State University of NY, Stony Brook. 2002.
11. Yaqoob U, Nawchoo IA. Conservation and cultivation of *Ferula jaeschkeana* Vatke: a species with deep complex morphophysiological dormancy. Proceedings of the National Academy of Sciences, India Section B: Biological. 2015;1-11.
12. Tabari H, Talaee PH. Analysis of trends in temperature data in arid and semi-arid regions of Iran. Global and Planetary Change. 2011;79:1-10.
13. Ghasemi AR. Changes and trends in maximum, minimum and mean temperature series in Iran. Atmospheric Sci Letters. 2015;16:366-372.
14. Aghajanlou F, Ghorbani A. A study on the effects of some environmental factors on the distribution of *Ferula gummosa* and *Ferula ovina* in Shilander mountainous rangelands of Zanjan, Iran. J Rangeland. 2016;9:407-419. [In Persian].
15. Shirvani A, Moradi-Choghamarani F, Zand-Parsa S, Moosavi AA. Analysis of long-term trends in air and soil temperature in a semi-arid region in Iran. Environmental Earth Science. 2018;77:173-177.
16. Baskin C, Baskin JM. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego: Elsevier/Academic Press. 2014.
17. Sommerville KD, Martyn AJ, Offord CA. Can seed characteristics or species distribution be used to predict the stratification requirements of herbs in the Australian Alps? Botanical J the Linnean Society. 2013;172:187-204.
18. Laurance WF, Dell B, Turton SM, Lawes MJ, Hutley LB, McCallum H, Dale P, Bird M, Hardy G, Prideaux G, Gawne B, McMahon CR, Yu R, Hero JM, Schwarzkopf L, Krockenberger A, Douglas M, Silvester E, Mahony M, Vella K, Saikia U, Wahren CH, Xu Z, Smith B, Cocklin C. The 10 Australian ecosystems most vulnerable to tipping points. Biological Conservation. 2011;144:1472-1480.
19. Hughes L. Climate change and Australia: trends, projections and impacts. Australian Ecololgy, 2003; 28: 423-443.
20. Nicholls N. Climate variability, climate change and the Australian snow season. Australian Meteorological Magazine. 2005;54:177-185.
21. Amiri MJ, Eslamian S. Investigation of Climate Change in Iran. J Environmental Science Technology. 2010;3:208-216.
22. Liu K, Baskin JM, Baskin CC, Bu H, Liu M, Liu W, Du G. Effect of storage conditions on germination of seeds of 489

species from high elevation grasslands of the eastern Tibet Plateau and some implications for climate change. American J Botany. 2011;98:12-19.

23. Hoyle GL, Daws MI, Steadman KJ, Adkins SW. Mimicking a semi-arid tropical environment achieves dormancy alleviation for seeds of Australian native Goodeniaceae and Asteraceae. Annals Botany. 2008;101:701-708.
24. Walck JL, Hidayati SN, Dixon KW, Thompson K, Poschlod P. Climate change and plant regeneration from seed. Global Change Biology. 2011;17:2145-2161.
25. Walck JL, Hidayati SN. Germination ecophysiology of the western North American species *Osmorhiza depauperata* (Apiaceae): implications of preadaptation and phylogenetic niche conservatism in seed dormancy evolution. Seed Sci Res. 2004;14:387-394.
26. Walck JL, Hidayati SN, Okagami N. Seed germination ecophysiology of the Asian species *Osmorhiza aristata* (Apiaceae): comparison with its North American congeners and implications for evolution of types of dormancy. American J Botany. 2002;89:829-835.
27. Shimono Y, Kudo G. Comparisons of germination traits of alpine plants between fellfield and snowbed habitats. Ecological Res. 2005;20:189-197.
28. Andersson L, Milberg P. Variation in seed dormancy among mother plants, populations and years of seed collection. Seed Science Research. 1998;8:29-38.
29. Podrug A, Gadžo D, Muminović Š, Grahić J, Srebrović E, and Đikić M. Dormancy and germination of johnsongrass seed (*Sorghum halepense* L.). Herbologia. 2014;14:1-10. doi: 10.5644/Herb.14.2.01.
30. Mahmoudi J, Mahdavi SKh, Mansouri B. Examination of effect of topography (elevation and aspect) on distribution of medicinal plant *Ferula gummosa* case study: rangelands of Khombi and Saraii Germeh city in Khorasan Shomali Province. Bull. Environ. Pharmacol. Life Science. 2015;4:108-113.
31. Mazangi A, Ejtehadi H, Mirshamsi O, Ghassemzadeh F, Hosseinian Yousefkhani SS. Effects of climate change on the distribution of endemic *Ferula xylorhachis* Rech.f. (Apiaceae: Scandiceae) in Iran: Predictions from ecological niche models. Russian J Ecology. 2016;47:349-354.
32. Mirzaei Mossivand A, Ghorbani A, Zare Chahoki MA, Keivan Behjou F, Sefidi K. Compare the environment factors affecting the distribution of species *Prangos ferulacea* and *Prangos pabularia* in rangelands of Ardabil Province. Iranian J Range Desert Res. 2018;25:200-210. (In Persian).
33. Aghajanlou F, Ghorbani A, Zare Chahoki, MA, Hashemi Majd K, Mostafazadeh R. The impact of environmental factors on distribution of *Ferula ovina* (Boiss.) Boiss. in northwest Iran. Applied Ecology Environ Res. 2018;16:977-992.
34. Haasis FW, Thrupp AC Temperature relations of lodgepole pine seed germination. Ecology. 1931;12:728-744.
35. Skordilis A, Thanos C. Seed stratification and germination strategy in the Mediterranean pines *Pinus brutia* and *P. halepensis*. Seed Sci Res. 1995;5:151-160.
36. McNaughton SJ. Ecotype functions in the *Typha* community-type. Ecological Monographs. 1966;36:297-325.
37. Lauer E. Über die Keimtemperatur von Ackerun-kräutern und deren Einfluß auf die Zusammensetzung von Unkrautgesellschaften. Flora. 1953;140:551-595.
38. Noroozi J, Akhani H, Breckle S. W. Biodiversity and phytogeography of the alpine flora of Iran. Biodiversity and Conservatio*n*. 2013;17:493-521.
39. Noroozi J, Dietmar M, Franz E. Diversity, distribution, ecology and description rates of alpine endemic plant species from Iranian mountains. Alpine Botany. 2015;126:1-9.
40. Giménez-Benavides L, Escudero A, Pérez-Garcia F. Seed germination of high mountain Mediterranean species: altitudinal, interpopulation and interannual variability. Ecological Research. 2005;20:433-444.
41. Venn SE. Plant recruitment across alpine summits in south-eastern Australia. DPhil Thesis, LaTrobe University. 2007.
42. Mondoni A, Probert R, Rossi G, Hay F, Bonomi C. Habitat-correlated seed germination behavior in populations of wood anemone (*Anemone nemerosa* L.) from northern Italy. Seed Sci Res. 2008;18:213-222.
43. Meyer SE, Kitchen SG, Carlson SL. Seed germination timing patterns in intermountain *Penstemon* (Scrophulariaceae). American J Botany. 1995;82:377-389.