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Impact of Seed Priming Treatments to Enhance Germination of Black Mustard Against Dormancy

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Abstract Black mustard (Brassica nigra L. Brassicaceae) represents an important source of raw materials for many agro-industry branches in Turkey and elsewhere in the world. Black mustard is a highly significant plant for its aromatic, medicinal, and therapeutic uses, as well as its potential as an alternative energy plant. In our country, cultivar development studies are carried out on wild genotypes of this species. One of the major challenges to black mustard growth and yield is its homogeneous, simultaneous, and fast emergence. Presoaking seeds in water (hydroprimed) and polyethylene glycol (PEG) (osmoprimed), has been demonstrated to enhance the germination of seeds of different species. The seeds of pure line black mustard originated in the Ankara, Turkey, and were used in this study. The purpose of the study was to measure the effects of durations (12, 24, 36, and 48 h); unprimed, hydroprimed, and five osmoprimed doses (-0.1, -0.2, -0.4, 0.6, and -0.8 MPa of PEG) treatments on seed germi-nation and seedling establishment characteristics of black mustard for 14 days. The present findings demonstrate that priming durations and treatments of black mustard seeds significantly affected final germination percentage, mean germination time, root and seedling length, and seedling fresh and dry weight among different treatments compared to the control. This positive effect on germination especially shortened the mean germination time. In terms of the properties studied, the optimal priming time is 12 and 24 h, for hyropriming and osmopriming doses (-0.1, -0.2, and -0.4 Mpa of PEG). The study indicated that the implemented priming treatments can be useful in improving the capability of black mustard on germination treatments.

Key words

Hydropriming; Osmopriming; Priming duration; Germination; Brassica nigra.

Introduction

Black mustard (*Brassica nigra* L. *Brassicaceae*) is an annual growth habit plant, is widely cultivated for its blackish brown-red seeds that are slightly more pungent than the other mustard seeds, such as white or brown mustard (Palle-Reisch et al., 2013; Kayacetin, 2020; Lietzow, 2021). It is important for its aromatic and medicinal uses (Darwesh, 2017; Nisar et al., 2018; Asaduzzaman et al., 2021; Mayekar et al., 2021) as well as its potential as an alternative energy plant (Kinay and Kayacetin, 2023).

In the entire life cycle of the plant, seed germination is the initial step for growth and development, which result in the emergence of a radicle to form a primary root (Kayacetin et al., 2018). Quantitative parameters like final germination percentage, mean germination time, root and seedling length, seedling fresh and dry weight, and synchronization of the whole germination process are thought to be highly significant. According to Kayacetin (2019), there are germination obstacles in the black mustard seeds, and thus, there are difficulties in homogeneous, rapid, and simultaneous emergence.

Dormancy is an internal state of the seed that inhibits its germination. It is examined in two groups as primary and secondary dormancy. Primary dormancy refers to innate dormancy, and secondary dormancy refers to a dormant state induced in the nondormant seed by conditions inconvenient for germination. These reasons may be the cause of dormancy in the seeds of many weed species (Benech-Arnold et al., 2000). Dormancy and germination are vital phenomena in the life cycle of all species. Although the seeds vary considerably according to the species, they have different germination and dormancy characteristics according to their genetic structure (Gupta, 2016). Plant growth regulators such as PEG have been recommended to break seed dormancy and enhance germination (Bao et al., 2010; Luo et al., 2022; Hassan et al., 2023)

Seed priming is a simple and effective technology to help ensure homogeneous, simultaneous, and rapid emergence, thus leading to better crop yields (Finch-Savage and Bassel, 2016; Raj and Raj, 2019). Recently published studies showed that priming treatment can positively affect seed germination and seedling growth in the *Brassicaceae* family (Guragain et al., 2023; Kayacetin, 2023). As primed seeds are physiologically close to the germination stage, they show an improved germination percentage, early and uniform germination, improved growth characteristics, and faster and more homogeneous emergence (Fu et al., 2022; Okello et al., 2022). The Priming technique is one of the most effective options to shorten germination time and advence germination percentage to increase yield (Kayacetin, 2021; Thakur et al., 2022).

For the uniform emergence of the seed, osmopriming and hydropriming are the most effective methods (Singh et al., 2017; Pandey et al., 2022). The positive contributions of osmopriming on germination in sorghum (Zhang et al., 2015), in savory (Vidak et al., 2022), in wheat (Farooq et al., 2022), in sesame (Biswas and Dutta, 2021) and in black cumin (Kayacetin, 2022); whereas hydropriming on germination in mustard (Thapa et al., 2022), in sorghum (Demb'el'e et al., 2021), and in lemon balm (Hatami et al., 2021) have been previously reported.

Current and future work while focusing on the different uses of mustard plants and seeds, the negative effects on germination and emergence will be tried to be eliminated both in terms of breeding and agriculture. Information on enhancing the germination of black mustard against dormancy is still limited. Therefore, it was thought that the purpose of the research was to identify the effects of priming durations (12, 24, 36, and 48 h); unpriming, hydropriming, and five osmopriming doses (-0.1, -0.2, -0.4, 0.6, and -0.8 MPa of PEG) on seed germination and seedling establishment characteristics of black mustard (*Brassica nigra* L.) for 14 days on the dormancy-breaking and germination indices.

Material and Method

This study was done using pure line seeds of the *Brassica nigra*, obtained from the Research Institute in Ankara, Turkey (Table 1). The experimental design consisted of two factors (priming duration \times seed treatment) regulated in a completely randomized design with three replicates. The osmotic potential of PEG-6000 was arranged at-0.2,-0.4,-0.6, and-0.8 MPa according to Michel and Kaufmann (1973). The priming durations (12, 24, 36, and 48 hours) were the main factors, seed treatments [unprimed (control), hydroprimed, and five osmoprimed doses (-0.1,-0.2,-0.4, 0.6, and-0.8 MPa of PEG)] was the subfactors. Samples of 150 seeds (three replicates of 50 seeds each) were placed in 9-cm Petri dishes containing two layers of filter paper.

Black mustard seeds were treated with immersed solution of -0.2, -0.4, -0.6, and -0.8 MPa polyethylene glycol for 12, 24, 36, and 48 hours and in distilled water (hydroprimed) for 12, 24, 36, and 48 hours. The unprimed seeds were used as control (unprimed). The seeds were carefully dried after priming to the first moisture level at 25 ± 3 °C and were and used 24 h after priming.

Seeds were kept at 22 ± 1 °C in the dark for germination for 14 days (Fallah-Toosi and Baki, 2013). Black mustard seed was considered to have germinated when >1 mm radicle emerged. Germination percentage was recorded every 24 h for 7 days. The mean germination time was numbered according to ISTA (2003). Shoot length, root length, seedling freshness and dry weight were

evaluated in five seedlings selected randomly from each replicate after the 14th day. Dry weight was evaluated after drying samples in an oven at 70 °C for 48 h (Böhm, 1979). The final germination percentage (FGP) and mean germination time (MGT) were determined according to the method of Kader (2005).

FGP (%) =
$$\frac{\text{Total number of germinated seeds}}{\text{Total number of observed seeds}} \times 100$$

MGT (day) =
$$\frac{n1 \times d1 + n2 \times d2 + n3 \times d3 + \cdots}{\text{Total number of days}}$$

n= number of seeds newly germinated at day;

d= days counted from the start of the germination test Data statistical analysis

Analysis of variance of the experimental data was computed using the JMP 13 Statistical Software. The differences among the means were performed by LSD Test (p < 0.05).

Results and Discussion

The interaction effect of seed priming durations and seed priming treatment on final germination percentage (FGP), mean germination time (MGT), root length (RL), seedling length (SL), seedling fresh weight (SFW), and seedling dry weight (SDW) was significant (Table 2). While the maximum GP (97.33%) was obtained with the treatment of 12 and 24 h durations at -0.1 and -0.2 MPa of PEG priming, the minimum GP (64.67%) was determined with the treatment of 48 h durations at -0.8 MPa of PEG priming (Table 2). The maximum MGT (2.28 d) was obtained with unprimed, while the minimum MGT (1.17 d) was determined at -0.6 MPa of PEG for 48 h durations. The maximum RL (3.23 cm) and SL (6.21 cm) were obtained at -0.6 MPa and 0.4 MPa of PEG for 12 h durations, respectively, whereas the minimum RL (0.74 cm) and SL (2.36 cm) were obtained at -0.8 MPa of PEG for 48 h durations. The maximum SFW (8.28 mg) and SDW (0.71 mg) were obtained at -0.4 MPa for 12 h durations. The minimum SFW (0.83 mg) was determined at -0.8 MPa of PEG for 48 h durations. 48 h \times -0.2 MPa of PEG priming and 48 h \times -0.4 MPa PEG priming were detected at the minimum SDW (0.25 mg), and no significant differences were observed (Table 2). At the beginning of germination, rapid water uptake slows down after seed-based metabolic activities, resulting in the emergence of radicles leading to germination (Kayacetin, 2022). These results may explain that priming durations and treatments could improve theemergence performance of black mustard seeds, which is approved by earlier studies on some cultured species like black cumin (Kayacetin, 2022), sunflower (Bourioug et al., 2020), caraway (Mirmazloum et al., 2020), and kenaf (Lee et al., 2018). All priming processes used in this research made it possible to break dormancy in black mustard seeds considered dormant under appropriate conditions. Kayacetin (2023) demonstrated that osmo-priming treatments of black cumin using -0.2 or -0.4 MPa PEG for 24 or 36 hours demonstrated improved germination ability. Also, Trisnawaty et al. (2021) reported that different seed priming treatments resulted in significant seedling growth and reduced MGT in capers. In stevia, Shahverdi et al. (2017) demonstrated that PEG positively affected many aspects of plant growth, including seed germination and seedling growth. The rise in seedling growth traits with priming treatment could play a vital role in regulating plants's primary seedling growth. The major influence of the priming treatments was improved germination; therefore, post-germination progress could also be improved by priming seedling treatments. Considering seed priming treatments, it was noted that priming treatments improved seedling growth, which ended up with the maximum RL and SL. Kayacetin (2023) in black cumin noted that the maximum RL and SL were determined with the treatment of 36 h priming time at -0.4 MPa osmoprimed. This supports the idea that with priming treatments, the seed performs faster water uptake than normal germination. Previous studies showed that osmo and hydropriming achieved faster emergence and germination compared to control for germination (Neamatollahi et al., 2009; Kartika et al., 2021; Kayacetin, 2022; Bahreininejad, 2023).

A significant difference was detected among the priming durations for all the investigated characteristics (Table 3). While the maximum GP (91.24%) was obtained with the application of 24 h durations, the minimum GP (75.62%) was detected with the application of 48 h durations (Table 3). The minimum MGT (1.40 d) was determined with the application of 48 h durations, while the maximum MGT (1.61 d) was obtained within a 12 h duration. The maximum RL (2.23 cm) and SL (4.96 cm) were obtained for 12 and 36 h durations, respectively, whereas the minimum RL (1.19 cm) and SL (3.49 cm) were obtained for 48 h durations, respectively. The maximum SFW (5.41 mg) and SDW (0.46 mg) were obtained for 24 and 12 h durations, respectively. The minimum SFW (3.27 mg) and SDW (0.33 mg) weights were determined for 48 h durations (Table 3). While priming durations significantly increased the FGP and seedling growth parameters in black mustard seedlings, these parameters decreased by 48 h for the priming duration. At the onset of germination, rapid water uptake slows down after seed-based metabolic activities, resulting in the emergence of radicles leading to germination (Kayacetin, 2022). Therefore, 12 and 24 h are considered optimum duration for priming (Table 3). Kayacetin (2023) in black cumin found that 24 or 36 h priming treatment durations improved germination and seedling growth. Sadeghi et al. (2011) demonstrated that soybean seed osmopriming for 12 h improved the FGP. Mirmazloum et al. (2020) in caraway showed that 24 h priming duration was recommended as the best treatment for improving the FGP when compared to unprimed seeds. Benadjaoud et al. (2022) in Lavandula stoechas and Bahreininejad (2023) in Thymus daenensis reported that germination was positively affected by priming treatments compared to the unprimed. Mehra et al. (2003) determined that seeds of brown mustard and field mustard subjected to aerated hydration for up to 24 h had the most suitable timing at 12 h, which increased the final germination, and reduced MGT. Similar findings were observed by OrzeszkoRywka and Podlaski (2003) in sugar beet; Kayacetin, 2022 in black cumin with washing and priming that MGT was shortened by seed treatments.

A significant difference was determined among the priming treatments for all the investigated characteristics (Table 4). While the maximum GP (96.67%) and MGT (2.28 d) were obtained with unprimed applications, the minimum GP (72.67%) and MGT (1.37 d) were obtained with applications at -0.8 MPa of PEG priming (Table 4). The maximum RL (2.22 cm) and SL (5.11 cm) were obtained at -0.4 MPa and -0.1 MPa of PEG priming, respectively, whereas the minimum RL (1.35 cm) and SL (3.15 cm) were obtained at 0.8 MPa of PEG priming. The maximum SFW (6.00 mg) and SDW (0.49 mg) were obtained at -0.1 MPa of PEG priming and unprimed, respectively. The minimum SFW (3.23 mg) and SDW (0.36 mg) were determined at -0.8 MPa of PEG priming (Table 4). Whereas hydro and osmopriming treatments significantly increased the FGP and seedling growth parameters in black mustard seedlings compared to the unprimed, these parameters decreased by -0.6 and -0.8 MPa PEG osmopriming treatments, respectively. MGT was decreased by seed priming treatments and priming durations compared to unprimed ones. In black cumin, Kayacetin (2023) found that a -0.2 or -0.4 MPa priming treatment improved germination and seedling growth. Faijunnahar et al. (2009) in wheat seeds determined the most successful seedling growth with -0.1 MPa osmoprimed in comparison to unprimed. These outcomes are aided by Trisnawaty et al. (2021), who detected that rice seeds primed with PEG both improved germination indices and reduced MGT. Furthermore, results revealed that priming treatments were successful techniques to improve seed germination. Hydro and osmopriming of black mustard seeds also increased germination traits and seedling growth in the study.

Conclusions

The results showed that both priming durations and priming treatments of black mustard seeds significantly affected GP, MGT, RL, SL, SFW, and SDW compared to the unprimed. This positive effect, especially on germination, shortened the MGT. In terms of the properties studied, the optimal priming durations are 12 and 24 h; and priming treatments are hyropriming and osmopriming doses (-0.1, -0.2, and -0.4 Mpa of PEG). Results revealed that the applied priming treatments can be useful in improving the ability of black mustard in terms of germination treatments. It may be concluded that priming could end up being a very effective treatment to increase fast and identical emergence to accomplish better vigor, ending up with a better stand and yield. Therefore, current findings confirm that seed priming with PEG can be employed as a novel approach for improving black mustard seed germination efficiency. This technique is a practical pretreatment for fast and uniform emergence in unsuitable climatic conditions and can be used by researchers and farmers.

Table 1. Characteristics of the mustard species used in the study

Scientific name	Common name	Other name	Origin	Seed color	Thousand seed weight (g)	Registration
Brassica nigra	black mustard	Sinapis nigra	Ankara/Turkey	black-brown	1.4-1.6	pure line

Table 2. Effect of	priming duration ×	priming treatment	t interaction on different	ent germination	parameters of black mustard

1 0	uration × priming treatment						
Priming durations	Priming treatment	GP (%)	MGT (day)	RL (cm)	SL (cm)	SFW (mg)	SDW (mg)
	Unprimed	96.67 a	2.28 a	1.44 ij	4.31 fg	4.23 hi	0.49 b
	Hydroprimed	96.67 a	1.61 b	1.51 ij	4.25 fgh	4.33 h	0.35 g
	-0.1 MPa PEG primed	97.33 a	1.56 bc	1.73 hi	4.53 f	5.78 de	0.37 efg
12	-0.2 MPa PEG primed	97.33 a	1.52 cd	2.65 bc	5.71 cd	6.01 cd	0.41 cde
	-0.4 MPa PEG primed	90.67 b	1.49 de	2.71 bc	6.21 b	8.29 a	0.71 a
	-0.6 MPa PEG primed	82.00 d	1.45 ef	3.23 a	5.99 bc	4.43 gh	0.49 b
	-0.8 MPa PEG primed	76.67 e	1.38 ghi	2.30 def	3.64 j	3.92 I	0.35 fg
	Unprimed	96.67 a	2.28 a	1.44 ijk	4.31 fg	4.25 hi	0.49 b
	Hydroprimed	97.33 a	1.55 cd	1.72 hi	4.38 fg	4.78 fg	0.41 cde
	-0.1 MPa PEG primed	97.33 a	1.45 ef	2.40 c-f	5.10 e	5.91 cd	0.45 c
24	-0.2 MPa PEG primed	97.33 a	1.53 cd	2.62 bc	4.88 e	5.95 cd	0.51 b
	-0.4 MPa PEG primed	90.67 b	1.51 cde	2.82 b	6.18 b	6.24 bc	0.43 cd
	-0.6 MPa PEG primed	82.67 cd	1.41 fgh	2.18 fg	5.90 bc	5.90 cd	0.41 cde
	-0.8 MPa PEG primed	76.67 e	1.45 ef	1.14 kl	3.48 jk	4.85 f	0.40 de
	Unprimed	96.67 a	2.28 a	1.44 ijk	4.31 fg	4.23 hi	0.49 b
	Hydroprimed	92.67 b	1.42 fg	2.58 bcd	6.80 a	5.12 f	0.39 def
	-0.1 MPa PEG primed	90.67 b	1.29 jkl	2.53 b-e	6.69 a	6.44 b	0.43 cd
36	-0.2 MPa PEG primed	85.33 c	1.24 lm	2.22 efg	5.54 d	5.48 e	0.51 b
	-0.4 MPa PEG primed	85.33 c	1.35 hij	2.10 fg	4.25 fgh	4.78 fg	0.41 cde
	-0.6 MPa PEG primed	76.00 e	1.29 kl	1.96 gh	3.97 hi	4.44 gh	0.21 j
	-0.8 MPa PEG primed	72.67 f	1.41 fgh	1.22 jkl	3.131	3.30 j	0.39 def
	Unprimed	96.67 a	2.28 a	1.44 ijk	4.31 fg	4.24 hi	0.49 b
	Hydroprimed	62.67 h	1.32 ijk	1.26 jkl	3.69 ij	4.34 h	0.35 fg
	-0.1 MPa PEG primed	83.33 cd	1.33 ijk	1.22 jkl	4.13 gh	5.85 d	0.41 cde
48	-0.2 MPa PEG primed	81.33 d	1.27 kl	1.29 jkl	3.47 jk	4.35 h	0.25 ij
	-0.4 MPa PEG primed	72.67 f	1.21 mn	1.26 jkl	3.24 kl	2.04 k	0.25 ij
	-0.6 MPa PEG primed	68.00 g	1.17 n	1.111	3.24 kl	1.261	0.30 h
	-0.8 MPa PEG primed	64.67 h	1.24 lm	0.74 m	2.36 m	0.83 m	0.27 hi
Summary of ANOVA	**	**	**	**	**	**	

** Significant at p < 0.05

Table 3. Effect of priming duration on different germination parameters of black mustard

Priming durations (h)	GP (%)	MGT (day)	RL (cm)	SL (cm)	SFW (mg)	SDW (mg)
12	91.05 a	1.61 a	2.23 a	4.94 a	5.30 a	0.46 a
24	91.24 a	1.60 a	2.05 b	4.89 a	5.41 a	0.44 a
36	85.62 b	1.47 b	2.01 b	4.96 a	4.83 b	0.41 b
48	75.62 c	1.40 c	1.19 c	3.49 b	3.27 c	0.33 c
Summary of ANOVA	**	**	**	**	**	**

** Significant at p < 0.05

Table 4. Effect of priming treatment on different germination parameters of black mustard

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Priming treatment	GP (%)	MGT (day)	RL (cm)	SL (cm)	SFW (mg)	SDW (mg)		
Unprimed	96.67 a	2.28 a	1.45 d	4.29 d	4.26 d	0.49 a		
Hydroprimed	87.33 d	1.48 b	1.77 c	4.78 c	4.64 c	0.38 d		
-0.1 MPa PEG primed	92.17 b	1.41 c	1.97 b	5.11 a	6.00 a	0.42 c		
-0.2 MPa PEG primed	90.33 c	1.39 cd	2.20 a	4.90 bc	5.45 b	0.42 c		
-0.4 MPa PEG primed	84.83 e	1.39 cd	2.22 a	4.97 ab	5.34 b	0.45 b		
-0.6 MPa PEG primed	77.17 f	1.33 e	2.12 ab	4.77 c	4.01 e	0.35 e		
-0.8 MPa PEG primed	72.67 g	1.37 d	1.35 d	3.15 e	3.23 f	0.36 de		
Summary of ANOVA	**	**	**	**	**	**		

** Significant at p < 0.05

Data Availability Statement

The data are available on request.

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Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

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