Synchronized Seed Germination and Seedling Growth of Black Cumin

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Summary. **Black cumin (*Nigella sativa*) is an important medicinal plant in the pharmacological industry. It is cultivated on a commercial scale, but its seeds have a slow, unsynchronized germination rate. Enhancing seed germination is crucial for improving the production of black cumin. The influence of presowing treatments [gibberellic acid (GA_3), potassium nitrate, salicylic acid, and stratification at 4 °C] on seed germination was assessed. Seed germination was determined daily for 30 days, and germination parameters, including final germination percentage (FGP), corrected germination rate, number of days to reach 50% of FGP, and seedling length vigor index, were evaluated. Endogenous contents of GA_3 and abscisic acid (ABA) in nonstratified and stratified seeds were estimated using high-performance liquid chromatography (HPLC) and seedling growth was determined in 45-day-old seedlings. All presowing treatments tended to boost early germination for the first 10 days compared with the control. Low concentrations of GA_3 at 0.25 $g \cdot L^{-1}$ also increased FGP (80%) compared with the control group (65.55%). Stratification for 4 weeks provided the greatest FGP value at 95.56%, and stratification for 3 weeks proved to be the most effective treatment for optimal seedling growth. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis patterns of stratified seeds revealed the alteration in intensities of 13 bands and the appearance of a new band (180 kDa) indicating a change in the synthesis of proteins during stratification. Moreover, stratification modulated the endogenous GA_3 and ABA contents of black cumin seeds, which alleviated the physiological dormancy and resulted in high and synchronized seed germination.**

Black cumin (*Nigella sativa*) is an annual plant grown in arid and semiarid lands and is native to the eastern Mediterranean (Ozer et al., 2020). Black cumin is considered one of the most important medicinal herbs around the world (Yimer et al., 2019). Forouzanfar et al. (2014) reported that black cumin is popular for its use in traditional medicine for treating diseases and disorders such as eczema, back pain, and asthma. This is in addition to its wide use as a spice in food products. The essential oil of black cumin is also widely used in the pharmaceutical and cosmetics industry due to its medicinal properties (Khan et al., 2011). A significant number of studies have confirmed the antioxidant, antihypertensive, antimicrobial, antibacterial, antifungal, antiviral, antiinflammatory, anticancer, and antidiabetic activities, neuroprotective, and analgesic impacts of black cumin (Abdallah, 2017; Abulfadl et al., 2018; Ozdemir et al., 2018; Yimer et al., 2019). The germination of black cumin seeds and the growth of seedlings present a big challenge for achieving high levels of growth and productivity of this crop (Papastylianou et al., 2018). Seeds of black cumin possess dormancy characteristics due to their underdeveloped embryos. In a laboratory investigation conducted during nongrowing season, Rouhi et al. (2012) postulated that black cumin seeds have morpho-physiological dormancy in addition to a physiological component. As a winter annual, black cumin seeds are usually sown in October and November and typically take 10 d or more to germinate under natural conditions. Low germination percentages and rates are common, resulting in variation of emergence under field conditions.

Stratification and temperature shocks are used to break dormancy in many species (Hidayati et al., 2012; Su et al., 2016). Numerous molecular and physiological changes occur during cold treatment among them, the transcriptional activation and repression of genes by low temperature (Thomashow, 1999). The reprogramming of gene expression results in the accumulation of protective proteins and metabolites which are known to have protective effects. It is also reported that cold treatment could improve the physiological metabolism

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
100	bar	kPa	0.01
29,574	fl oz	µL	3.3814×10^{-5}
29.5735	fl oz	mL	0.0338
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
1	micron(s)	µm	1
28.3495	oz	g	0.0353
0.001	ppm	$g \cdot L^{-1}$	1000
0.1	ppm	mg/100 g	10
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

of the plant, such as increased activities of dehydrogenase, superoxide dismutase (Yin et al., 2005), peroxidase (Jiang et al., 2014), photosynthetic pigments, photosynthetic efficiency, and nitrate reductase activity (Wu et al., 2007). Changes in the synthesis of constitutive proteins and the synthesis of new proteins under low-temperature have confirmed the relationship between protein expression and low temperature (Evstigneeva et al., 2001). Various presowing treatments including chemical and mechanical scarification (Shaik et al., 2008), soaking in plant growth regulators (Chauhan et al., 2009; Dewir et al., 2011; Elhindi et al., 2016; El-Nashar and Dewir, 2019) or other germination promoting substances such as nitrogen-containing compounds (Bethke et al., 2007) are used to overcome seed dormancy and improve seed germination. This investigation explored options to accelerate black cumin seed germination using presowing seed treatments to enhance the uniformity of emergence in field conditions.

Materials and methods

Seed materials and presowing treatments. Seeds of an inbred-selected black cumin line [self-pollinated for 3 years (El-Mahrouk et al., 2015)] were used in this investigation and subjected to different presowing treatments (chemical and stratification). The experiment took place during Oct. 2018 at the farm of Kafrelsheikh University (Kafr El-Sheikh, Egypt). For the chemical treatments, dry seeds were soaked in one of 14 aqueous solutions of salicylic acid [SA (Winlab, Market Harborough, UK)] at 0.05, 0.1, 0.2, and 0.4 $g{\cdot}L^{-1}$, potassium nitrate [KNO_3 (PanReac AppliChem, Darmstadt, Germany)] at 0.5, 1, 1.5, 2.5, and 3 $g{\cdot}L^{-1}$, or gibberellic acid [GA_3 (Merck, Darmstadt, Germany)] at 0.25, 0.5, 1.0, 1.5, and 2 $g{\cdot}L^{-1}$. The soaked seeds were shaken in an orbital shaker for 24 h under dark conditions, then washed with distilled water and air-dried. In addition to chemical treatments, we explored the use of cold stratification. Dry seeds were wrapped in aluminum foil sheets and transferred to a refrigerator (4 °C) for different time intervals (0, 1, 2, 3, 4, and 5 weeks). Seeds without treatments served as the negative control, and seeds soaked in distilled water alone for 24 h served as the positive control treatment.

Germination medium and experimental design. A mixture of peatmoss and vermiculite [1:1 v/v (Egyptian Company for Mineral Resources, Cairo, Egypt)] was used as the germination medium. The medium was fertilized with 1 $g{\cdot}L^{-1}$ solution containing 19N–8.3P–15.7K water-soluble fertilizer (Rosasol; Rosier, Moustier, Belgium) and sterilized in 1 $g{\cdot}L^{-1}$ commercial fungicide solution [20% tolclofos-methyl, 30% thiram (Rizolex; Kafr El-Zayat Company, El-Gharbia, Egypt)]. The pH of the medium was adjusted to 6 ± 1 with calcium carbonate powder using a pH meter to measure pH (3510; Jenway, Stafford, UK). The germination mixture was covered with a plastic cover for 24 h before filling the tray and sowing the seeds. Seeds were sown, one seed in each cell, in expanded polyurethane foam trays (3.0 × 3.0-cm cells; 209 cells per tray). Only 100 seeds were sown in each tray to facilitate data recording. The trays were kept in a greenhouse at a temperature of 25 ± 2 °C and light intensity of 300 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ after sowing. The trays were manually watered every week and the same water amount (1 L) was applied to each tray. All treatments were covered with plastic sheets until the first germinated seeds were visible and then the plastic covers were removed.

Data collection. Germination was assessed for 30 d and daily germination percentages were summed up to obtain the cumulative germination percentage for each treatment on each assessment date. A seed was considered germinated when the cotyledons were visible above the surface and the following five germination parameters were calculated: 1) final germination percentage (FGP) = number of germinated seeds (30 d from sowing/number of sown seeds) × 100; 2) germination rate index (GRI) = [(G1/1) + (G2/2) + (G_x/X)] where, G = germination percentage on each alternate day after placement 1, 2, x = corresponding day of germination (Esechie, 1994); 3) corrected germination rate index (CGRI) = (GRI/FGP) × 100; 4) GT_{50} = number of days lapsed to reach 50% of FGP (Hsu et al.1985); 5) seedling length vigor index (SLVI) = (mean shoot length + mean root length) × FGP (Ashkan and Jalal, 2013).

After 45 d of sowing, 20 seedlings were randomly chosen to evaluate seedling growth and the following parameters were recorded for each seedling: lengths (centimeters) of shoot and root (seedlings were washed and the longest root was measured), number of leaves, and fresh and dry weights of shoots and roots (grams). Dry weight was measured after drying for 48 h at 60 °C.

HPLC estimation of phytohormones. HPLC extraction procedure was performed according to Shindy and Smith (1975) and abscisic acid (ABA) and GA_3 analyses were performed with the modifications described by Dewir et al. (2015). Samples of both stratified (3 weeks), and nonstratified seeds were used for these analyses. Five grams of fresh weight seeds was ground and soaked for 72 h in aqueous methanol (80% v/v, Merck). The extracts were filtered through Whatman filter paper (No. 42; Sigma-Aldrich Chemie, Taufkirchen, Germany). The filter paper and the residue were reextracted twice with a fresh volume of methanol and filtered. The combined extracts were evaporated to the aqueous phase using a rotary evaporator and the aqueous phase was adjusted to pH 2.8 with 1% hydrochloric acid (Merck) and extracted three times with ethyl acetate (Merck). It was evaporated to dryness, dissolved in 1 mL of HPLC methanol, and used for of ABA and GA_3 quantification at 254 nm. The extract was filtered through a membrane filter (0.45 μm) before injecting (10 μL) into a HPLC system (746 data module, 510 pump; Waters, Millford, MA) equipped with a 300 × 3.9-mm column (μBondapak C18 column, Waters). A mobile phase consisting of methanol HPLC containing 2% of glacial acetic acid was used at a flow rate of 1.0 $mL{\cdot}min^{-1}$. ABA, and GA_3 concentrations in the sample were calculated using the response ratio of the target compound and the appropriate internal standards (GA_3 = 48880; ABA = A1049).

Protein extraction and sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE electrophoresis was employed to elucidate the effect of stratification (3 weeks) on protein synthesis. Total proteins were extracted from nonstratified and stratified seeds. Briefly, each sample (0.5 g) was individually ground into powder with liquid nitrogen. Then, 0.5 mL of the protein extraction buffer (62.5 mM tris hydrochloride, pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, 5 M urea and 0.01% bromophenol blue) was mixed by vortexing. Protein extracts were centrifuged at 14,000 g_n for 10 min at 4 °C and separated by 12% SDS-PAGE according to Laemmli (1970). Molecular weights of different bands were calibrated with a mixture of standard protein markers [11-245 kDa molecular weight marker (BLUEstain; GoldBio, St. Louis, MO)]. The banding profile was stained by Coomasie blue dye then photographed and scored.

Experimental design and statistical analysis. The experiment was arranged in a completely randomized design. There were 21 treatments replicated three times and each replicate (one tray) consisted of 100 seeds. The seedling growth parameters were recorded from 10 randomly selected seedlings from each tray. Data of ABA, GA_3, and protein analysis in nonstratified and stratified seeds were analyzed separately. The mean and one-way analysis of variance were calculated using statistical software (SAS ver. 9.13; SAS Institute, Cary, NC). The mean separations were performed using Duncan's multiple range test and least significant difference test.

Results and discussion

The treatments of water soak for 24 h (Fig. 1A), 0.25 g·L^{-1} GA_3 (Fig. 1B), and stratification for 3 to 5 weeks (Fig. 1E) improved the time-course changes in the germination percentage of black cumin compared with the control. The treatments using KNO_3 and SA had reduced effects on seed germination (Fig. 1C–D). All presowing treatments tended to boost earlier germination compared with the control. The treatments with GA_3,

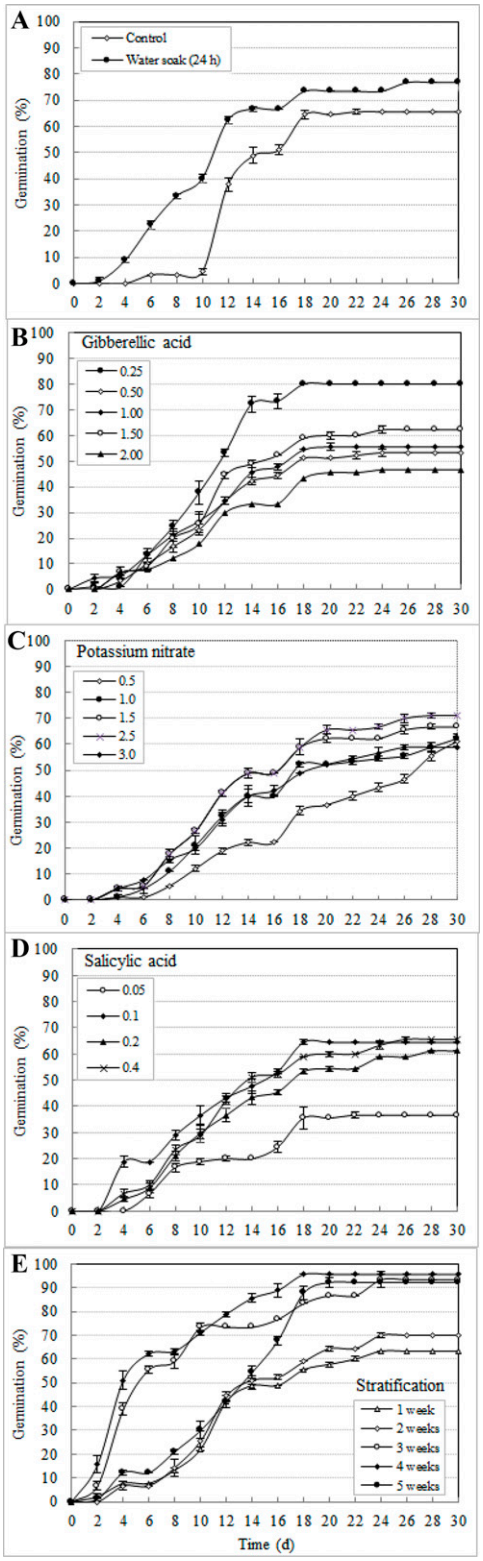

Fig. 1. Time-course changes in germination percentage for black cumin seeds with different presowing treatments: water soak (24 h), salicylic acid (0.05–0.4 g·L^{-1}), potassium nitrate (0.5–3.0 g·L^{-1}), gibberellic acid (0.25–2.0 g·L^{-1}), and stratification at 2 °C [35.6 °F (1–5 weeks)] during Oct. 2018 at the farm of Kafrelsheikh University (Kafr El-Sheikh, Egypt). Data presented are mean ± SE. Nontreated seeds are the control; 1 g·L^{-1} = 1000 ppm.

KNO_3, and SA concentrations, as well as stratification duration, also caused variations in seed germination over the full culture period. Water soak for 24 h increased the FGP from 65.55% (control) to 76.66% (Table 1). GA_3 at 0.25 $g{\cdot}L^{-1}$ also promoted FGP to 80%. The highest FGP (93.44, 95.56, and 92.22) were recorded for stratification treatments (Fig. 1E) at 3, 4, and 5 weeks, respectively. The relationships between FGP and the concentrations of pre sowing treatments revealed negative impact of SA [r^2 = 0.010 (Fig. 2A)] and KNO_3 [r^2 = 0.004 (Fig. 2B)] while high correlations with GA_3 [r^2 = 0.56 (Fig. 2C)] and stratification [r^2 = 0.79 (Fig. 2D)] were observed. The highest CGRI and the lowest GT_{50} were observed at 4 weeks of stratification while the highest seedling vigor occurred with 2 to 4 weeks of stratification (Table 1). Germination speed (CGRI and GT_{50}) of the seeds because of stratification treatments was significant for black cumin. The seeds reached 50% of their final germination in a minimum time (7.2 d) compared with control (13.53 d). High GA_3 concentrations ($\geq$0.5 $g{\cdot}L^{-1}$) as well as all SA concentrations showed inhibitory effects on seed germination.

The impermeability of resistant seeds to both water and oxygen is a major constraint on germination in many species. Speedy germination with high germination rates can frequently be achieved by soaking seeds in water for 12 to 48 h. However, the response is species dependent. For example, high seed germination (98.6%) of tomato (*Solanum lycopersicum*) could be obtained using water soaking for 12 h (Sabongari and Aliero, 2004). However, in this study, the water-soaking (24 h) treatment improved seed germination of black cumin by only 10%. This is consistent with the investigation by Shaik et al. (2008) in which germination capacity of cancer bush (*Sutherlandia frutescens*) was similar to that of the control after soaking (24 h) in water. For black cumin, high GA_3 concentration (1.25 $g{\cdot}L^{-1}$) recorded maximum FGP (76%) (Rouhi et al., 2012). However, our results indicated that low concentrations of GA_3 (0.25 $g{\cdot}L^{-1}$), rather than higher concentrations (>0.25 $g{\cdot}L^{-1}$), had stimulatory effects (80% FGP). Moreover, GA_3 (0.25 $g{\cdot}L^{-1}$) stimulated germination by 14.45% as compared with nontreated seeds. These different responses indicate the variable dormancy state of black cumin seeds through storage until the sowing. Gibberellin accumulation is often associated with dormancy release and seed germination (Finkelstein et al., 2008) by overcoming the effects of growth inhibitors (Rehman and Park, 2000). GA_3

Table 1. Effect of presowing seed treatments on final germination percentage (FGP), corrected germination rate index (CGRI), time taken to reach 50% of final germination percentage (GT_{50}), and seed length vigor index (SLVI) in black cumin after 30 d in culture during Oct. 2018 at the farm of Kafrelsheikh University (Kafr El-Sheikh, Egypt).

	Seed germination parameters[z]			
Treatment	**FGP (%)**	**CGRI**	**GT_{50} (d)**	**SLVI**
Control	65.55 def[y]	46.98 g	13.53 bc	18.156 ab
Water soak (24 h)	76.66 bc	66.61 cd	10.50 g	17.77 abc
Salicylic acid ($g{\cdot}L^{-1}$)[x]				
0.05	36.66 k	55.77 ef	12.38 cde	11.24 h
0.1	64.45 efg	69.56 c	10.31 g	13.82 fg
0.2	58.89 ghi	56.03 ef	12.75 cd	14.33 fg
0.4	65.56 def	58.22 ef	12.09 cdef	14.08 fg
Potassium nitrate ($g{\cdot}L^{-1}$)				
0.5	57.78 hi	29.52 h	20.00 a	16.58 bcde
1.0	62.23 fgh	46.68 g	14.69 b	17.63 abcd
1.5	66.67 def	53.89 efg	12.82 cd	14.17 fg
2.5	71.12 cd	51.86 fg	13.34 bc	13.38 fgh
3.0	58.89 ghi	52.50 fg	13.32 bc	14.43 efg
Gibberellic acid ($g{\cdot}L^{-1}$)				
0.25	80.00 b	60.72 de	11.08 efg	14.47 efg
0.5	53.34 i	58.69 def	11.67 defg	14.03 fg
1.0	55.56 i	66.29 cd	10.88 fg	14.39 efg
1.5	62.23 fgh	59.37 def	11.60 defg	15.63 cdef
2.0	46.67 j	57.93 ef	12.15 cdef	13.06 gh
Stratification (weeks)				
1	63.34 fgh	57.50 ef	12.41 cde	15.24 efg
2	70.00 de	53.60 efg	12.90 cd	17.87 abc
3	93.44 a	85.67 b	8.72 h	19.27 a
4	95.56 a	97.04 a	7.20 i	17.63 abcd
5	92.22 a	54.67 efg	12.86 cd	15.43 def
Significance				
LSD value	5.74*	7.99*	1.47*	2.24*

[z]FGP = number of germinated seeds (30 d from sowing/number of sown seeds) × 100; CGRI = (GRI/FGP) × 100; GT_{50} = number of days lapsed to reach 50% of FGP (Hsu et al., 1985); SLVI = (mean shoot length + mean root length) × FGP (Ashkan and Jalal, 2013).

[y]Values followed by the same letter in the same column are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

[x]1 $g{\cdot}L^{-1}$ = 1000 ppm.

*Significant at $P \leq 0.05$.

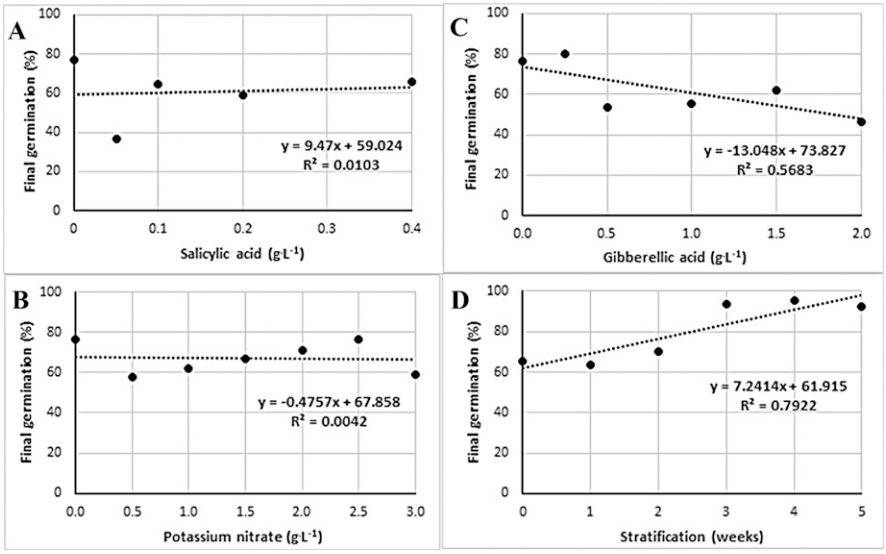

Fig. 2. Relationship between final germination percentages of black cumin seeds and concentrations of (A) salicylic acid, (B) potassium nitrate, (C) gibberellic acid, and (D) stratification at 2 °C [35.6 °F (1–5 weeks)] presowing treatments during Oct. 2018 at the farm of Kafrelsheikh University (Kafr El-Sheikh, Egypt); 1 g·L^{-1} = 1000 ppm.

promotes the activity of enzymes such as endo-β-mannanase, which loosen cell walls within the endosperm, thereby reducing resistance to radicle emergence (Yamaguchi and Kamiya, 2002). Moreover, GA_3 activates α-amylase, which digests the available carbohydrate into simpler sugars, so that energy and nutrition are easily available for faster growth of seedlings (Wani et al., 2014). Dormant seeds that require chilling, dry storage after ripening, and light as a germination stimulator, are often combined with GA_3 to overcome their dormancy with varied responses to its concentrations. Al-Hawezy (2013) reported that GA_3 concentration (>0.25 g·L^{-1}) quickly diminished the germination rate of loquat (*Eriobotrya japonica*).

Priming of black cumin seeds with 3% KNO_3 improved seed germination by 20% compared with nonprimed seeds under osmotic stress (–3 bar) conditions (Balouchi et al., 2015). Nitrogen-containing compounds have been shown to improve seed germination and seed vigor by breaking seed dormancy (Bethke et al., 2007). However, their effects are concentration and plant species dependent. For example, Dewir et al. (2011) reported that 90% seed germination of cabbage palmetto (*Sabal palmetto*) was obtained at 1% KNO_3 but higher concentrations reduced seed germination. Conversely, for key thatch palm (*Thrinax morrisii*), high KNO_3 concentrations (4%) increased seed germination (80%), whereas low concentrations had negative effects. These compounds also proved to be effective germination stimulants under stress conditions. It has been documented that KNO_3 raises the ambient oxygen levels by making less oxygen available for the citric acid cycle (Bewley and Black, 1983). Previous investigations on seed germination of black cumin during the nongrowing season by Rouhi et al. (2012) reported that KNO_3 (0.3%) stimulated germination by 76.6%, during off-season, whereas nontreated seeds did not germinate. In the present investigation, KNO_3 (2.5 g·L^{-1}) slightly stimulated seed germination by 5.57%, and nontreated seeds achieved 65.55% germination. Clearly, KNO_3 treatment could be used to break the dormancy of black cumin seeds after harvesting and during storage (Rouhi et al., 2012), but in the present study, it was not shown to be effective to obtain synchronized germination during the sowing and cultivation seasons. In the present investigation, the presowing treatment of black cumin seeds with SA did not improve germination. However, previous studies pointed out that SA facilitates seed germination under saline and drought stresses (Anaya et al., 2018; Carvalho et al., 2007; Farhadi et al., 2016).

Cold stratification has been reported to stimulate de novo GA biosynthesis leading to seed germination (Oh et al., 2006). In this study, ABA and GA_3 contents in stratified black cumin increased compared with nonstratified seeds (Fig. 3A and B). Previous studies addressed the positive effects of stratification on seed germination of several plant species, including devil's dung [*Ferula assafoetida* (Raisi et al., 2013)] and wild celery [*Kelussia odoratissima* (Shaykhi et al., 2015)]. In arabidopsis (*Arabidopsis thaliana*), exposure of imbibed seeds to cold conditions resulted in increased expression of *GA20ox1* and *GA20ox2* (Yamauchi et al., 2004). Moreover, it has been demonstrated that a cold stratification period can synchronize germination (Baskin and Baskin, 2014). For black cumin, Rouhi et al. (2012) reported that cold stratification for 3 weeks at 5 ± 1 °C resulted in 82% seed germination, whereas in our investigation, 95.56% seed germination was recorded for 4 weeks cold stratification at 4 °C. We also noted that prolonged cold stratification for 5 weeks resulted in a decline in germination (92.22%). SDS-PAGE banding patterns revealed 20 scorable bands with different molecular weights (Table 2, Fig. 4). Among them, 14 bands showed high variability. However, the other six bands were commonly detected in the stratified and nonstratified seeds. The most visible alterations in SDS-PAGE patterns of stratified seeds were the alteration in band intensities (100, 75, 72, 68, 63, 62, 50, 48, 40, 39, 35, 34, and 33 kDa) and the appearance of a new band (180 kDa). Many of the

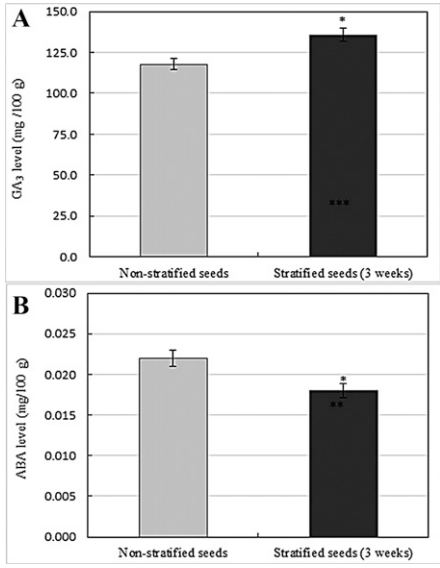

Fig. 3. High-performance liquid chromatography (HPLC) level of (A) gibberellic acid (GA_3) and (B) abscisic acid (ABA) in nonstratified vs. stratified (3 weeks) seeds of black cumin during Oct. 2018 at the farm of Kafrelsheikh University (Kafr El-Sheikh, Egypt). Data presented are mean ± SE; *Significantly different at $P \leq 0.05$; 1 mg/100 g = 10 ppm.

physiological, biochemical, and molecular changes caused by low temperature are triggered by changes in gene expression, and the transcriptional activation and repression of genes. The early transient response to cold stress encompasses genes encoding transcription factors, cell signaling components, and those involved in detoxification processes (Rihan et al., 2014; Sun et al., 2007), whereas the genes active during the late response played a role in metabolism, cell structure, and transport systems (Kreps et al., 2002).

Growth of black cumin seedlings was significantly improved by presowing treatments and their concentrations (Table 3). Although low concentration of GA_3 at 0.25 g·L^{-1} enhanced seed germination, it did not favor seedling growth. Stratification treatment for 3 weeks resulted in statistically greater fresh and dry weights than the control. However, no significant differences were recorded for shoot and root length, or number of leaves compared with control. Number of leaves was not significantly influenced by stratification treatments. The highest seedling length and dry weight were attained from stratification of wild celery seeds for 12 weeks (Shaykhi et al., 2015). Dhupper (2013) reported that cold water pre-treatment (24 h) resulted in the best

Table 2. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis banding patterns of total protein isolated from 3 weeks stratified and nonstratified (control) black cumin seeds during Oct. 2018 at the farm of Kafrelsheikh University (Kafr El-Sheikh, Egypt).

Band no.	Molecular mass (kDa)	Nonstratified seeds	Stratified seeds
1	180	–	+
2	100	+	++
3	75	+	++
4	72	+	++
5	68	++	+++
6	63	+	++
7	62	+	++
8	50	+	++
9	48	++	+++
10	40	+++	++++
11	39	+++	++++
12	35	+	++++
13	34	++	++++
14	33	++++	++++
15	32	+	+
16	30	+	+
17	25	+	+
18	24	+	+
19	23	+	+
20	20	+	+

– = absent; + = weak; ++ = intermediate; +++ = strong; ++++ = very strong.

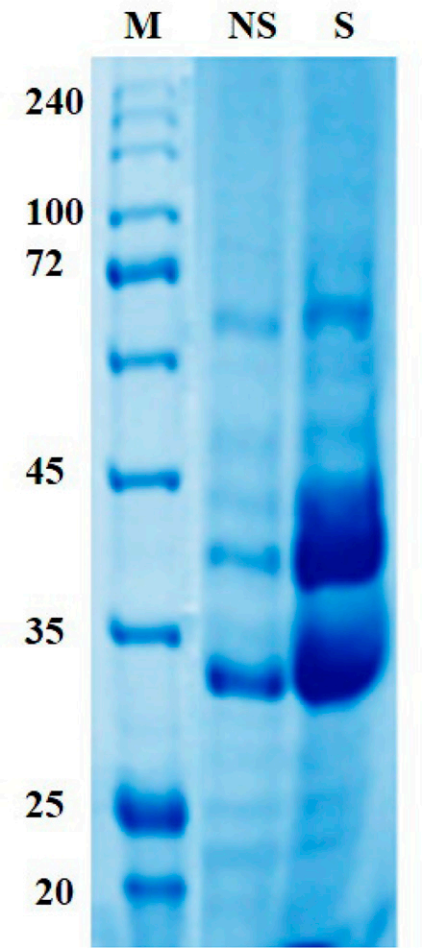

Fig. 4. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis showing protein bands patterns of 3 weeks stratified (S) and nonstratified [NS (control)] black cumin seeds during Oct. 2018 at the farm of Kafrelsheikh University (Kafr El-Sheikh, Egypt); M = 11–245 kDa molecular weight marker (BLUEstain; GoldBio, St. Louis, MO).

Table 3. Effect of presowing seed treatments on seedling growth of black cumin after 45 d in culture during Oct. 2018 at the farm of Kafrelsheikh University (Kafr El-Sheikh, Egypt).

Treatment	Seedling growth parameters[z]				
	Shoot length (cm)	Root length (cm)	Leaves (no./seedling)	Fresh wt (g/seedling)	Dry wt (g/seedling)
Control	8.00 ab[y]	9.5 abc	4.7 abc	0.2715 d	0.047de
Water soak (24 h)	7.67 ab	9.34 abc	5.00 a	0.323 c	0.057 b
Salicylic acid ($g \cdot L^{-1}$)[z]					
0.05	5.34 fgh	5.54 h	4.16 bcd	0.214 fgh	0.036 hi
0.1	6.17 defg	7.0 efgh	4.33 bcd	0.230 fg	0.037 hi
0.2	6.24 cdefg	7.50 defg	4.00 cd	0.206f-j	0.035 i
0.4	7.00 bcde	6.44 gh	4.17 bcd	0.205f-j	0.040 g
Potassium nitrate ($g \cdot L^{-1}$)					
0.5	7.00 bcde	9.00 abcd	4.66 abc	0.236 ef	0.039 gh
1.0	6.34 cdef	10.67 a	5.00 a	0.264 de	0.043 f
1.5	5.5 fgh	8 0.0 cdefg	4.34 bcd	0.180 ijk	0.035 i
2.5	6.00 efg	6.67 fgh	3.67 de	0.179 ijk	0.030 jk
3.0	5.167 gh	8.67 bcde	4.66 abc	0.163k	0.026lm
Gibberellic acid ($g \cdot L^{-1}$)					
0.25	5.84 fg	7.84 cdefg	4.16 bcd	0.185 h-k	0.037 hi
0.5	5.167 gh	8.34 bcdef	4.17 bcd	0.175jk	0.028 kl
1.0	5.34 fgh	8.5 bcde	3.84 de	0.208 f-i	0.032 j
1.5	6.34 cdef	8.67 bcde	3.67 de	0.159 k	0.027lm
2.0	4.60 h	8.00 cdefg	3.16 e	0.190 g-k	0.025 m
Stratification (weeks)					
1	6.27 cdefg	8.34 bcdef	4.67 abc	0.224 f	0.035 i
2	7.17 bcd	10.00 ab	5.00 a	0.362 b	0.052 c
3	8.50 a	9.84 ab	5.00 a	0.42 8 a	0.064 a
4	7.34 bc	9.34 abc	4.83 ab	0.305 c	0.048 d
5	7.84 ab	6.67 fgh	5.00 a	0.220 fg	0.045 ef
Significance					
LSD value	1.12*	1.71*	0.719*	0.031*	0.002*

[z]1 cm = 0.3937 inch, 1 g = 0.0353 oz, 1 $g \cdot L^{-1}$ = 1000 ppm.
[y]Values followed by the same letter in the same column are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.
*Significant at $P \leq 0.05$.

growth behavior of arabic gum tree (*Acacia nilotica*), lebbeck (*Albizzia lebbeck*), and ghaf (*Prosopis cineraria*). It has been generally noted that presowing chemical treatments did not enhance seedlings growth compared with water soaking treatment or the control treatment. Application of presowing cold stratification treatment has been reported to improve seedlings growth.

Conclusions

Our investigation confirmed that black cumin seeds possess physiological dormancy. Stratification treatments (3 and 4 weeks) increased final seed germination and speed of germination leading to synchronization of seed germination during the sowing season. Cold stratification modulated GA_3 and ABA levels and proved effective to release seed dormancy. This could positively improve the total yield of this medicinally important plant.

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