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Efficacy of Priming Technique to Enhance Germination of Cumin (*Cuminum cyminum*) Seeds of Different Lifespans

Ali Moradi[*1], Sajad Sohrabiani[1], Ramin Piri[2], Bahman Fazeli-Nasab[3,4], Muhammad Farooq[*5]

[1]*Department of Agronomy and Plant Breeding, College of Agriculture, University of Yasouj, Yasouj, Iran*
[2]*Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Tehran, Tehran, Iran*
[3]*Department of Biotechnology and Plant Breeding, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad 9177948978, Iran*
[4]*Department of Agronomy and Plant Breeding, Agriculture Institute, Research Institute of Zabol, Zabol, Iran*
[5]*Department of Plant Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, Al-Khoudh, Muscat, Oman*

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ABSTRACT

Optimum germination and seedling establishment are important stages in the life cycle of plants with dormant seeds. This research was conducted to evaluate the impact of seed priming on the germination indices and seedling growth of cumin seeds with different lifetimes. Cumin seeds of different lifetimes (freshly harvested seeds, 12 and 24 months old) were soaked in distilled water (hydropriming), KH_2PO_4 (1 and 2%), NaH_2PO_4 (1 and 2%), ascorbic acid (AA; 100 and 200 mg L^{-1}) and Gibberellic acid (GA; 100 and 200 mg.L^{-1}) at 4 °C and 20 °C for 12 or 24 h. Seed priming treatments caused significant improvement in the germination of seeds with all three lifetimes. In 12 and 24 months old seeds, seed priming with NaH_2PO_4 and AA were more effective in improving the germination indices compared to other priming composition. However, in freshly harvested seeds, due to relative dormancy, treatment with GA was more effective compared to the other priming treatments. In the most of germination indices, seed priming at 20 °C along with 24 h showed higher effectiveness compared to other priming temperature and duration combinations. The overall results showed that the response of cumin seeds to different priming treatments depends on seed age.

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1. Introduction

Cumin (*Cuminum cyminum* L.) is known to be the one of most important cultivated medicinal plants. This plant is cultivated extensively in regions with arid and semi-arid climates such as Iran, China, Türkiye, Algeria, and Morocco (Piri *et al.*, 2021).

Cumin is used to increase urine flow and relieve bloating, as well as in spices, foods, and beverages as a flavoring component (Janipour *et al.*, 2017). In other manufacturing processes, cumin oil is used as a fragrance in cosmetics (Janipour *et al.*, 2018). In Iranian traditional medicine, cumin seeds were used for their therapeutic effects on gastrointestinal, gynecological and respiratory disorders, and also for the treatment of toothache, diarrhea and epilepsy. The seeds were also documented as stimulant, carminative and astringent. The production of cumin plants is challenging because of the poor germination of seed lots and weak establishment of seedlings (Janipour *et al.*, 2018; Moradi and Piri, 2018).

The high oil content (10%) of cumin seeds in addition to volatile oils such as cumin aldehyde, cymene, and terpenoids (Merah *et al.*, 2020) leads to acceleration of seed aging, increasing the deterioration rate followed by decreasing seed vigor (Kumar *et al.*, 2019). Furthermore, cumin seeds are reported to have different degrees of dormancy (Namjoo *et al.*, 2022).

Most of the seeds are usually stored from several days to several years after the harvest (De Vitis *et al.*, 2020). Temperature, relative humidity, seed moisture

* Corresponding author.
E-mail addresses: amoradi@yu.ac.ir; farooqcp@squ.edu.om

content, and storage period are the most important factors affecting the quality of the seed in storage (Bakhtavar *et al.*, 2019). During ageing, a series of biochemical changes happen inside a seed. These changes include decreased metabolic activities, changes in enzyme activities and decreased synthesis of proteins and nucleic acids, damage to the cell wall and membrane lipids, and increased electrolyte leakage (Gebeyehu, 2020; Thirusendura Selvi and Saraswathy, 2018). Physiological changes during the seed deterioration include increased susceptibility to pathogens and a decrease in the germination capability of the seeds at the time of sowing and seedling growth and establishment (Farooq *et al.*, 2019; Fazeli-Nasab and Sayyed, 2019; Santos *et al.*, 2021).

The application of artificial physicochemical treatments could help more rapid and uniform emergence by improving the seed performance and seed germination rate (Bose *et al.*, 2018; Shelar *et al.*, 2021). The common priming compounds include water, hormones, salts, vitamins and matrix components, etc. which have diverse effects on the improvement of the seed performance. Seed priming reverses some adverse events of seed ageing. Seed priming techniques also lead to changes in the amounts of proteins, however, the type of the proteins is usually not altered. The increase in tRNA are tangible and the amount of mRNA is fixed. Higher production of ATP and energy during priming at the tip of the root leads to increased protein synthesis (Liu *et al.*, 2022).

Seed priming treatment causes a decrease in the average germination time and an increase in the germination percentage of deteriorated sunflower seeds. In addition, the priming treatments' performance also depends on the physiological state of the seed (Pagano *et al.*, 2023). The seed age and use of gibberellic acid (GA) as a priming agent significantly affected the cumin seed germination (Ipek *et al.*, 2008).

The effectiveness of seed priming depends on the physiological state of the seed. The freshly harvested cumin seed lots have some levels of physiological dormancy (Soltani *et al.*, 2019). In nature, this dormancy is removed by the factors such as thermal cycles and washing germination inhibitors. However, in laboratory conditions, seed treatments such as GA, moist chilling, and potassium nitrate help break the dormancy. In research on 40 species of medicinal plants, it was revealed that cumin seed has some levels of physiological dormancy and the GA seed treatment was the most effective to break the dormancy of cumin seeds (Aghilian *et al.*, 2014).

Cumin seed is prone to deterioration during storage due to its oil and active ingredients such as volatile oils. Few studies are carried out regarding the relationship between the treatments of improving germination or breaking dormancy of cumin seeds having different seed lifetimes. Selecting the treatment suitable to the physiological state of the seed could help improve the germination of the freshly harvested seeds as well as the cumin seeds with a different lifetime. Therefore, this study was conducted to answer the question of whether the selection of the seed priming treatment is related to the seed age (the initial physiological state of the seed).

2. Materials and methods

A laboratory experiment was carried out on cumin seeds (collected from the Sabzevar region of the Khorasan Province, Iran) in the Seed Science and Technology Laboratory of Agriculture Faculty at Yasouj University, Iran. Seeds used consisted of different lifetimes i.e., freshly harvested (harvest of 2014), 12-month lifetime (harvest of 2013), and 24-month lifetime (harvest of 2012) which were stored at 25°C until the time of the experiment. At the beginning of the experiment, the initial germination percentage of the seeds was measured based on the ISTA method (ISTA, 2010). The seed viability was evaluated using the tetrazolium test (Table 1).

Table 1. Basic attributes of seeds used in the study

Seed age (month)	1000 seed weight (g)	Seed moisture content (% of fresh weight)	Viability (%)	Germination (%)
Fresh	4.22	5.99	98	49
12	4.15	5.7	97	76
24	4.11	5.56	91	66

For priming, seeds were soaked in distilled water (hydropriming), 1% and 2% solutions of potassium dihydrogen phosphate (KH_2PO_4) and sodium dihydrogen phosphate (NaH_2PO_4), and 100 and 200 mg L^{-1} solutions of ascorbic acid (AA) and gibberellic acid (GA) at 4 or 20°C for 12 or 24 hours. After the priming, the seeds were removed from the solutions, washed thoroughly and then dried between two layers of paper for 24 h at room temperature. Before the germination test, to prevent seed contamination, the seeds were disinfected with a fungicide (carboxyinthiram). The germination test was carried out in four 25-seed replicates inside 9 cm Petri dishes with filter paper and 5 ml of distilled water. The Petri dishes were kept inside a germinator with alternating temperatures of 20-30°C for 14 days (Piri *et al*., 2021; ISTA, 2010). In this test, untreated seeds were used as control samples. During the test, germination counting was done on a daily basis at a certain time. The germination criterion was a 2 mm emergence of the radicle. At the end of the experiment, the total germination percentage (GP) (Eq. 1), germination rate (Eq. 2), mean germination time (Eq.3), seedling length, seedling dry weight, and seedling vigor index (Eq.4) were measured.

$$GP = \frac{germinated\ seeds}{total\ seeds\ in\ the\ replication} \times 100 \quad (1)$$

(ISTA, 1999)

$$Germination\ rate = \sum \left(\frac{n_i}{d_i}\right) \quad (2)$$

(Ellis and Roberts, 1981)

Where n_i is the germinated seeds in the day i and d_i days after the germination began.

$$\text{Mean germination time (days)} = \sum(Ni \times Di)/N \quad (3)$$

Where N_i the germinated seeds are in the ith day, D_i is the number of days after the germination began, and N is the total number of germinated seeds.

Seedling vigor index= (Standard germination percentage × average seedling weight)/100 (4)

Statistical analyses of the results of the experiment were carried out based on a completely random design (CRD) with four replications using SAS (Version 9.4) software. For drawing the diagrams, excel software was used. Comparing the averages was conducted using the LSD test at a 5% probability level.

3. Results

3.1. Germination percentage

Comparing the interaction of priming, temperature and priming duration for cumin seeds with different lifetimes showed that in freshly harvested seeds (production of 2014), and GA treatment showed the highest impact on germination. The GA seed treatment increased the seed germination by 14% to 33%, although. In the primed seeds with 12 months' lifetimes, the highest germination percentage was observed for AA seed priming (100 mg L^{-1} at 20°C for 24 h). In seeds with a 24-months lifetime, most of the priming treatments had higher germination compared to unprimed seeds. In this condition, the highest germination was observed for seed priming with 1% $NaH_2PO_4$1% (at 20°C for 24 h), 1% NaH_2PO_4 at 4°C for 24 h and AA (100 mg L^{-1} at 20°C for 12 h). These treatments enhanced seed germination from 49% to 89% (Table 2).

3.2. Germination rate

The freshly harvested seeds (production of 2014) showed the highest germination rate in seed GA priming treatment (100 mg L^{-1} at 20°C for 24 h) (3.12 seed d^{-1}). The same treatments had the highest germination rate for the seeds with lifetimes of 12 and 24 months. In seeds with 12 months lifetime, seed priming with NaH_2PO_4 increased the germination rate to a greater extent compared to other priming treatments. The highest germination rate in these seeds was observed for NaH_2PO_4 (1% at 4°C for 24 h) (3.42 seed d^{-1}). In most of the treatments, the combination of the temperature of 20°C and duration of 24 h had a higher germination rate compared to other temperature and priming duration combinations. In seeds with a 24-month lifetime, the highest germination rate was observed for NaH_2PO_4 (1% at 20°C for 12 h) (Table 2). However, this treatment combination could increase the germination rate from 1.7 seeds per day in unprimed seeds to 3.4 seeds per day.

3.3. Seedling dry weight

The results concerning the interaction of priming treatment, temperature, and priming duration in seeds with different lifetimes showed that the seedling dry weight was significantly affected by different priming treatments (Table 3). In freshly harvested seeds (harvested in 2014), the highest seedling dry weight was obtained from the seed priming with GA (200 mg L^{-1} at 20°C for 12 h). In seeds with 12 months lifetime (production 2013), the highest seedling dry weight was obtained from GA seed priming (200 mg L^{-1} at 4°C for 24 h). In seeds with a lifetime of 24 months, the highest seedling dry weight was observed for the treatment with GA (200 mg L^{-1} at 4°C for 12 h). However, the combination of temperature 20°C with 24-hour priming duration showed higher seedling dry weights compared to other combinations.

3.4. Seedling vigor index

The results showed that priming treatments, temperature and priming time and their interaction had a significant effect on seedling vigor index in cumin. In freshly harvested seeds, the highest seedling vigor index was obtained for the treatment with GA100 mg. L^{-1} -24 h- 20°C, such that the seedling vigor index was increased from 0.003 in unprimed seeds to 0.0065. In seeds with lifetimes of 12 months, the highest seedling vigor index was observed in primed seeds with gibberellic acid. GA200- 24 h-20 °C had the highest seedling vigor index. Meanwhile, for the seeds with 24-month lifetimes the highest seedling vigor index (weight) was observed in GA200- 24 h- 4 °C (Table 3). However, this treatment did not have a meaningful difference from some of the other priming treatments. The combination of a temperature of 20 °C with 24-h duration led to a higher seedling vigor index compared to other temperatures and priming durations. The unprimed seeds had the lowest seedling vigor index.

Table 2. The effects of seed priming treatments on germination percentage and germination rate of cumin seeds with different lifetime.

Seed priming	Temperature and duration of the priming		Germination percentage			Germination rate (seed d^{-1})		
			Seed Age (months)			Seed Age (months)		
			Fresh	12	24	Fresh	12	24
Hydropriming	4 °C	12 h	78^{de}	77^{i}	60^{f-g}	2.29^{m}	2.20^{o}	1.40^{j-l}
		24 h	85^{a-d}	83^{g-i}	60^{f-g}	2.83^{c-j}	2.61^{j-n}	1.52^{g-k}
	20 °C	12 h	83^{a-d}	88^{d-h}	65^{c-f}	2.49^{i-m}	2.86^{e-k}	1.69^{d-i}
		24 h	85^{a-d}	87^{e-h}	71^{b-d}	2.88^{b-i}	2.48^{k-o}	1.85^{b-d}
GA_3 100 mg L^{-1}	4 °C	12 h	81^{b-e}	84^{f-i}	63^{e-g}	2.76^{e-k}	2.67^{i-n}	1.63^{d-j}
		24 h	84^{a-d}	88^{d-h}	72^{bc}	2.82^{c-j}	2.82^{f-l}	1.81^{c-e}
	20 °C	12 h	87^{a-c}	90^{b-g}	74^{b}	2.89^{b-h}	3.11^{a-h}	2.04^{bc}
		24 h	88^{ab}	93^{a-e}	82^{a}	3.12^{a-e}	3.31^{a-c}	2.30^{a}
GA_3 200 mg L^{-1}	4 °C	12 h	85^{a-d}	81^{hi}	64^{d-f}	2.69^{f-m}	2.46^{l-o}	1.55^{f-k}
		24 h	85^{a-d}	79^{i}	70^{b-e}	2.89^{b-i}	2.28^{no}	1.74^{d-h}
	20 °C	12 h	84^{a-d}	81^{hi}	70^{b-e}	2.77^{e-j}	2.45^{l-o}	1.79^{c-f}
		24 h	85^{a-d}	90^{b-g}	82^{a}	3.04^{a-g}	2.79^{g-l}	2.09^{ab}
KH_2PO_4 1%	4 °C	12 h	83^{a-d}	92^{a-e}	63^{e-g}	2.60^{h-m}	2.69^{i-m}	1.49^{h-l}
		24 h	84^{a-d}	89^{c-g}	65^{c-f}	2.55^{h-m}	2.65^{i-n}	1.52^{g-k}
	20 °C	12 h	83^{a-d}	89^{c-g}	71^{b-d}	2.56^{h-m}	2.73^{h-m}	1.71^{d-i}
		24 h	86^{a-c}	95^{a-d}	71^{b-d}	2.61^{h-m}	2.88^{e-j}	1.79^{c-f}
KH_2PO_4 2%	4 °C	12 h	80^{c-e}	93^{a-e}	67^{b-f}	2.46^{j-m}	2.81^{g-l}	1.59^{e-k}
		24 h	78^{de}	90^{b-g}	56^{g}	2.36^{k-m}	2.84^{f-l}	1.25^{l}
	20 °C	12 h	80^{c-e}	90^{b-g}	56^{g}	2.58^{h-m}	2.70^{i-m}	1.34^{kl}
		24 h	85^{a-d}	95^{a-d}	60^{f-g}	2.73^{e-l}	2.91^{d-j}	1.52^{g-k}
NaH_2Po_4 1%	4 °C	12 h	87^{a-c}	94^{a-e}	65^{c-f}	3.20^{a-d}	3.20^{a-f}	1.74^{d-h}
		24 h	89^{a}	96^{a-c}	65^{c-f}	3.08^{a-f}	3.42^{a}	1.72^{d-i}
	20 °C	12 h	86^{a-c}	96^{a-c}	65^{c-f}	3.41^{a}	3.32^{a-c}	1.73^{d-h}
		24 h	89^{a}	97^{ab}	70^{b-e}	3.24^{ab}	3.39^{a}	1.80^{c-f}
NaH_2Po_4 2%	4 °C	12 h	88^{ab}	93^{a-e}	69^{b-e}	2.87^{b-i}	3.29^{a-d}	1.66^{d-j}
		24 h	88^{ab}	88^{d-h}	65^{c-f}	3.02^{a-g}	2.96^{c-j}	1.68^{d-i}
	20 °C	12 h	87^{a-c}	96^{a-c}	68^{b-e}	3.22^{a-c}	3.35^{ab}	1.88^{b-d}
		24 h	82^{a-e}	94^{a-e}	64^{d-f}	3.02^{a-g}	3.33^{a-c}	1.64^{d-j}
AA 100 mg L^{-1}	4 °C	12 h	84^{a-d}	81^{hi}	74^{b}	2.77^{e-j}	2.36^{m-o}	1.85^{b-d}
		24 h	87^{a-c}	93^{a-e}	56^{g}	2.83^{c-j}	2.95^{c-j}	1.34^{kl}
	20 °C	12 h	89^{a}	95^{a-d}	70^{b-e}	2.80^{d-j}	3.00^{b-i}	1.78^{c-f}
		24 h	88^{ab}	91^{b-f}	71^{b-d}	2.85^{b-j}	3.23^{a-e}	1.71^{d-i}
AA 200 mg L^{-1}	4 °C	12 h	75^{e}	91^{b-f}	64^{d-f}	2.35^{lm}	2.75^{g-m}	1.47^{i-l}
		24 h	84^{a-d}	91^{b-f}	70^{b-e}	2.64^{g-m}	2.95^{c-j}	1.74^{d-h}
	20 °C	12 h	81^{b-e}	89^{c-g}	64^{d-f}	2.89^{b-i}	2.88^{e-j}	1.46^{i-l}
		24 h	88^{ab}	99^{a}	67^{b-f}	3.07^{a-f}	3.13^{a-g}	1.75^{d-g}
Control†	-		66	76	49	1.70	2.29	1.21

Means sharing the same letters for a parameter don't have different significance at $p < 0.05$. †; not included in data analysis.

3.5. Seedling length

The results revealed that the seedling length was affected by the experimental treatments. In freshly harvested seeds, the maximum seedling length was related to the NaH_2PO_4 treatment at 12 h and 4 °C, so that seedling length increased from 3 cm in unprimed seeds to 5.3 cm in this treatment. Also, the lowest seedling length was observed for hydro-primed seeds at 4°C for 12 hours. In seeds primed for 12 months with NaH_2PO_4 1 to 4°C - 24 h increased seedling length from 4.12 cm in unprimed seeds to 7.7 cm. However, in seeds primed for 24 months with NaH_2PO_4, longer seedlings were produced compared to other treatments (Table 4). In this condition, the longest seedling was observed in this combination with 2% concentration with 24 h duration and temperature 20 °C, such that the length of the seedling was increased from 3.6 cm in unprimed seeds to 8.5 cm. KH_2PO_4 1% treatment at a temperature of 20 °C with a duration of 12 h led to the shortest seedling. Among the priming temperature and duration, the longest seedlings were observed in combination with 20°C and 12 and 24 hours.

Table 3. The effects of seed priming on seedling dry weight and seedling vigor index of cumin seeds with different lifetime.

Seed priming	Temperature and duration of the priming		Seedling dry weight (g)			Seedling vigor index		
			Seed Age (months)			Seed Age (months)		
			Fresh	12	24	Fresh	12	24
Hydropriming	4 °C	12 h	0.0089d^{e}	0.00847^{h-j}	0.00642^{k-o}	0.00694^{d-k}	0.00652^{g}	0.00385^{n-p}
		24 h	0.00857^{e-g}	0.00975^{c-g}	0.00627^{m-o}	0.0073^{d-h}	0.00812^{a-f}	0.00376^{op}
	20 °C	12 h	0.00832^{e-i}	0.00935^{e-i}	0.00752^{c-g}	0.00691^{d-k}	0.00823^{a-f}	0.00488^{d-k}
		24 h	0.00895d^{e}	0.01072^{a-c}	0.00645^{k-o}	0.0076^{c-e}	0.00933^{ab}	0.00457^{h-l}
GA_3 100 mg L^{-1}	4 °C	12 h	0.0094^{cd}	0.0091^{g-j}	0.00762^{b-f}	0.00761^{c-e}	0.00765^{d-g}	0.00481^{e-k}
		24 h	0.01017^{ab}	0.01042^{a-e}	0.00792^{bc}	0.00857^{ab}	0.00919^{a-c}	0.00569^{bc}
	20 °C	12 h	0.0101^{ab}	0.01025^{b-}f	0.00725^{d-j}	0.00878^{a}	0.0092^{a-c}	0.00536^{c-f}
		24 h	0.0094^{cd}	0.0094^{e-i}	0.008^{bc}	0.00827^{a-c}	0.00874^{a-f}	0.00655^{a}
GA_3 200 mg L^{-1}	4 °C	12 h	0.0107^{a}	0.01112^{ab}	0.008^{bc}	0.00907a	0.00901^{a-d}	0.00512^{c-i}
		24 h	0.0103^{ab}	0.01147^{a}	0.00857^{a}	0.00877^{a}	0.00911^{a-c}	0.006^{ab}
	20 °C	12 h	0.0085^{e-h}	0.01112^{ab}	0.0086^{a}	0.00713^{d-i}	0.00905^{a-c}	0.00602^{ab}
		24 h	0.0089^{de}	0.01052^{a-d}	0.0078^{b-d}	0.00756^{c-f}	0.00948^{a}	0.00641^{a}
KH_2PO_4 1%	4 °C	12 h	0.00817^{f-j}	0.00925^{f-j}	0.00722^{e-j}	0.00678^{e-k}	0.00853^{a-f}	0.00454i^{-m}
		24 h	0.00867^{ef}	0.00885^{g-j}	0.00672^{j-n}	0.0073^{d-h}	0.00787^{c-g}	0.00436^{k-o}
	20 °C	12 h	0.00755^{jk}	0.00895^{g-j}	0.00642^{k-o}	0.00627i^{-k}	0.008^{b-f}	0.00457^{h-l}
		24 h	0.00895^{de}	0.0092^{f-j}	0.00762^{b-f}	0.0077^{b-d}	0.00873^{a-f}	0.00541^{b-e}
KH_2PO_4 2%	4 °C	12 h	0.00835^{e-h}	0.00907^{g-j}	0.00772^{b-f}	0.00667^{f-k}	0.00842^{a-f}	0.00517^{c-h}
		24 h	0.0079^{h-j}	0.00927^{f-j}	0.0081^{ab}	0.00617^{k}	0.00834^{a-f}	0.00453^{i-m}
	20 °C	12 h	0.00857^{e-g}	0.0094^{e-i}	0.00792^{bc}	0.00686^{d-k}	0.00845^{a-f}	0.00445^{j-n}
		24 h	0.00837^{e-h}	0.009^{g-j}	0.00685^{i-l}	0.00712^{d-i}	0.00856^{a-f}	0.00402^{l-p}
NaH_2Po_4 1%	4 °C	12 h	0.00712^{k}	0.00915^{f-j}	0.00745^{c-h}	0.0062^{jk}	0.0086^{a-f}	0.00482^{e-k}
		24 h	0.00975^{bc}	0.00937^{e-i}	0.00757^{b-f}	0.00866^{a}	0.009^{a-d}	0.00492^{d-k}
	20 °C	12 h	0.00767^{i-k}	0.00837^{ij}	0.00697^{g-k}	0.0066^{g-k}	0.00803^{b-f}	0.00454^{i-m}
		24 h	0.0101^{ab}	0.00852^{h-j}	0.00717^{f-j}	0.00898^{a}	0.00826^{a-f}	0.00502^{d-j}
NaH_2Po_4 2%	4 °C	12 h	0.00865^{ef}	0.00942^{d-i}	0.0069^{h-l}	0.00763^{c-e}	0.00876^{a-f}	0.00475^{f-k}
		24 h	0.00797^{g-j}	0.0094^{e-i}	0.00745^{c-h}	0.007^{d-k}	0.00827^{a-f}	0.00485^{d-k}
	20 °C	12 h	0.00815^{f-j}	0.0085^{h-j}	0.0078^{b-d}	0.00711^{d-j}	0.00815^{a-f}	0.00529^{c-g}
		24 h	0.00802^{f-j}	0.00947^{d-i}	0.0068i^{-m}	0.00659^{g-k}	0.0089^{a-e}	0.00435^{k-o}
AA 100 mg L^{-1}	4 °C	12 h	0.0081^{f-j}	0.00915^{f-j}	0.0064^{l-o}	0.0068^{d-k}	0.00741^{f-g}	0.00474^{f-k}
		24 h	0.00855^{e-h}	0.00822^{j}	0.00615^{o}	0.00744^{c-g}	0.00759^{e-g}	0.00344^{p}
	20 °C	12 h	0.00865^{ef}	0.00952^{d-h}	0.00617^{no}	0.00769^{b-e}	0.00906^{a-c}	0.00432^{k-o}
		24 h	0.00997^{bc}	0.00925^{f-j}	0.00677^{i-m}	0.00877^{a}	0.00842^{a-f}	0.0048^{e-k}
AA 200 mg L^{-1}	4 °C	12 h	0.00855^{e-h}	0.00982^{c-g}	0.0073^{d-i}	0.0064^{h-k}	0.00894^{a-e}	0.00467^{g-k}
		24 h	0.00852^{e-h}	0.00972^{c-g}	0.00777^{b-e}	0.00714^{d-i}	0.00884^{a-e}	0.00544^{b-d}
	20 °C	12 h	0.00857^{e-g}	0.000379^{e-i}	0.00615^{o}	0.00693^{d-k}	0.00834^{a-f}	0.00394^{m-p}
		24 h	0.00997^{bc}	0.00892^{g-j}	0.00722^{e-j}	0.00877^{a}	0.00883^{a-e}	0.00483^{d-k}
Control		-	0.0065	0.00917	0.0069	0.00428	0.00688	0.00338

Means sharing the same letters for a parameter don't different significant at p 0.05. †; not included in data analysis.

Table 4. The effects of seed priming on seedling length of cumin with different lifetime.

Seed priming	Temperature and duration of the priming		Seedling length (cm) Seed Age (months) Fresh	12	24
Hydropriming	4°C	12 h	4.78^{jk}	4.23^{u}	2.17^{v}
		24 h	6.77^{de}	6.40e^{f}	2.62^{s-u}
	20°C	12 h	4.77^{jk}	5.35^{m-r}	3.80^{h-k}
		24 h	5.85^{hi}	5.83^{h-l}	3.32^{l-p}
GA_3 100 mg L^{-1}	4°C	12 h	5.80^{hi}	6.10^{f-i}	3.97^{g-i}
		24 h	5.74^{i}	6.22^{e-h}	3.56^{i-n}
	20 °C	12 h	5.99^{g-i}	5.50^{k-q}	3.86^{h-j}
		24 h	7.11^{cd}	5.42^{l-q}	3.72^{h-l}
GA_3 200 mg L^{-1}	4 °C	12 h	5.73^{i}	5.19^{o-s}	3.11^{o-r}
		24 h	5.94^{g-i}	5.17^{p-s}	3.62^{h-m}
	20 °C	12 h	7.70^{b}	5.34^{n-r}	3.74^{h-l}
		24 h	6.69^{d-f}	6.31^{e-g}	3.37^{l-p}
KH_2PO_4 1%	4 °C	12 h	4.97^{j}	4.56^{tu}	3.54^{i-n}
		24 h	4.85^{j}	4.93^{r-t}	2.86^{q-t}
	20 °C	12 h	4.34^{k}	5.04^{q-s}	4.29^{d-g}
		24 h	4.86^{j}	5.81^{h-m}	3.89^{g-i}
KH_2PO_4 2%	4 °C	12 h	5.14^{j}	5.91^{g-k}	3.40^{k-p}
		24 h	4.79^{jk}	5.84^{h-l}	2.59^{s-v}
	20 °C	12 h	4.89^{j}	5.67^{i-n}	3.19^{n-q}
		24 h	4.87^{j}	6.35^{e-g}	4.91^{bc}
NaH_2Po_4 1%	4 °C	12 h	6.62^{d-f}	7.42^{a-c}	5.34^{a}
		24 h	7.09^{cd}	7.75^{a}	4.39^{d-f}
	20 °C	12 h	7.91^{b}	7.61^{ab}	4.68^{b-d}
		24 h	6.25^{f-h}	7.19^{bc}	5.02^{ab}
NaH_2Po_4 2%	4°C	12 h	6.92^{d}	6.96^{cd}	4.02^{f-h}
		24 h	5.76^{hi}	6.00^{f-j}	3.24^{m-q}
	20 °C	12 h	7.48^{bc}	7.32^{a-c}	4.03^{e-h}
		24 h	8.58^{a}	6.60^{de}	4.51^{cd}
AA 100 mg L^{-1}	4° C	12 h	6.83^{de}	4.73^{st}	3.10^{o-r}
		24 h	5.97^{g-i}	5.65^{i-n}	2.70^{r-t}
	20 °C	12 h	6.13^{g-i}	5.64^{i-o}	3.44^{j-o}
		24 h	6.35^{e-g}	5.48^{k-q}	3.01^{p-s}
AA 200 mg L^{-1}	4 °C	12 h	5.23^{j}	5.45^{l-q}	2.53^{t-v}
		24 h	4.83^{jk}	6.63^{de}	4.45^{de}
	20 °C	12 h	6.70^{d-f}	5.35^{m-r}	2.27^{uv}
		24 h	5.15^{j}	5.59^{j-p}	3.69^{h-l}
Control		-	3.61	4.12	3.04

Means sharing the same letters for a parameter don't have different significance at $p < 0.05$. †; not included in data analysis.

4. Discussion

The seeds of some medicinal plants, such as cumin, have physiological dormancy after harvesting. In order to release seed dormancy, the use of hormonal treatments, the after-ripening period, and stratification treatments can be very effective. One year after harvesting, an increase in the germination process was observed in the seeds. The exogenous application of plant growth regulators can break dormancy, but the obtained results show that in order to stimulate the beginning of cumin seed germination, in addition to the presence of growth regulators, a temperature of 20 ºC is also necessary. In this study, the effects of seed pretreatment were different on the germination indices of cumin seeds with different lifetimes. In general, the results showed that cumin seeds have low germination in the early months after the harvest reaching the maximum rate in an approximately one-year period. During more than one-year storage periods, the quality of the seed as well as its germination ability is affected and decreases considerably. The freshly harvested seeds (49%) as well as the seeds with a 24-month lifetime (66%) had lower germinations compared to the seeds with a 12-month lifetime (76%). The variations of germination with increasing the seed lifetime reflect the fact that the maximum quality of the seed is in close relationship with the seed lifetime. In a similar study on cumin, it was shown (Ipek *et al.*, 2008) that the germination of the freshly harvested seeds was 56% which increased to 64% after 9 months and then decreased by 32% after 24 months. The germination diversity in the seeds with different lifetimes and storage periods such as soybean (Mohammadi *et al.*, 2011), *Moringa oleifera* (Mubvuma *et al.*, 2013), and fenugreek (Ipek *et al.*, 2008) are reported which show the favorable time after the harvest to obtain the maximum germination.

The seed dormancy could be pointed out among the factors which led to low germination (49% in the control treatments) in the freshly harvested seeds (produced in 2014), despite high viability (98%). The dormancy of the seeds in freshly harvested seeds is also reported in other studies on cumin (Hammami *et al.*, 2018; Soltani *et al.*, 2019) as a physiological survival mechanism. In these seeds, seed pretreatments of GA100-20 ºC-24 h and GA200-20 ºC-24 h could improve the germination percentage by 33% compared to unprimed seeds. The combined effect of temperature and the use of gibberellic acid increases the activity of the alpha-amylase enzyme and the permeability of the seed coat, failing seed dormancy (Mousavi Naserabad *et al.*, 2020). A somewhat similar situation is observed for the seedling dry weight and seedling vigor index of these seeds. Considering the effectiveness of these hormones, it can be concluded that the freshly harvested seeds have physiological dormancy. The positive effect of gibberellic acid is reported in previous studies in improving the germination components of the cumin seeds (Ipek *et al.*, 2008) and Arabidopsis seeds (Yamauchi *et al.*, 2007) and 40 medicinal plant species (Aghilian *et al.*, 2014). In previous results, it was shown (da Silva *et al.*, 2005) that gibberellins are needed for both elongating the plant embryo cells and endosperm weakening during the germination of the seed. Gibberellic acid leads to the growth of the cell by increasing the xyloglucan endotransglucosylase enzyme which results in the infiltration of the expansin protein into the cell wall. Gibberellic acid also leads to the mobilization of storage materials of the seed by increasing the release of carbohydrate hydrolyzing enzymes and proteins (Amri *et al.*, 2016) as well as increasing the cell division rate leading to increased dry weight of the seedling (Castro-Camba *et al.*, 2022).

The results of the viability test showed that survival rates were higher than 90% in all three seed groups. However, in all three seed groups, the maximum observed germination and seedling vigor index was less than for the viability test. By increasing the lifetime of the seed to 12 and 24 months, germination was increased by 27% and 17%, respectively. Although, the seeds with a lifetime of 24 months showed decreased germination compared to the seeds with a 12-month lifetime due to vigor loss at the storage conditions. A decrease in cumin seed vigor is also reported by increasing the storage time to 20 months, in previous studies (Aghilian *et al.*, 2014). It seems that higher germination in seeds with a 12-month lifetime compared to the seeds with a 24-month lifetime could on one hand be due to less lifetime, and on the other hand, be due to complete breaking of the dormancy of the seed. Physiological changes during the deterioration of the seed include decreased germination and growth rate of the seedling, decreased germination ability of the seed at the time of harvest, increased ionic leakage in the seed and increased susceptibility to pathogens. It was stated (Mohammadi *et al.*, 2011) that decreased seed germination indices in deteriorated seeds could be due to the decreased consumption rate of the seed storage materials and consequently decreased transfer of the storage materials to the embryo and decreased growth of the embryo. According to these reports, the decrease in the cumin seed germination indices under the storage condition could be related to the decrease in the consumption of the storage materials. So, the improved dry weight of the seedling in the treated seeds with GA could be related to the positive role of this hormone in the stimulation of the storage materials of the seeds.

Different priming treatments have a different impacts on the cumin seeds germination indices. In most germination indices, the combination of temperature 20°C with 24-hour priming duration showed better performance compared to other temperature and priming duration combinations. In freshly harvested seeds, KH_2PO_4, NaH_2PO_4, and ascorbic acid priming treatments also had positive impacts on germination attributes and seedling vigor index. The impact of these combinations on the improvement of the germination of the seed with 12 and 24 months lifetimes was higher than the freshly harvested seeds. The success of using the KH_2PO_4 compound is also reported in the improvement of the germination of watermelon seeds (Nascimento, 2003) and rapeseed (Abdolahi *et al.*, 2012). Priming with phosphorus compounds could be effective in supplying the need for phosphorous for the plant during germination. This impact could occur through DNA replication and increased ATP production (Seyyedi *et al.*, 2015). As a treatment, the researchers relate the reason for the more rapid sprouting of the root and plumule in osmoprimed seeds to the higher efficiency of the water absorption and metabolic activity during the germination and believe that the higher ability of water absorption in primed seeds compared to unprimed seeds lead to positive impact on the germination percentage and rate (Biswas *et al.*, 2023). 1.5 to 2 times increase of the length of the seedling in osmoprimed seeds with 1% NaH_2PO_4 could also be caused by these effects. The increased length of the seedling in osmoprimed *Vigna unguiculata* seeds is reported, too (Singh *et al.*, 2014).

Treated seeds with 1% NaH_2PO_4 in two seed groups with 12 and 24 months lifetimes had the highest germination rate and seedling length. However, in freshly harvested seeds the germination rate in primed seeds was higher with GA. The disrupted membrane structure is among the effects of seed ageing. In general, seed osmopriming could be an important mechanism for starting the preparation of the membrane and internal metabolism of the seed for germination by controlling the seed water absorption. Water absorption damage control and restoring the damages to the membrane during storage (seed deterioration) could be pointed out as a major reason for the improved germination rate for the seeds with a 24-months lifetime. It is reported that priming could help improve water absorption by improving the plasma membrane structure (Biswas *et al.*, 2023). In fact, priming protects the plant against unfavorable effects of stress by increasing the level of the required enzymes for germination, increasing the germination percentage and rate, maintaining the ionic balance and providing hormonal balance and improves the growth of the plant under this condition (Huang *et al.*, 2021). Increasing the rate of DNA repair, construction of RNA, protein synthesis, enzymes activation, removing active oxygen radicals, increasing the cell expansion as well as the progress of the germination stages of the primed seeds compared to the unprimed seeds are pointed out as the most important reasons for increased germination rate (Kiran *et al.*, 2020).

5. Conclusion

The overall experiment results assert the positive role of hydropriming treatments, hormonal priming with gibberellic acid and ascorbic acid and osmopriming with KH_2PO_4 and NaH_2PO_4 in improving the cumin seedling germination and growth. However, seeds with different lifetimes (freshly harvested, 12 and 24-month lifetimes) had rather a different affectability compared to priming treatments. In the freshly harvested seeds, hormonal treatment with gibberellic acid meaningfully improved germination percentage, germination rate, seedling dry weight, and seedling vigor index compared to other priming and control treatments. Considering the role of gibberellic acid in improving the germination of freshly harvested seeds, physiological dormancy in these seeds seems possible. However, for seeds with a 24-month lifetime, priming treatments could improve the unfavorable effects of storage (seed ageing). But, seeds with a 12-month lifetime showed the highest seed quality which asserts the need for after-ripening in this plant.

Conflict of Interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed Consent

The authors declare not to use any patients in this research.

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