JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2023, VOL. 6(3), 235-246

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Physical, plant growth regulators and TiO_2 nanoparticles priming treatments to improve seed germination of endangered asafoetida (*Ferula assafoetida* L.)

Amir Saadatfar[1], Effat Ahmadi Mousavi[2], Iraj Tavassolian[3*]

[1]Department of Medicinal Plants, Research and Technology Institute of Plant Production (RTIPP), Shahid Bahonar University of Kerman, Kerman, Iran.
[2]Department of Biology, Faculty of Science, Shahid Bahonar University of Kerman, Kerman, Iran.
[3]Department of Horticultural Science and Landscape Design, Shirvan Faculty of Agriculture, University of Bojnord, Bojnord, Iran

ARTICLE INFO

Original Article

Article history:
Received 27 January 2023
Revised 17 April 2023
Accepted 22 April 2023
Available online 29 May 2023

Keywords:
Carrageenan
Cold treatment
Germination rate
Gibberellin
Seed dormancy

DOI: 10.22077/jhpr.2023.6064.1309
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Department of Horticultural Science and Landscape Design, Shirvan Faculty of Agriculture, University of Bojnord, Bojnord, Iran.

Email: iraj.tavassolian@ub.ac.ir

ABSTRACT

Purpose: *Ferula assafoetida* (L.) is one of the most important medicinal plants with many applications in food, pharmaceutical and cosmetic industries. It has been endangered due to overharvesting from natural habitat and long period of seed dormancy. Knowledge of seed germination behavior leads to the development of its conservation and cultivation. **Research methods:** We conducted this research as a factorial experiment in Completely Randomized Design (CRD) to evaluate seed germination in response to low temperature, plant growth regulators (kinetin, gibberellin, carrageenan as plant bio-stimulant) and TiO_2 nanoparticles (TiO_2 NPs). The germination percentage and rate, mean germination time, and radicle elongation were measured. **Findings:** The results showed that the cold (4 °C), GA_3, carrageenan, kinetin and TiO_2 NPs increased seeds germination rate and percentage. Maximum seed germination percentage (86% or 23% more than control) and minimum mean germination time (26 days or 12.6 days shorter than control) obtained with seeds pretreated by kinetin soaking and TiO_2 NPs treatment at 4 °C. Furthermore, most treatments produced healthier and stronger radicles compared to the control which is vital for better establishment and growth. **Research limitations:** No limitations were found. **Originality/Value:** The price and demand of asafoetida products have been increased dramatically. The most important constrain to hinder reliable supply of the products is the shortage of plant or difficulty to access its products. Here, we showed the cost effective and environmentally friendly methods to provide high seeds germination with vigorous roots.

INTRODUCTION

Asafoetida (*Ferula assafoetida* L.) is a valuable perennial herbaceous medicinal plant indigenous to Iran, Tajikistan and Afghanistan. The main compound of the plant is an oleo-gum resin, called asafoetida (Anghozeh- in Persian), which is obtained by incision of the roots (Sadraei et al., 2003). Asafoetida has several medicinal properties such as antispasmodic, digestive, expectorant, sedative, laxative, analgesic (Abd El-Razek et al., 2001). Asafoetida has been widely used as spice or in traditional medicine mainly in west, central Asia and India. This plant belongs to the Apiaceae family with sweet and bitter types. Oleo gum resin is extracted from 4-7-year-old plants before flowering. Its chemical compositions contain 40-64% resin, 25% gum and 10-17% essential oil (Amalraj & Gopi, 2016). Demand for asafoetida has been increased due to the public acceptance of herbal medicines and its effective properties. The native habitats are being quickly diminished by human activities. Thus, there is an urgent need to develop the conservation strategies to restore its habitat and potential cultivation (Abd El-Razek et al., 2001). Asafoetida in its habitat propagates by dispersing its seeds through wind. This endemic plant is also endangered due to the weak seed germination. Seed germination of asafoetida is poor both in the native arid regions and under laboratory conditions (Nadjafi et al., 2006). The regeneration of this plant is possible only by seed, therefore, studies of breaking seed dormancy by chemical and physical factors are paramount importance.

The effects of prechilling at 4 °C for 10 to 90 days and gibberellic acid at 200 to 800 ppm and the combination of both treatments on *Ferula asafoetida* and *Ferula gummosa* seeds dormancy breaking was investigated. The results indicated that the higher the time of exposure to cold treatment and the higher concentration of GA_3, the better seed germination percent and rate, radicle length and plumule and seed vigor are become. The combination of cold for 70 days with GA_3 at 400 ppm was the best treatment (Sharifi et al., 2017). The effects of different temperature at 10, 15, 20 °C, gibberellic acid at 1000 ppm and kinetin on seed dormancy breaking of asafoetida seeds showed that pretreatment with kinetin at 250 ppm and 10 °C significantly improved seed germination parameters such as germination percentage and decreased germination time compared to the control (Malek et al., 2022). Plant growth regulators are found to play an important role in the germination process. It was reported that 0.1 µL of 24-epibrassinolide significantly enhanced gladiolus corm germination up to 300% compared to the control (Mollaei et al., 2018). It has been stated that other plant growth regulators, particularly those with cytokinin activity can be effective on breaking seed dormancy (Kabar, 1998). Carrageenans are the major polysaccharides exist in Rhodophyta red algae that may improve seed germination (Gonzàlez, 2013). It has been reported that TiO_2 NPs application can improve seed germination rate and percentage (Zheng et al., 2005; Castiglione et al., 2011). It is imperative to investigate the effects of natural and synthetic products on seed germination of each specific plant such as asafoetida.

The aims of this study were to evaluate the effects of low temperature, GA, kinetin, carrageenan and TiO_2 NPs treatments on asafoetida seed germination. Furthermore, to optimize the most effective combination of treatments suitable for seed germination of this rare plant.

MATERIALS AND METHODS

Seed collection and analysis

The seeds of Asafoetida [*Ferula assafoetida* (L.)] were collected from five healthy and robust mature eight-year-old plants in natural habitat which were labeled and separated by the fence. The asafoetida herbal parts and seeds that were collected from the natural habitats were taken to the Department of Botany at Shahid Bahonar University of Kerman, Iran and the samples were identified and confirmed by herbarium specialist. The seeds were mixed and randomly selected from composite samples for the experiments. The site is located at 25 km west of Chatrod city, Kerman province, Iran (between eastern longitudes of 30° 38´ to 30° 39´ and northern latitudes of 55° 0´ to 55° 2´) (Fig. 1). The experiments were conducted at the Faculty of Agriculture, Shahid Bahonar University of Kerman, Iran during 2021 for 6 months.

The seeds were stored in small cotton bag at room temperature (23 ± 2 °C) for six months before the experiments. To overcome seed dormancy, several treatments have been used: Temperature [4 °C (cold temperature), and -17 °C (freezing temperature)], plant growth regulators [Kinetin (5 mg L^{-1}, Duchefa Biochemie, Netherland), gibberellic acid 1500 mg L^{-1}, Merck, Germany) and kappa-carrageenan (0.5 g L^{-1}, Sigma-Aldrich, Germany) and TiO_2 NPs (10 mg L^{-1}, Sharif Co., Iran)].

All seeds were immersed in water and the blank seeds floated on top of the water were separated before the start of any treatments. Seeds were sterilized with 70% ethanol for two min and then washed with sterilized distilled water three times. The seeds were divided into two groups: The first group was soaked in 50 ml sterilized distilled water and placed in the refrigerator, which was replaced every 5 hours with fresh distilled water. The second group was soaked in 50 ml sterilized kinetin solution and placed in the refrigerator and was replaced with fresh kinetin solution every 5 hours. The soaking and leaching of two groups of seeds continued for 72 hours and then seeds were transferred for the next stages of germination test. For each treatment, five replications and in each replication 12 seeds were used and covered by film sheet. Seeds without drying were placed on double layered Whatman No.1 filter paper moistened with 5 ml of sterilized treatment solution [Water (control), GA_3 (1500 mg L^{-1}), carrageenan (0.5 g L^{-1})] and TiO_2 NPs (10 mg L^{-1}) in sterilized Petri dishes for ten days (Table 1) and 40 days in refrigerator, then 40 days more for germination tests had been performed up to 93rd day.

Fig. 1. The asafoetida (Yellow plants) natural habitats, the hillsides around Chatrod city (Kerman province, Iran).

Table 1. Treatment designations and conditions for asafoetida seed dormancy breaking.

Treatment Codes	Soaking and Leaching Treatments (72 h)	Temperature Treatments (24 h)	Treatment Solution (9 days in 4 °C)
0CW	(0) Water	4 °C (Cold)	Water
0FW	(0) Water	-17 °C (Freezing)	Water
0FCg	(0) Water	-17 °C (Freezing)	Carrageenan
0FG	(0) Water	-17 °C (Freezing)	Gibberellic acid
0FTi	(0) Water	-17 °C (Freezing)	TiO_2 NPs
0CCg	(0) Water	4 °C (Cold)	Carrageenan
0CG	(0) Water	4 °C (Cold)	Gibberellic acid
0CTi	(0) Water	4 °C (Cold)	TiO_2 NPs
1FW	(1) Kinetin	-17 °C (Freezing)	Water
1FCg	(1) Kinetin	-17 °C (Freezing)	Carrageenan
1FG	(1) Kinetin	-17 °C (Freezing)	Gibberellic acid
1FTi	(1) Kinetin	-17 °C (Freezing)	TiO_2 NPs
1CW	(1) Kinetin	4 °C (Cold)	Water
1CCg	(1) Kinetin	4 °C (Cold)	Carrageenan
1CG	(1) Kinetin	4 °C (Cold)	Gibberellic acid
1CTi	(1) Kinetin	4 °C (Cold)	TiO_2 NPs

0: Soak-leaching in water, 1: Soak-leaching in kinetin, C: Cold stratification, Cg: Carrageenan, F: Freezing stratification, G: GA_3 and Ti: TiO_2 NPs treatments, W: Water (control)

Cold stratification

Seeds were kept at 4 °C (refrigerator) in Petri dish for ten days. Freezing stratification: Seeds were kept at -17 °C (freezer) in Petri dish for one day, and then thawed and the seeds were placed in a refrigerator for nine days. After this treatment (ten days), seeds were washed with sterilized water and transferred to a new Petri dish containing filter paper and sterilized distilled water and stored for 40 days in a refrigerator at 4 °C, filter papers were kept moist with sterilized distilled water during the experiments.

Germination analysis

Germinated seeds were counted every 24 h for 40 days. A seed was considered as germinated when the radicle tip had grown free of its coat (when the radicle showed at least 2 mm in length). Counting was continued until the cumulative value of the germinated seeds reached a constant level.

Germination percentage was calculated with the following formula (1) (Nadjafi et al., 2006).

$$GP = N/Nt \times 100 \qquad (1)$$

Where N = Number of germinated seeds, and Nt = Total number of seeds.

Mean germination time (MGT) was measured for seed germination of time period, after applying each treatment (2) (Ellis & Robert, 1981).

$$MGT = \Sigma n \times x/\Sigma n \qquad (2)$$

Where n is the number of seeds newly germinated at the day of x, x is the number of days from sowing. Σn is the total number of germinated seeds during the test.

Germination rate (GR) is the number of germinated seeds per day, which was determined according to Yang et al. (2007) (3).

$$GR = \sum_1^i ni/di \qquad (3)$$

Where ni is the number of germinated seeds after i days from the start of imbibition (di).

Statistical analysis

This research was carried out as a factorial experiment in Completely Randomized Design (CRD) with five replications. All experiments were conducted twice and the mean results of the experiments were presented. To detect significant differences among means, the data were statistically calculated according to the analysis of variance (ANOVA) by Duncan's multiple range test ($P \leq 0.05$) using the SPSS 23 software (IBM, Armonk, NY, USA). The results were shown as mean ± SE (standard error of the mean).

RESULTS AND DISCUSSION

Asafoetida seed germination percentage under different treatments showed positive correlations with germination rate. In other words, when germination rate of seeds increased, their germination percentage also significantly increased. Therefore, fast seed germination was associated with high germination percentage (Fig. 2).

We observed that the cold stratifications stimulated the germination of asafoetida seeds. The cold treatment was effective to overcome seed dormancy and germination percentage. In the control (0CW: soaking in water under 4 °C) these results were observed: seed germination percentage (63.3%), germination rate (no/day; 0.23), mean germination time (day; 38.5) and root elongation (cm; 3.3).

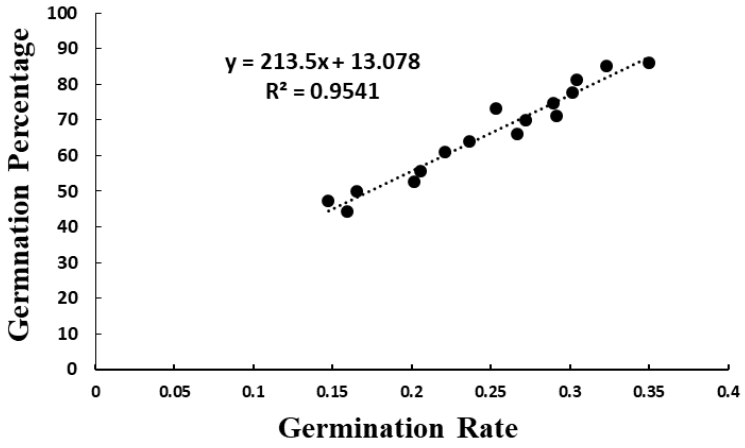

Fig. 2. Correlation between asafoe tida seed germination percentage and rate.

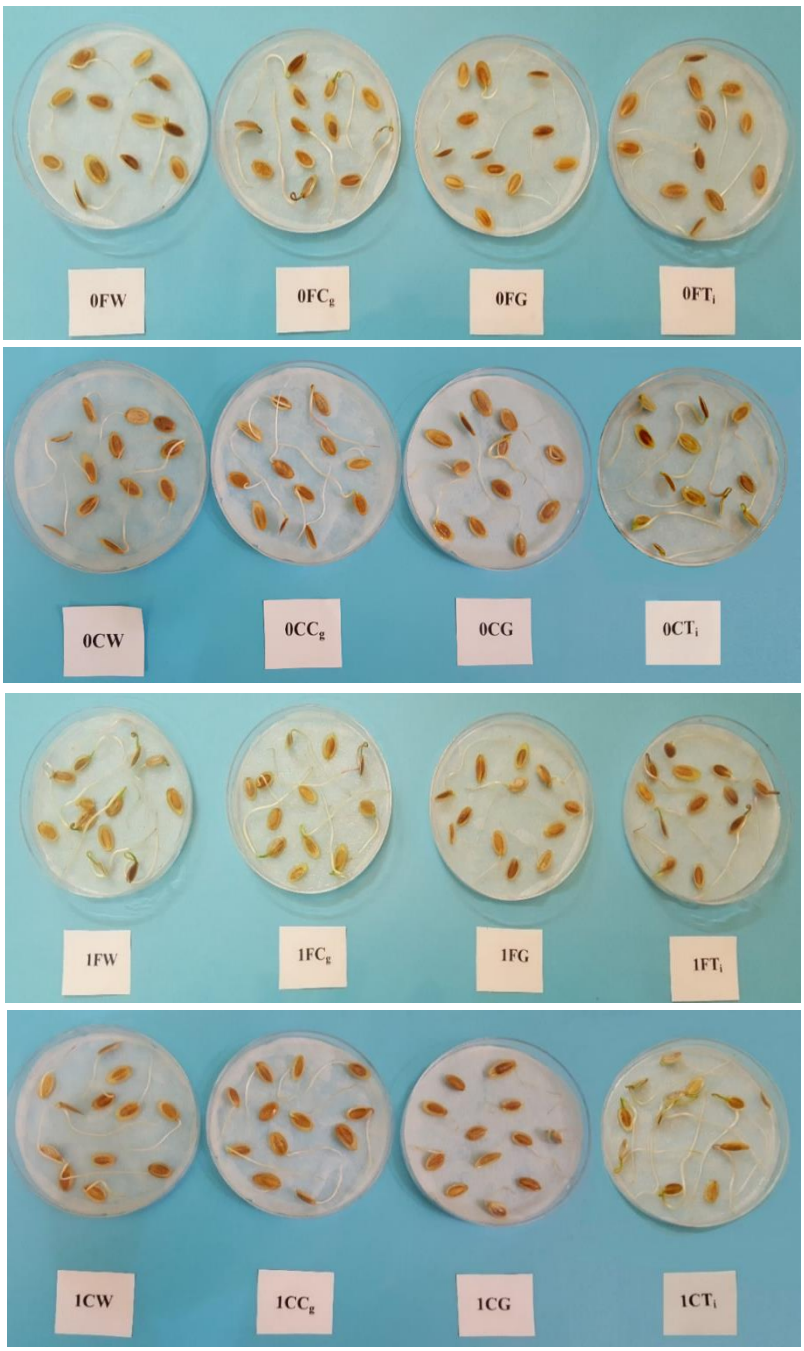

Fig. 3. Effect of seed germination treatments on radicle emergence of germinated seedlings.
Seed treatments are abbreviated as follows:
0: Soak-leaching in water, 1: Soak-leaching in kinetin, C: Cold stratification, Cg: Carrageenan,
F: Freezing stratification, G: GA_3 and Ti: TiO_2 NPs treatments, W: Water (control).

The seeds that were soaked in kinetin and exposed to cold temperatures improved germination percentage and rate (69.9% and 0.27, respectively), and reduced mean germination time (32.7 days, Table 2). In the present study, pre-treatment and soaking of the asafoetida seeds by kinetin solution (under freezing or cold treatments) increased root length (5.1 cm, Table 2). Results showed that soaking of the seeds in kinetin solution which were under freezing temperature did not improve seed germination percentage and rate notably although, the root growth significantly improved (63.9%, 0.24 and 5.5 cm, respectively).

Table 2. Seed germination of asafoetida under physical, plant growth regulators and TiO_2 NPs treatments.

Treatments	Germination percentage (%)	Germination rate (n/day)	Mean germination time (day)	Root elongation (cm)
0CW	63.3±2.3^{fg}	0.23±0.008^{fg}	38.5±1.02^{a}	3.3±0.09^{g}
0FW	44.4±3.9^{k}	0.16±0.001^{j}	32.3±0.57^{ef}	3.6±0.12^{g}
0FCg	52.8±3.9^{ij}	0.20±0.008^{i}	29.9±0.42^{g}	4.8+0.12^{d}
0FG	50±0.4^{ijk}	0.16±0.007^{j}	34.8±0.58^{bc}	4.4±0.15^{e}
0FTi	47.2±3.9^{jk}	0.15±0.016^{j}	38.1±0.66^{a}	4.0±0.14^{f}
0CCg	81.2±2.9^{abc}	0.30±0.003^{bc}	36.3±0.54^{b}	5.3±0.12^{bc}
0CG	71.2±3.8^{def}	0.29±0.01^{cd}	30.2±0.62^{g}	3.6±0.09^{g}
0CTi	77.8±3.9^{bcd}	0.30±0.008^{bc}	30.7±0.51^{fg}	5.3±0.14^{bc}
1FW	63.9±3.9^{fg}	0.24±0.16^{gh}	36.1±0.39^{b}	5.5±0.13^{ab}
1FCg	66.2±0.9^{efg}	0.27±0.12^{ef}	29.7±1.14^{g}	5.8±0.17^{a}
1FG	55.6±3.9^{hi}	0.21±0.15^{i}	34.4±1.2^{cd}	4.0±0.15^{f}
1FTi	61.1±3.9^{gh}	0.22±0.007^{hi}	29.8±0.85^{g}	4.8±0.13^{d}
1CW	69.9±4.9^{def}	0.27±0.005^{def}	32.7±1.2^{de}	4.1±0.1^{f}
1CCg	85±4.9^{ab}	0.32±0.005^{b}	31.2±1.2^{efg}	5.1±0.08^{cd}
1CG	74.7±4.5^{cd}	0.29±0.16^{cde}	32.6±0.87^{de}	2.8±0.09^{h}
1CTi	86.1±3.9^{a}	0.41±0.009^{a}	25.9±0.81^{h}	5.1±0.11^{cd}

0: Soak-leaching in water, **1**: Soak-leaching in kinetin, **C**: Cold stratification, **Cg**: Carrageenan, **F**: Freezing stratification, **G**: GA_3 and **Ti**: TiO_2 NPs treatments, **W**: Water (control).
Mean values ± standard error; different letters in the same column indicate significant differences among treatments ($p \leq 0.05$).

Seed germination and dormancy play important roles in plant reproduction. Seed germination is the process, in which physiological and morphological changes culminated in the activation of embryo. If the seed dormancy is broken, the expansion and development of embryo will occur successfully. The mechanism of seed germination is completed when the radicle has grown out of the seed coat (Hermann et al., 2007). Most of the medicinal plants suffer from low seed germination including those in Apiaceae family that possess morpho-physiological seed dormancy (Hassani et al., 2010). There are many environmental factors that may affect seed germination and dormancy: light, temperature, water, plant growth regulators, smoke, oxygen and carbon dioxide (Taiz et al., 2015). Many seeds respond to more than one environmental factor. Different dormancy breaking treatments can either substitute for each other or work in combinational treatments (Taiz et al., 2015). Asafoetida is originated in cold climate; therefore, stratification is helpful to break seed dormancy. In this study, we observed that the cold stratification improved the germination of asafoetida seeds. Temperature is the most important environmental factor for breaking seed dormancy among medicinal plant seeds from the temperate or cold regions (Baskin & Baskin, 1998). The balance between inhibitors and stimulators determines the status of embryo dormancy. The most important inhibitor of seeds germination is abscisic acid (ABA) (Taiz et al., 2015). Before seed to germinate, these inhibitors must be removed; therefore, seeds need to have enough moisture to wash out these inhibitors. Seed soaking and leaching treatment releases the inhibitors and improves germination. The action of low temperatures in breaking dormancy may be due to the reduction of inhibitors level, and/or the increasing level of stimulator hormones. Cold stratification of *Acer morrisonense* seeds for 12 weeks showed the substantial changes in biochemicals and ultrastructure traits of embryo. ABA content reduced up to 3.3-fold, proteins and lipids decreased significantly but total soluble sugars and amino acids increased markedly. Sucrose increased considerably during the stratification period. However, after radicle emergence it catabolized significantly. It was suggested that hydrolyzed lipids, sugars and oligopeptides and amino acids supply needed energy for cell division and seed germination. Large vacuoles formed in cotyledons and true leaves of germinated seeds indicated that the depleted lipid and protein bodies provide space to absorb more water in organelles for growth and development (Chen et al., 2015).

Freezing acts as the abiotic stress and probably produced chemicals such as osmolytes and oxidants that impede seed germination. Although we did not measure these compounds, it seems that freezing especially in combination with other treatments works as drought stress that led to the longer roots. The most important functions of plant growth regulators are controlling and coordinating cell division, growth, differentiation and dormancy breaking (Majumdar & Kar, 2021). Cytokinins are plant growth regulators that control wide range of plant processes including seed germination. They are active in all germination steps (Riefler et al., 2006). Asafoetida seed has a rudimentary embryo, cytokinins assist the growth and development of the embryo and therefore, the seeds were soaked in kinetin solution. The results showed that in most cases, soaking of asafoetida seed with kinetin solution under cold was more effective than freezing temperatures to enhanced seed germination rate and percentage. Also, seeds soaking in kinetin at cold temperatures significantly reduced mean germination time. Soaking and leaching of asafoetida seeds in kinetin solution improved root growth. Cytokinin regulates different processes related to the seed physiology and development such as the development of embryo by affecting the cell division, seed size and germination, radicle growth, hypocotyl and shoot growth, and minerals uptake (Heyl et al., 2012). Santner et al. (2009) reported that the cytokinins improve seed germination by increasing the activities of meristemic cells in both radicle and plumule.

In our study, it was observed that GA_3 treatment in water-soaked seeds under cold treatment, significantly increased germination percentage and decreased mean germination time (71.2% or 30.2 days). GA_3 treatment in the seeds that were soaked in water and were under freezing treatment decreased germination percentage and rate but increased root length (50%, 0.16 and 4.4 cm). GA_3 treatment in the seeds that were soaked in kinetin and were under freezing temperature did not increase percentage of seed germinations (55.6%, Table 2). In this study, GA_3 treatment in combination with cold and kinetin improved asafetida seed germination and rate but not with freezing treatment.

It has been shown that stratification causes an increase in internal GA concentration. GA stimulates hydrolytic enzyme activity and concentration (Apipinis et al., 2012). It probably induces respiratory systems contain citric acid cycle, glycolysis, and pentose phosphate pathway which provide enough ATP for germination process (Norastehnia et al., 2007). It was reported that GA_{4+7} at the concentration of 100 ppm and 500 ppm significantly increased seed germination and establishment of pennycress. Both treatments as soaking in GA or seed pelleting with GA showed very promising results compared to the control (Koirala et al., 2022). It seems that exogenous GA_3 application enhanced seed germination by inhibiting ABA activity. It is caused by the activation of hydrolase enzymes and inhibition of the related biosynthesis pathways for ABA, which decreases seed ABA content (Atia et al., 2009). Moreover, GA stimulates the production of proteins and catabolizing enzymes (hydrolases), especially amylase, glucanases and proteases resulting in seed germination (Atia et al., 2009, Kavandi et al., 2018; Yamaguchi, 2008). Proteins involve in the conversion of cell wall like expansins and xylo glucanendo trans glycosylase/hydrolases can improve embryo growth (Voegele et al., 2011). The study of the effects of GA and moist prechilling on asafoetida seed dormancy showed that the simultaneous application of GA and stratification significantly improved seed germination. However, GA treatment alone at 400 ppm failed to improve seed dormancy noticeably. It was suggested that although one function of cold treatment is to stimulate GA production, moist stratification must have other mechanisms irrelevant to GA increase in asafoetida for seed dormancy breaking (Sharifi et al., 2017).

In our experiments, carrageenan application on the asafoetida seeds that were exposed to cold treatment increased the germination percentage and rate (81.2% and 0.3). Also, carrageenan in seeds that were soaked in kinetin and were treated under cold temperature

significantly improved both the germination percentage and root elongation (85% and 5.1 cm, Table 2). Carrageenan in seeds that treated with freezing temperature and kinetin produced the longest roots (5.8 cm, Fig. 3). Carrageenan treatment increased root growth in almost all groups of the asafoetida seeds. In the seeds that were soaked in water and exposed to freezing temperature and carrageenan both the germination percentage and rate were low (52.8% and 0.2, respectively) but roots were longer compared to the control (4.4 cm). We observed that in seeds that were soaked in water and exposed to freezing temperature, carrageenan treatment was not significant.

The marine red algae oligosaccharide trigger seed germination by increasing the metabolic activity (Hu et al., 2004). The kappa, iota and lambda-carrageenan increased plants growth by enhancing photosynthesis, rubisco and glutamate dehydrogenase activity, which is involved in cell proliferation, nitrogen assimilation and basal metabolism (Vera et al., 2012). Ahmadi Mousavi et al. (2017 and 2018) reported that the application of kappa-carrageenan induced beneficial effects in plants such as increasing ions uptake, regulation of water absorption, growth and antioxidant enzyme activities.

TiO_2 NPs treatment (10 mg L^{-1}) on the asafoetida seeds that were soaked in water and were under cold temperature, increased germination percentage and rate (77.8% and 0.3, respectively; Table 2). Nevertheless, TiO_2 NPs treatment in the seeds that were soaked in water and under freezing temperature showed one of the lowest seed germination percentage and rate (47.2% and 0.15, respectively) (Table 2). TiO_2 NPs treatment of the seeds that were soaked and leached with kinetin solution and under freezing temperature did not significantly improved seed germination percentage, rate and mean germination time (61.1%, 0.22 and 29.8 days). The highest percentage of germination (86%) was observed in kinetin pre-treated seeds under cold and TiO_2 NPs. Moreover, the mean germination time of these seeds decreased up to 25 days. TiO_2 NPs treatment increased the root length of asafoetida seeds under all treatments (Table 2, Fig. 3).

TiO_2 NPs treatment stimulates seed germination and seedlings growth. Lu et al. (2002) showed that TiO_2 NPs could increase soybean (*Glycine max*) abilities to absorb water and fertilizers which accelerate the seed germination and growth (Lu et al., 2002). The engineered nanoparticles could sequester nutrients on their surfaces and serve as a nutrient stock to the organisms, particularly those with high specific surface area (Navarro et al., 2008). It has been stated that the germination rate of spinach (*Spinacia oleracea*) was enhanced with TiO_2 NPs treatments (Zheng et al., 2005). It seemed that TiO_2 NPs increase embryo growth, rubisco activity, chlorophyll formation, and the photosynthetic rate. It was reported that in treated *S. oleracea* by TiO_2 NPs, rubisco activity was 2.67 times higher than the control (Gao et al., 2006).

CONCLUSION

The results of this study showed that the seed of asafoetida is temperature dependent. To break seed dormancy in asafoetida, usually low temperature is used. Although, this technique is simple and cheap, it takes long time and produce weak roots. Here, we report the combinational treatments that are cost effective and need less time to improve seed germination. It is concluded that seed soaking in water or kinetin then carrageenan or TiO_2 NPs treatments under cold temperature at 4 °C were the most effective treatments to break asafoetida seed dormancy to improve seed germination percentage, rate and root growth. These treatments also produce stronger roots.

Conflict of interest

The authors declare no conflict of interest to report.

Acknowledgments

This study was supported by Research and Technology Institute of Plant Production (RTIPP) grant no. P/900/106.

REFERENCES

Abd El-Razek, M. H., Ohta, S., Ahmed, A. A., & Hirata, T. (2001). Sesquiterpene coumarins from the roots of *Ferula assa-foetida*. *Phytochemistry*, *58*, 1289-1295. https://doi.org/10.1016/S0031-9422(01)00324-7.

Ahmadi Mousavi, E., Nasibi, F., Manouchehri Kalantari, K., & Oloumi H. (2017). Stimulation effect of carrageenan on enzymatic defense system of sweet basil against *Cuscuta campestris* infection, *Journal of Plant Interactions*, *12*, 286-294. https://doi.org/10.1080/17429145.2017.1341560.

Ahmadi Mousavi, E., Manouchehri Kalantari, K., Nasibi, F., & Oloumi, H. (2018). Effects of carrageenan as elicitor to stimulate defense responses of basil against *Cuscuta campestris* Yunck. *Acta Botanica Croatia*, *1*, 62- 69. https://doi.org/10.2478/botcro-2018-0005.

Amalraj, A., & Gopi, S. (2016). Biological activities and medicinal properties of Asafoetida: A review. *Journal of Traditional and Complementary Medicine*, *7*(3), 347-359. http://doi.org/10.1016/j.jtcme.2016.11.004.

Atia, A., Debez, A., Barhoumi, Z., Smaoui, A., & Abdelly, C. (2009). ABA, GA_3, and nitrate may control seed germination of *Crithmum maritimum* Apiaceae under saline conditions. *Comptes Rendus Biologies*, *332*, 704-710. https://doi.org/10.1016/j.crvi.2009.03.009.

Baskin, C., & Baskin, J. (1998). Seed ecology, biogeography and evolution of dormancy and germination. New York: Academic Press.

Castiglione, M. R., Giorgetti, L., Geri, C., & Cremonini, R. (2011). The effects of nano-TiO_2 on seed germination, development and mitosis of root tip cells of *Vicia narbonensis* L. and *Zea mays* L. *Journal of Nanoparticle Research*, *13*, 2443-2449. https://doi.org/10.1007/s11051-010-0135-8.

Chen, S. Y., Chou, S. H., Tsai, C. C., Hsu, W. Y., Baskin, C. C., Baskin, J. M., Chien, C. T., & Kuo-Huang, L. L. (2015). Effects of moist cold stratification on germination, plant growth regulators, metabolites and embryo ultrastructure in seeds of *Acer morrisonense* (Sapindaceae), *Plant Physiology and Biochemistry*, *94*, 165-173. https://doi.org/10.1016/j.plaphy.2015.06.004.

Ellis, R. A., & Roberts, E. H. (1981). The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology*, *9*, 373-409.

Gao, F., Hong, F., Liu, C., Zheng, L., Su, M., Wu, X., Yang, F., Wu, C., & Yang, P. (2006). Mechanism of nano-anatase TiO_2 on promoting photosynthetic carbon reaction of spinach. *Biological Trace Element Research*, *111*, 239-253. https://doi.org/10.1385/BTER:111:1:239.

Gonzàlez, A., Castro, J., & Vera, J. (2013). Seaweed oligosaccharides stimulate plant growth by enhancing carbon and nitrogen assimilation, basal metabolism, and cell divition. *Journal of Plant Growth Regulation*, *32*, 443-448. https://doi.org/10.1007/s00344-012-9309-1.

Hassani, S. B., Saboora, A., Radjabian, T., & Fallah Husseini, H. (2010). Effects of Temperature, GA3 and cytokinins on breaking seed dormancy of *Ferula assa-foetida* L. *Iranian Journal of Science and Technology*, *33*, 75-85.

Hermann, K., Meinhard, J., Dobrev, P., Linkies, A., Pesek, B., Heb, B., Machackova, I., Fischer, U., & Leubner-Metzger, G. (2007). 1-Aminocyclopropane-1-carboxylic acid and abscisic acid during the germination of sugar beet (*Beta vulgaris* L.) - A comparative study of fruits and seeds. *Journal of Experimental Botany*, *58*, 3047–3060. https://doi.org/10.1093/jxb/erm162.

Heyl, A., Riefler, M., Romanov, G., & Schmulling, T. (2012). Properties, functions and evolution of cytokinin receptors. *European Journal of Cell Biology*, *91*, 246–256. https://doi.org/10.1016/j.ejcb.2011.02.009.

Hooley R. (1994). Gibberellins: perception, transduction and responses. *Plant Molecular Biology*, *26*, 1529-1555. https://doi.org/10.1007/BF00016489.

Hu, X. K., Jiang, X. L., Hwang, H. M., Liu, S. L., & Guan, H. S. (2004). Promotive effects of alginate-derived oligosaccharide on maize seed germination. *Journal of Applied Phycology*, *16*, 73-76.

Kabar, K. (1998). Comparative effects of kinetin, benzyladenine, and gibberellic acid on abscisic acid inhibited seed germination and seedling growth of Red Pine and Arbor Vitae. *Turkish Journal of Botany*, *22*, 1-6. https://journals.tubitak.gov.tr/botany/vol22/iss1/1.

Kavandi, A., Ashraf Jafari, A., & Jafarzadeh, M. (2018). Effect of seed priming on enhancement of seed germination and seedling growth of annual sainfoin (*Onobrychis crista-galli* (L.) Lam.) in medium and long-term collections of gene bank. *Journal of Rangeland Science*, *2*, 117-128. https://doi.org/10.22092/ijrdr.2018.117817.

Koirala, N., Barker, D., Helfer, C. A., Phippen, W. B., Heller, N., Hard, A. W., Wells, S., & Lindsey, A. J. (2022). A process to enhance germination of a wild pennycress variety. *Seed Science and Technology*, *50*(2), 195-205. https://doi.org/10.15258/sst.2022.50.2.03.

Lu, C. M., Zhang, C. Y., Wen, J. Q., & Tao, M. X. (2002). Research of the effect of nanometer on germination and growth enhancement of *Glycine max* and its mechanism. Soybean Science, 21, 168-172.

Majumdar, A., & Kar, R. K. (2021). Seed Germination: Explicit Crosstalk Between Hormones and ROS. In *Hormones and Plant Response* (Gupta, D.K., Corpas, F.J. (eds).). Plant in Challenging Environments, vol 2. Springer, Switzerland, 67-90. https://doi.org/10.1007/978-3-030-77477-6-3.

Malek, M., Hassani, F., Rezvani, E., Mahmoodi, V., & Khosravi, M. (2022). Optimal conditions determination for standard germination test of asafoetida (*Ferula assa-foetida)* seeds. *Iranian Journal of Seed Science and Technology*. *11*(5), 1-18. https://doi.org/10.22092/ijsst.2022.359018.1438.

Mollaei, S., Farahmand, H., & Tavassolian, I. (2018). The effects of 24-epibrassinolide corm priming and foliar spray on morphological, biochemical and postharvest traits of sword lily. *Horticulture, Environment, and Biotechnology*, *59*(3), 325-333. https://doi.org/10.1007/s13580-018-0033-z.

Nadjafi, F., Bannayan, M., Tabrizi, L., & Rastgoo, M. (2006). Seed germination and dormancy breaking techniques for *Ferula gummosa* and *Teucrium polium*. *Journal of Arid Environments*, *64*, 542-547. https://doi.org/10.1016/j.jaridenv.2005.06.009.

Navarro, E., Baun, A., Behra, R., Hartmann, N. B., Filser, J., Miao, A., Quigg, A., Santschi, P. H., & Sigg, L. (2008). Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology, 17, 372-386. https://doi.org/10.1007/s10646-008-0214-0.

Norastehnia, A., Sajedi, R., & Nojavan-Asghari, M. (2007). Inhibitory effects of methyl jasmonate on seed germination in maize (*Zea mays*): Effect on α-amylase activity and ethylene production. *General and Applied. Plant Physiology*, *33*, 13-23.

Pipinis, E., Milios, E., Kiamos, N., Mavrokordopoulou, O., & Smiris, P. (2012). Effects of stratification and pre-treatment with gibberellic acid on seed germination of two *Carpinus* Species. *Seed Science and Technology*, *40*, 21-31. https://doi.org/10.15258/sst.2012.40.1.03.

Riefler, M., Novak, O., Strnad, M., & Schmulling, T. (2006). *Arabidopsis* cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development, and cytokinin metabolism. *Plant Cell*, *18*, 40–54. https://doi.org/10.1105/tpc.105.037796

Sadraei, H., Ghannadi, A., & Malekshahi, K. (2003). Composition of the essential oil of *Ferula assafoetida* and its spasmolytic action. *Saudi Pharmaceutical Journal*, *11*, 136-140.

Santner, A., Calderon-Villalobos, L., & Estelle, M. (2009). Plant hormones are versatile chemical regulators of plant growth. *Nature Chemical Biology*, *5*, 301-307. https://doi.org/10.1038/nchembio.165.

Sharifi, H., Nemati, A., & Gerdakaneh, M. (2017). Breaking seed dormancy and improve germination of four medicinal species of Apiaceae under gibberellic acid and prechilling treatments. *Iranian Journal of Seed Science and Research*, *4*(3), 27-38. https://doi.org/10.22124/jms.2017.2505.

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). Plant Physiology. Massachusetts U.S.A.: Sinauer Associates.

Vera, J., Castro, J., Contreras, R. A., González, A., & Moenne, A. (2012). Oligo-carrageenans induce a long-term and broad-range protection against pathogens in tobacco plants (var. Xanthi). *Physiological and Molecular Plant Pathology*, *79*, 31-39. https://doi.org/10.1016/j.pmpp.2012.03.005.

Voegele, A., Linkies, A., Muller, K., & Leubner-Metzger, G. (2011). Members of the gibberellin receptor gene family *GID1* (GIBBERELLIN INSENSITIVE DWARF1) play distinct roles during *Lepidium sativum* and *Arabidopsis thaliana* seed germination. *Journal of Experimental Botany, 155,* 1851–1870. https://doi.org/10.1093/jxb/err214.

Yamaguchi, S. (2008). Gibberellin metabolism and its regulation. *Annual Review of Plant Biology, 59,* 225-251. https://doi.org/10.1146/annurev.arplant.59.032607.092804.

Yang, Q. H., Ye, W. H., & Yin, X. J. (2007). Dormancy and germination of *Areca triandra* seeds. *Scientia Horticulturae, 113,* 107-111. https://doi.org/10.1016/j.scienta.2007.01.028.

Zheng, L., Hong, F., Lu, S., & Liu, C. (2005). Effect of nano-TiO_2 on strength of naturally aged seeds and growth of spinach, *Biological Trace Elements Research, 105,* 83-91. https://doi.org/10.1385/BTER:104:1:083.