Appl Biochem Biotechnol (2016) 180:26–40
DOI 10.1007/s12010-016-2082-z

Seed germination of medicinal plant, fennel (*Foeniculum vulgare* Mill), as affected by different priming techniques

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Received: 29 January 2016 / Accepted: 7 April 2016 /
Published online: 14 April 2016

Abstract Reduced seed germination is among the most important factors adversely affecting crop stand and subsequent plant growth. Fennel (*Foeniculum vulgare* Mill) is an important medicinal plant with poor seed germination rate, occasionally. It is accordingly pertinent to find methods which can enhance fennel seed germination and remove the barriers of dormancy breaking. The present experiments studied the effects of two different priming (cold moist stratification and osmopriming) and 14 dormancy breaking techniques (hormonal, osmopriming, biopriming, chemical priming, and hydropriming) on the seed germination and seedling growth of two different fennel genotypes under growth chamber conditions. In the first and second experiment, the priming techniques including the time lengths of cold moist stratification (0, 15, 30, and 45 days) and the concentrations of polyethylene glycol 6000 (PEG_{6000}, osmopriming at −0.99, −1.35, and −2.33 MPa) were used as the main plots. However, in both experiments, the dormancy breaking techniques and fennel genotypes were factorially combined and used as the subplots. Different seed- and seedling-related parameters including germination (%), plumule, radicle and seedling length, average germination time, rate and homogeneity of germination, and seed vigor index were determined. Both priming techniques were efficient on the enhancement of seed germination and seedling growth. Among the dormancy breaking techniques, Aminol Forte (biopriming), kadostim (biopriming), benzyl adenine + kinetin (biopriming), distilled water (hydropriming), gibberellin + kinetin (hormonal priming), and benzyl adenine + kinetin + gibberellin (biopriming) were the most effective ones. The related concentrations were equal to 100 mg/l, 10^{-5} M, and 0.4 %. The fennel genotypes reacted significantly different under priming conditions. It is possible to

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enhance seed germination and seedling growth of fennel using priming and dormancy breaking techniques, which is useful for the increased production of fennel under different conditions. The results indicate that bio and hydropriming techniques were among the most effective ones, which significantly increased seed germination and seedling growth, and removed the seed dormancy barriers.

Keywords Priming techniques · Fennel (*Foeniculum vulgare* Mill) · Seed germination · Seedling growth

Introduction

Fennel (*Foeniculum vulgare* Mill) is a 2-year odor herb from the Apiaceae family, which may grow up to 2 m. The pimpernel fruit is small with an average width of 3 mm with odor smell and amiable taste [1]. Fennel is an important medicinal plant produced in different parts of the world including Europe, China, India, Iran, and Pakistan [2]. The plant can be used as an expectorant, carminative, stimulant, stomachic, antispasmodic, and diuretic. Among the most limiting factors which may delay fennel growth and yield production is the decreased rate of seed germination. Accordingly, it is pertinent to find the most suitable techniques, strategies, and methods that can increase fennel seed germination under different conditions including stress [3, 4].

Different parameters including seed genetics and physiology as well as stress can affect seed germination. Accordingly, it is possible to use different strategies including seed priming and dormancy breaking techniques to enhance seed germination under different conditions including stress. Such techniques and treatments may also importantly affect plant growth and physiology [5–8].

Seed priming, which is treating the seeds before germination using natural and synthetic compounds, results in the induction of physiological activities in seed. Seed priming induces physiological processes in plant including the activation of different signaling pathway under stress, and hence, enables the plant to react more quickly and efficiently under stress. Accordingly, plant cells become strengthened under stress. The plants, which are the result of primed seeds, have uniform and early seed germination and subsequent increased plant growth [9, 10].

With respect to the importance of fennel as a medicinal plant and due to its reduced rate of germination [11], we hypothesized that it is possible to enhance the rate of seed germination in fennel as well as the subsequent plant growth using a set of priming and dormancy breaking techniques including cold moist stratification, hormonal, and bio-, chemical, osmo- and hydropriming. It is important to find the most suitable technique to prime the seeds and to break the seed dormancy so that the highest rate of germination is resulted. The objective was to find the effects of different seed priming and dormancy breaking techniques on the seed germination and seedling growth of two different genotypes of fennel.

Materials and methods

The experiments

Two different experiments were conducted in the biotechnology laboratory of Islamic Azad University, Isfahan, Iran. In the first and in the second experiment, the length of cold moist

stratification and osmopriming (polyethylene glycol) were used as the main plots, respectively, and in both experiments, the combination of genotype and the seed dormancy breaking techniques including hormonal priming, biopriming, chemical priming, osmopriming, and hydropriming were used as the subplots.

The healthy seeds of similar size, which had been collected from the fennel fields in the cities of Nahavand and Malayer, Hamadan Province, Iran, in 2012, were selected for the experiments using a loop. The seeds were then sterilized using sodium hypochlorite 1.5 % for 1 min, and then were rinsed with distilled water several times. The experiments were conducted under sterilized conditions and the experimental tools were also sterilized using an autoclave and oven. The seed germinator used for the experiments was the model Light Control, by the Grouc Company, Iran. The germinator was first sterilized and then regulated for the light and dark period of 8 and 16 h, at the temperature of 30 and 20 °C, respectively, resembling the natural conditions during the season [12]. For each experiment, 25 seeds were placed on Whatman no. 5 and inserted in 8-cm Petri dishes.

Experiment 1

For the main experimental treatment, cold moist stratification was used at four levels including control, 15 days, 30 days, and 45 days of treatment. The seeds were first floated in distilled water for 24 h and were then placed in sterilized cotton bags, which had been moistened and placed in an isolated and dark place in the fridge, at 4 °C. After the period of moist cold stratification, the seeds were treated with the following 14 dormancy breaking treatments [13, 14]. The compounds and solvents were purchased from Sigma Company.

1. Gibberellic acid (100 mg/l) using 96 % ethanol as the solvent (hormonal priming)
2. Benzyl adenine (10^{-5} M) using NaOH 1 M as the solvent (biopriming)
3. Kinetin (10^{-5} M) using NaOH 1 M as the solvent (biopriming)
4. Gibberellic acid (100 mg/l) + Benzyl adenine (10^{-5} M) (hormonal priming)
5. Gibberellic acid (100 mg/l) + Kinetin (10^{-5} M) (hormonal priming)
6. Benzyl adenine (10^{-5} M) + Kinetin (10^{-5} M) (biopriming)
7. Benzyl adenine (10–5 M) + Kinetin (10^{-5} M) + Gibberellic acid (100 mg/l) (biopriming)
8. Concentrated sulfuric acid (90 %) for 15S (chemical priming)
9. Potassium nitrate (0.4 %) (osmopriming)
10. Distilled water as the control treatment (hydropriming)
11. Aminol Forte (0.4 %), growth stimulator (biopriming)
12. Kadostim (0.4 %), growth stimulator (biopriming)
13. Fosnutren (0.4 %), growth stimulator (biopriming)
14. Humiforte (0.4 %), growth stimulator (biopriming)

Experiment 2

Polyethylene glycol 6000 (PEG_{6000}) was used as the main plots and the interaction of genotype and dormancy breaking treatment was used as the subplots. The seeds were rinsed with distilled water, dried, and were then treated with the osmotic solutions of −0.99, −1.35, and −2.33 MPa (osmotic potential of PEG [15, 16]) at the temperature of 25 °C and then treated with the 14 abovementioned treatments. The osmotic solutions were prepared using the table of osmotic potentials and the essential

rate of product was solved in water and brought up to volume. For example, for the treatments of this experiment 25, 30, and 35 g of polyethylene glycol were dissolved in water with the final volume of 100 ml.

Method of sampling

In both experiments, the seeds were counted each 24 h to determine the percentage and rate of germination. The seeds were considered germinated when the length of radicle was at 2–3 mm. The germinated seeds were counted (until end of germination) and when the number of seeds was exactly similar for all treatments. Then, the following traits were determined:

1. Percentage of germination using the following equation [17], $G = (n/N) \times 100$ in which "G" is the percentage of germination, "n" is the number of germinated seeds, and "N" is the total number of seeds in each petri dish.
2. The average time of germination: it is an indicator of rate and acceleration of germination and is calculated using the following equation [18, 19]. MTG = $(D * N)/$, in which N is the number of germinated seeds at d^{th} day and D is the number of days from the beginning of germination.
3. Rate of germination: it is proportional to the inverse of the essential time for germination.

RG = 1 / MTG, in which "RG" is the rate of germination, and "MTG" is the average time, essential for germination.

4. The uniformity of germination: it is the essential time for the germination of 10–90 % of seeds [20] and is calculated using the following equation. The higher rate of germination is the indicator of higher homogeneity.

Seed homogeneity = $1/ (D - d) \times N/ N$ in which "D" is the number of days from the beginning of germination, "d" is the average number of days from the beginning of germination, "N" is the number of germinated seeds in each day, and "N" is the total number of germinated seeds.

5. Seed vigor index is calculated using the following equation:

Seed vigor index = (germination percentage × the average of radicle and plumule length) / 100

Statistical analyses

Data were analyzed using MSTATC. Means were compared using Duncan's multiple range test ($P = 0.05$).

Results

Experiment I

Analysis of variance indicated that stratification, dormancy breaking treatments, and genotype and the interaction of stratification and dormancy breaking treatment and genotype and

dormancy breaking treatments significantly affected germination percentage and the length of plumule, radicle, and seedling. However, the interaction of genotype and stratification just significantly affected seed germination. The interaction of stratification, dormancy breaking, and genotype treatments significantly affected different parameters with the exception of seedling length (Table 1).

Among the different stratification treatments, the 15-day treatment had the highest effect on seed germination, the length of plumule, radicle, and seedling significantly differing from the other treatments. Genotype Malayer was the more effective, significantly increasing germination rate and the length of plumule, radicle, and seedling, compared with the Nahavand genotype (Table 2).

Treatment Aminol Forte (29.25a) was the most effective treatment significantly increasing seed germination compared with the other dormancy breaking treatments, although not significant from distilled water (28.75a). Treatments benzyl adenine + kinetin (28.13a), kadostim (26.88ab), and Fosnutren (26abc) were also among the superior treatments. The highest plumule length was resulted by kadostim (2.523a), distilled water (2.513a), and benzyl adenine + kinetin + gibberellin (2.505a). Interestingly, distilled water (2.095a) was the most effective, significantly increasing the length of radicle followed by Aminol Forte (1.813ab). This was also the case for seedling length as distilled water resulted in the highest length (4.608a) followed by Aminol Forte (4.139ab), kadostim (4.005abc), and benzyl adenine + kinetin + gibberellin (3.926a–d) (Table 2).

Analysis of variance indicated that seed germination parameters including the average germination time, rate of germination, and vigor index were significantly affected by stratification; dormancy breaking treatments, the interaction of stratification, and dormancy breaking treatments; and the interaction of genotype, dormancy breaking treatment, and stratification. The effect of genotype was significant on the homogeneity of germination and seed vigor index. Rate of germination and seed vigor index were significantly affected by the interaction

Table 1 Analysis of variance indicating the effects of different experimental treatments on seed germination and seedling growth

S.V.	S.S.				
	d.f.	Germination (%)	Plumule length	Radicle length	Seedling length
S	3	14557.952[b]	158.901[b]	85.435[b]	475.404[b]
Error A	12	112.119	2.806	1.203	7.510
D	13	1161.978[b]	7.715[b]	5.135[b]	23.765[b]
S × D	39	358.850[b]	5.200[b]	3.266[b]	14.592[b]
G	1	41195.571[b]	33.431[b]	11.578[b]	85.741[b]
G × S	3	2532.238[b]	2.578	0.631	5.897
G × D	13	425.187[b]	3.178[a]	1.460[b]	8.046[b]
G × D × S	39	202.264[b]	2.294[a]	0.806[a]	4.480
Error B	324	92.551	1.508	0.546	3.231

s.v. source of variation, *S.S.* sum of squares, *d.f.* degree of freedom, *S* stratification, *D* dormancy breaking, *G* genotype

[a] Significant at 5 % level of probability

[b] Significant at 1% level of probability

Table 2 The effects of stratification, genotype, and seed dormancy breaking treatments on seed germination and seedling growth

Priming treatments	Germination (%)	Plumule length (cm)	Radicle length (cm)	Seedling length (cm)
Stratification (day)				
0	26.54b	2.436b	1.448b	3.884b
15	36.86a	3.343a	2.428a	5.771a
30	16.21c	1.612c	1.017c	2.616c
45	11.18d	0.5469d	0.3432d	0.8873d
Genotype				
Nahavand	13.11b	0.35a	1.148b	2.852b
Malayer	32.29a	0.47a	1.470a	3.727a
Seed dormancy breaking treatments				
Kinetin (10^{-5} M)	19.5de	1.673bc	1.033e	2.71e
Gibberellin (100 mg/l)	18.25e	1.741bc	1.098de	2.842e
Benzyl adenine (10^{-5} M)	20.75cde	1.599c	0.9997e	2.599e
Fosnutren (0.4 %)	26abc	2.196abc	1.385cde	3.582b–e
Humiforte (0.4 %)	24.38a–d	1.962abc	1.62bc	3.577b–e
Aminol Forte (0.4 %)	29.25a	2.366ab	1.813ab	4.139ab
Kadostim (0.4 %)	26.88ab	2.523a	1.482bcd	4.005abc
Potassium nitrate (0.4 %)	23.88a-d	1.856abc	1.087de	2.943de
Gibberellin + benzyl adenine	22b-e	1.868abc	1.216cde	3.084cde
Gibberellin + kinetin	22.13b–e	2.054abc	1.272cde	3.326b–e
Benzyl adenine + kinetin	28.13a	2.244abc	1.378cde	3.621b–e
Benzyl adenine + kinetin + gibberellin	22.38b–e	2.505a	1.421b–e	3.926a–d
Distilled water	28.75a	2.513a	2.095a	4.608a
Sulfuric acid (90 %)	5.5f	0.6819d	0.4209f	1.093f

Means in similar columns followed by similar letters are not significantly different using Duncan's multiple range test

of genotype and stratification. The interaction of genotype and dormancy breaking treatments significantly affected just the seed vigor index (Table 3).

There were no significant differences between the control treatment (8.919a) and the first level of stratification (8.262a) on seed germination although significantly higher than the other stratification treatments. The highest rate of seed germination was from the stratification treatment at 45 days (0.3103a), which is significantly higher than the other treatments. Although there were no significant differences among different treatments on the homogeneity of germination, the control treatment resulted in the highest rate of germination homogeneity. Stratification at 15 days resulted in the highest rate of seed vigor index being significantly higher than the other treatments and followed by the control treatment (Table 4).

There were no significant differences between the two genotypes; however, Nahavand (0.1231a) resulted in the higher homogeneity of germination significantly different from Malayer (0.04330b). The higher rate of seed vigor index was given by Malayer (1.661a) which is significantly different from Nahavand (0.5454b). The highest average germination time was related to gibberellin + kinetin (9.04a), followed by benzyl adenine + kinetin +

Table 3 Analysis of variance indicating the effects of different experimental treatments on different seed germination parameters

S.V.	d.f.	Average germination time	Rate of germination	Homogeneity of germination	Seed vigor index
S	3	622.11[b]	1.026[b]	0.106	115.829[b]
Error A	12	6.345	0.007	0.156	1.811
D	13	65.055[b]	0.026[b]	0.092	4.880[b]
S × D	39	17.747[b]	0.020[b]	0.110	2.800[b]
G	1	1.175	0.032	0.713[a]	139.304[b]
G × S	3	12.144	0.046[b]	0.125	27.957[b]
G × D	13	5.261	0.012	0.090	3.225[b]
G × D × S	39	7.527[a]	0.017[a]	0.111	1.385[b]
Error B	324	5.193	0.012	0.111	0.720

s.v. source of variation, *S.S.* sum of squares, *d.f.* degree of freedom, *S* stratification, *D* dormancy breaking, *G* genotype

[a] Significant at 5 % level of probability

[b] Significant at 1 % level of probability

gibberellin (8.3ab), and distilled water (8.01abc), which are significantly higher than the other treatments. The least rate of average germination time was resulted by sulfuric acid (Table 4).

Gibberellin (0.2138a) and benzyl adenine (0.2022a) resulted in the highest rate of germination followed by Fosnutren (0.1937ab), Humiforte (0.1928ab), and kinetin (0.1881abc). There were not significant differences among different treatments on the homogeneity of germination, although potassium nitrate resulted in the highest rate (0.2a). The highest rate of seed vigor index was resulted by distilled water (1.576a), kadostim (1.57a), and Aminol Forte (1.558a) (Table 4).

Experiment II

According to the analysis of variance, the effect of PEG_{6000} was significant on seed germination (%), plumule length, and seedling length. All seed-related parameters were significantly affected by dormancy breaking treatments and its interaction with PEG_{6000}. With the exception of plumule length, the other seed parameters were significantly affected by genotype; however, its interaction with PEG_{6000} was significant on seed germination. The interaction of genotype and dormancy breaking treatment significantly affected germination (%), radicle, and seedling length. However, the interaction of genotype, dormancy breaking treatments, and PEG_{6000} just significantly affected the radicle length (Table 5).

There were no differences between the first (36.86a) and second (36.11a) levels of PEG_{6000} on the rate of germination (%). This was also the case for plumule length as the highest ones were given by the second (4.681a) and first (4.556a) levels of PEG_{6000} which are significantly higher than the third level. Although radicle length was not affected by PEG_{6000}, the first (7.78a) and second level of (7.924a) PEG_{6000} resulted in significantly higher seedling length than did the third level (7.019b) (Table 6).

Malayer (47.21a) resulted in significantly higher seed germination (%) than Nahavand (21.71b) did. Plumule length was not significantly affected by genotype. Malayer also resulted

Table 4 The effects of stratification, genotype, and seed dormancy breaking treatments on different parameters of seed germination

Priming treatments	Average germination time	Rate of germination	Homogeneity of germination	Seed vigor index
Stratification (day)				
0	8.919a	0.1124b	0.09634a	1.297b
15	8.262a	0.1115b	0.05152a	2.443a
30	7.091b	0.1368b	0.06518a	0.5507c
45	3.626c	0.3103a	0.1197a	0.1212d
Genotype				
Nahavand	7.026a	0.1593a	0.1231a	0.5454b
Malayer	6.923a	0.1762a	0.04330b	1.661a
Seed dormancy breaking treatments				
Kinetin (10^{-5} M)	6.507d	0.1881abc	0.07437a	0.7722c
Gibberellin (100 mg/l)	7.15bcd	0.2138a	0.07813a	0.8141c
Benzyl adenine (10^{-5} M)	6.696d	0.2022a	0.04437a	0.7688
Fosnutren (0.4 %)	6.677d	0.1937ab	0.05562a	1.295ab
Humiforte (0.4 %)	6.791cd	0.1928ab	0.07125a	1.398ab
Aminol Forte (0.4 %)	6.879cd	0.1791a–d	0.05906a	1.558a
Kadostim (0.4 %)	6.438d	0.1578a–d	0.04062a	1.57a
Potassium nitrate (0.4 %)	7.659bcd	0.1578a–d	0.2a	1.079bc
Gibberellin + benzyl adenine	7.152bcd	0.1622a–d	0.06219a	0.9534bc
Gibberellin + kinetin	9.04a	0.1259cd	0.05906a	1.098abc
Benzyl adenine + kinetin	7.583bcd	0.15abcd	0.04312a	1.347ab
Benzyl adenine + kinetin + - gibberellin	8.3ab	0.1359bcd	0.1937a	1.011bc
Distilled water	8.01abc	0.1678a–d	0.04688a	1.576a
Sulfuric acid (90 %)	2.765e	0.1213d	0.1363a	0.2013d

Means in similar columns followed by similar letters are not significantly different using Duncan's multiple range test

in a significantly higher radicle and seedling length (3.319a, 7.866a) compared to Nahavand (3.003b, 7.282b). Aminol Forte resulted in the highest rate of seed germination (%) which was significantly higher than the other treatments with the exception of kadostim (43ab). The highest plumule length was resulted by distilled water (6.278a), Aminol Forte (6.017a), and kadostim (5.765a), which were significantly higher than the other treatments. The highest rates of radicle length (cm) were related to Aminol Forte (4.831a) and distilled water (4.972a), which were significantly different from the other treatments. Seedling length was the highest by distilled water (11.25a) followed by Aminol Forte (10.85ab), both significantly higher than the other treatments (Table 6).

According to the analysis of variance, PEG_{6000} and its interaction with dormancy breaking treatments were just significant on the rate of seed vigor index. Different seed parameters including average germination time, rate of germination, and seed vigor index were significantly affected by dormancy breaking treatments. Just rate of germination was not significantly affected by genotype. The interactions of genotype and PEG_{6000} and genotype and dormancy

Table 5 Analysis of variance indicating the effects of different experimental treatments on seed germination and seedling growth

S.V.	S.S.				
	d.f.	Germination (%)	Plumule length	Radicle length	Seedling length
P	2	1383.857[a]	14.525[a]	1.786	26.457[a]
Error A	9	217.952	2.175	1.247	5.821
D	13	1930.480[b]	35.377[b]	28.259[b]	118.811[b]
P × D	26	280.985[b]	4.933[b]	3.728[b]	15.330[b]
G	1	54621.00[b]	6.083	8.348[b]	28.601[a]
G × P	2	1713.000[b]	3.192	1.778	9.761
G × D	13	470.846[b]	2.881	2.552[b]	10.547[b]
G × D × P	26	160.077	2.679	1.408[a]	6.736
Error B	243	143.286	2.080	0.783	4.388

s.v. source of variation, *S.S.* sum of squares, *d.f.* degree of freedom, *S* stratification, *P* polyethylene glycol, *D* dormancy breaking, *G* genotype

[a] Significant at 5 % level of probability

[b] Significant at 1 % level of probability

breaking treatments significantly affected average germination time and seed vigor index. However, the interaction of genotype, dormancy breaking treatments, and PEG_{6000} was significant on the rate of seed vigor index (Table 7).

There were no significant differences among different treatments of PEG_{6000} on average germination time, rate of germination, and homogeneity of germination. However, seed vigor at the first (3.121a) and second (3.281a) levels of PEG_{6000} were significantly higher than that at the third level (2.382b). Higher average germination rate was resulted by Malayer (7.778a) compared to by Nahavand (7.194b); however, there were no significant differences between the two genotypes on the rate of germination. Nahavand resulted in a significantly higher homogeneity of germination (0.182a) related to Malayer (0.05179b). A significantly higher seed vigor index was related to Malayer (4.06a) compared with Nahavand (1.796b) (Table 8).

Gibberellin resulted in the highest average germination time (8.648a) followed by kadostim (8.064ab) and kinetin (7.832abc). Rate of germination was the highest by Fosnutren (0.165a) followed by benzyl adenine + kinetin (0.1517ab). The highest homogeneity of germination was resulted by sulfuric acid (0.4004a), which is significantly higher than the other treatments. Aminol Forte resulted in the highest seed vigor index, followed by distilled water (4.825ab) and kadostim (4.412b) (Table 8).

Discussion

The priming techniques including cold moist stratification and osmopriming (PEG_{6000}) and seed dormancy breaking techniques including osmopriming, biopriming, chemical priming, and hydropriming tested in these experiments significantly affected fennel seed germination and seedling growth indicating the effectiveness of the treatments. The significant interaction of stratification and genotype on seed germination indicated that the response of the two genotypes to stratification priming was different.

Table 6 The effects of polyethylene glycol, genotype, and seed dormancy breaking treatments on seed germination and seedling growth

Priming treatments	Germination (%)	Plumule length (cm)	Radicle length (cm)	Seedling length (cm)
Polyethylene glycol (MPa)				
−0.99	36.86a	4.556a	3.224a	7.78a
−1.35	36.11a	4.681a	3.243a	7.924a
−2.33	30.43b	4.004b	3.016a	7.019b
Genotype				
Nahavand	21.71b	4.279a	3.003b	7.282b
Malayer	47.21a	4.548a	3.319a	7.866a
Seed dormancy breaking treatments				
Kinetin (10^{-5} M)	39bc	4.6bc	3.45c	8.051cd
Gibberellin (100 mg/l)	26ef	4.088bcd	2.73d	6.818def
Benzyl adenine (10^{-5} M)	33.67cd	3.341d	1.957ef	5.297g
Fosnutren (0.4 %)	33.33cde	3.851cd	3.463c	7.313de
Humiforte (0.4 %)	40.17bc	4.572bc	4.151b	8.723c
Aminol Forte (0.4 %)	49.33a	6.017a	4.831a	10.85ab
Kadostim (0.4 %)	43ab	5.765a	4.227b	9.992b
Potassium nitrate (0.4 %)	37.5bc	4.57bc	2.701d	7.272de
Gibberellin + benzyl adenine	24.33f	3.549d	2.158e	5.706fg
Gibberellin + kinetin	33cde	4.816b	3.148cd	7.963cd
Benzyl adenine + kinetin	39.5bc	4.758bd	2.92cd	7.676cd
Benzyl adenine + kinetin + gibberellin	27.33def	4.089bcd	2.073e	6.162efg
Distilled water	41.5b	6.278a	4.972a	11.25a
Sulfuric acid (90 %)	14.83g	1.498e	1.472f	2.97h

Means in similar columns followed by similar letters are not significantly different using Duncan's multiple range test

Compared with the other treatments, the 15-day treatment was the most effective, significantly increasing seed germination and seedling growth. Genotype Malayer was also the superior treatment compared with Nahavand with respect to seed germination and seeding growth. Under different conditions, the germination of seeds are delayed or reduced. Hence, it is important to increase the rate of seed germination using different techniques such as priming [21].

The effects of different priming techniques including salicylic acid (SA) at 50 mmol L^{-1}, $CaCl_2$ at 2.2 % and moringa leaf extract (MLE) at 3.3 % on the growth and yield of linola was investigated by Rehman et al. [22]. The osmopriming technique with $CaCl_2$ decreased the time of emergence and resulted in the highest fresh and dry weights of seedlings and increased chlorophyll a content. The growth, yield, and oil content of linola was also significantly affected by $CaCl_2$ and MLE indicating the positive effects of such treatments on the early growth and yield of linola. Such results are comparable with the results of this research work [22].

Aminol Forte, as a biological fertilizer (a combination of amino acids), was among the most effective treatments significantly increasing seed germination and seedling growth. It is because the compound is easily absorbable by plant and seed and is incorporated into plant

Table 7 Analysis of variance indicating the effects of experimental treatments on different seed germination parameters

S.V.	d.f.	Average germination time	Rate of germination	Homogeneity of germination	Seed vigor index
P	2	0.497	0.000	0.244	25.766[b]
Error A	9	3.899	0.002	0.115	2.218
D	13	7.027[a]	0.010[b]	0.213	40.631[b]
P × D	26	3.837	0.001	0.111	5.394[b]
G	1	28.671[b]	0.001	1.425[b]	430.735[b]
G x P	2	20.285[b]	0.003	0.211	22.557[b]
G × D	13	9.371[b]	0.002	0.212	8.037[b]
G × D × P	26	2.931	0.001	0.103	3.121[a]
Error B	243	3.352	0.001	0.142	1.748

s.v. source of variation, *S.S.* sum of squares, *d.f.* degree of freedom, *S* stratification, *P* polyethylene glycol, *D* dormancy breaking, *G* genotype

[a] Significant at 5 % level of probability

[b] Significant at 1 % level of probability

physiology quickly. Accordingly, plant metabolic rate and the synthesis of RNA increase. Aminol forte can also increase plant growth by enhancing the rate of nutrient uptake, resulted by improving root growth [23].

Benzyl adenine is able to increase seed germination and seedling growth by enhancing the rate of cellular growth division by affecting the cytokinin pathway. After the absorption of benzyl adenine by plant, it is converted into some other metabolites including 6-benzylamino-9-glucopyranosylribosyl-purine and its novel phosphorylated form, 6-benzylamino-9-glucopyranosylribosyl-purine. Benzyl adenine by itself can decrease the rate of endogenous cytokinin, which indicates the activity of compound in plant [24].

The results of this and other research work has indicated that kinetin by itself is not among the most effective treatments on seed germination and seedling growth [10, 25]; however, the results of this research work show that kinetin in combination with benzyl adenine was the most effective treatment significantly increasing seed germination and seedling growth. They might have positive interaction effects on each other.

Kadostim and Fosnutren, which are a combination of amino acids and oligopeptides with potassium and phosphorous, respectively, and with micronutrients, were also among the effective treatments. They can be quickly absorbed by plant and increase plant growth by enhancing root growth and hence higher nutrient and water uptake. Hydropriming was also indicated to be among the effective treatments on seed germination and seedling growth [26, 27].

Analysis of variance indicated that the priming treatments significantly affected the seed germination parameters including the average germination time, the rate of germination, and seed vigor index. However, the only treatment, which increased the rate of germination uniformity, was fennel genotype. Stratification at 15 and 45 days resulted in the highest and least average germination time and vigor index, respectively [8, 28]. Although Nahavand had a significantly higher rate of germination uniformity, Malayer indicated significantly higher seed vigor.

Table 8 The effects of polyethylene glycol, genotype, and seed dormancy breaking treatments on different parameters of seed germination

Priming Treatments	Average germination time	Rate of germination	Homogeneity of germination	Seed vigor index
Polyethylene glycol (MPa)				
−0.99	7.516a	0.1334a	0.1377a	3.121a
−1.35	7.41a	0.1338a	0.1496a	3.281a
−2.33	7.532a	0.1316a	0.06348a	2.382b
Genotype				
Nahavand	7.194b	0.1342a	0.182	1.796b
Malayer	7.778a	0.1317a	0.05179b	4.06a
Seed dormancy breaking treatments				
Kinetin (10^{-5} M)	7.832abc	0.1321bc	0.08458b	3.398cd
Gibberellin (100 mg/l)	8.648a	0.11d	0.06542b	1.992fg
Benzyl adenine (10^{-5} M)	7.209bc	0.1458bc	0.2283ab	1.805fg
Fosnutren (0.4 %)	6.968bc	0.165a	0.075b	2.426ef
Humiforte (0.4 %)	7.731abc	0.1337bc	0.06667b	3.645c
Aminol Forte (0.4 %)	7bc	0.1454bc	0.0525b	5.379a
Kadostim (0.4 %)	8.064ab	0.1271cd	0.06792b	4.412b
Potassium nitrate (0.4 %)	7.719abc	0.1329bc	0.0725b	2.98cde
Gibberellin + benzyl adenine	7.46abc	0.1262cd	0.09125b	1.535gh
Gibberellin + kinetin	7.865abc	0.1304c	0.1596b	2.805de
Benzyl adenine + kinetin	6.852bc	0.1517ab	0.07417b	3.02cde
Benzyl adenine + kinetin + gibberellin	7.431bc	0.1425bc	0.1233b	1.797fg
Distilled water	7.405bc	0.1388bc	0.075b	4.825ab
Sulfuric acid (90 %)	6.619c	0.07958e	0.4004a	0.9704h

Means in similar columns followed by similar letters are not significantly different using Duncan's multiple range test

The least rate of average germinating time was resulted by sulfuric acid [29–31]. Potassium nitrate (osmopriming) resulted in the highest rate of germination uniformity, although it was not significantly different from the other treatments. Potassium nitrate influences seed germination by affecting the activity of nitrate reductase, antioxidant enzymes, and chlorophyll content [32–34].

Gibberellin and Humiforte were among the most effective treatments on the rate of seed germination. Humiforte is a complex of synthesized amino acids and oligopeptides, as well as nutrients and humic acting as a biostimulant and with a high rate of kinetin [23]. Gibberellin is able to affect seed germination by affecting cellular growth and development. The interactions between plant hormones and plant genes affect the process of seed germination. Accordingly, it is possible to regulate seed germination by modifying the expression of genes. Gibberellins are able to activate dormant seeds, although they do not control seed dormancy [35–37]. Hormonal priming with gibberellins can increase the rate of seed germination by increasing the activity of α-amylase resulting in the improvement of starch metabolism and solubility of sugar [38–41].

Analysis of variance indicated that PEG_{6000} significantly increased seed germination and seedling growth. PEG_{6000} at −0.99 and −1.35 MPa were the most effective ones, significantly

differing from −2.33 MPa. However, among the seed germination parameters, only seed vigor was significantly affected by PEG_{6000} at −0.99 and −1.35 MPa, significantly differing from −2.33 MPa. These results are similar to the results by Rahimi [21].

Conclusion

Plants including the medicinal plant, fennel, face the limited rate of germination under different conditions. We accordingly hypothesized that it is possible to increase seed germination and seedling growth of fennel using different priming and seed dormancy breaking techniques. Such methods are able to alter plant morphology and physiology of primed seeds and hence increase seed vigor, plant growth, and crop production. Different priming and seed dormancy breaking methods including cold moist stratification, hormonal, osmopriming, biopriming, chemical priming, and hydropriming were tested on the germination parameters and seedling growth of fennel using two different experiments. The results indicated both priming techniques including cold moist stratification and PEG_{6000} (osmopriming) affected seed germination parameters and the seedling growth of fennel. Among the seed dormancy breaking methods biopriming and hydropriming techniques were among the most effective ones, which significantly increased seed germination and removed the seed dormancy barriers. It is possible to enhance seed germination and seedling growth of fennel using priming techniques tested in this research work. Such seed pretreatments can be useful for the increased production of fennel under different conditions, especially stress.

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