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Improvement of germination of low viable pepper seeds by seed priming under optimal and suboptimal temperatures

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Abstract The study reported to enhance germination performance of two seed lots (high 94.5%, and low 51.5% viability) of pepper cultivar Çarliston by means of different concentrations of GA_3 and KNO_3 (hydration, 100, 250, and 500 ppm) for 24 hours at 25 ℃ under optimum (25 ℃) and cool (18 ℃) temperatures. The results showed that all the seed treatments improved germination and seedling growth of pepper at 18 ℃ and 25 ℃. Low temperature restricted germination percentage, index, and seedling growth, while seed priming alleviated the adverse effects of low temperature. The superiority of seed priming was obvious in low viable seeds. Germination of the seeds with low viability treated with 500 ppm GA_3 resulted in an increase of 31.0% at 25 ℃ and 66.7% at 18 ℃. The highest germination index and the shortest mean germination time were obtained from 500 ppm GA_3. The effect of GA_3 was more prominent on both low and high-viable seeds than KNO_3. The optimum dose of GA_3 varied significantly with seed viability and temperature. Also, seedling growth was induced by 500 ppm GA_3 for low-viable seeds and 100 ppm GA_3 for high viability. In comparison to hydration, GA_3 and KNO_3 were found more helpful treatments for increasing germination and seedling growth. It was concluded that the low-viable seeds primed with 500 ppm GA_3 may be used for seedling production under optimum temperature, and the application of 100 ppm GA_3 to the seeds with high viability should be advised under cool stress.

Keywords: *Capsicum annuum* L., GA_3, Germination, Hydration, KNO_3

Introduction

Seed production of vegetable crops has gained importance in Turkey during the last decade. The amount of seed production has changed between 2,000 to 4,000 tons between 2010 and 2020 years, and approximately 300-700 tons of seeds per year have been exported (Anonymous, 2021). These fluctuations in production and export adversely affect the use of seeds. Occasionally, overproduction of vegetable seeds exists, and they are stored until the next production season due to expensive production costs.

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Pepper (*Capsicum annuum* L.), which is widely grown in greenhouses, outdoor fields, and net houses, is mostly consumed by humans as a fresh, spice, pickle, sauceboat, and dried (Bosland and Votava, 2000). Furthermore, it has an important place in our country's economy and supplies important income for farmers and it is extensively produced in large quantities in Turkey. Although it can be directly sown in the field, young seedling transplantation is commonly preferred to prove a uniform plant density, earliness, and high-yielding fruit production by producers (Wien, 1997). Since pepper is a warm-season vegetable, it is susceptible to low temperature which is hazardous for pepper plants from germination, emergence, and early seedling growth to harvest (Stoffella *et al.*, 1988; Korkmaz and Korkmaz, 2009).

To overcome the low-temperature stress and to increase seed viability, vigor, and stand establishment, several seed priming techniques have been developed (McDonald, 2000). Therefore, the success of seed priming may differ among seed lots (Brocklehurst and Dearman, 1983) due to variation in seed vigor. Bradford *et al.* (1990) announced that slowly germinating seed lots in pepper gained the maximum benefit from priming. Hydropriming and osmopriming with KNO_3, GA_3, PEG, and various salt solutions (NaCl, $CaCl_2$, and $Mg(NO_3)_2$) have been mostly utilized for priming agents. Bradford (1986) and Bennett *et al.* (1992) found that primed seeds germinated faster and more uniformly under low temperatures than non-primed seeds. Tombegavani *et al.* (2020) reported that seed priming of pepper with plant hormones (GA_3 and NAA) improved germination and seedling growth. Similarly, Yogananda *et al.* (2004) recommended that priming with GA_3 (200 ppm) caused a significant increase in germination and seedling growth of bell pepper seeds. Also, Nascimento and Aragão (2004) demonstrated that seeds primed with 0.35 g L^{-1} KNO_3 increased germination performance in low vigorous muskmelon seeds. On the other hand, hydration promoted germination of aubergine and pepper seeds under low temperature and after 4 months of storage (Demir and Okcu, 2004). This study aimed to determine the possibilities of improvement in low viable seeds in pepper by using GA_3 and KNO_3 and to search if germination performance under low temperatures can be increased by seed priming.

Materials and methods

This study focused on increasing the germination ability of low viable pepper seeds and enhancing seed vigor of high viable seeds under suboptimal temperatures. The study was conducted in the facilities of the seed science and technology laboratory of the Department of Field Crops, Eskişehir Osmangazi University. Two seed lots with low viability (51.5%) and high viability (94.5%)

pepper cultivar Çarliston were selected from eleven lots purchased from seed suppliers in 2020.

Seed priming

These seeds were treated with 100, 250, and 500 ppm of GA_3 and KNO_3 solutions at 25 ℃ for 24 hours in the dark. To determine the efficiency of GA_3 and KNO_3, the seeds were hydrated in distilled water under the same conditions. Also, non-treated seeds were used as a control. After the treatments, the seeds were surface-dried with paper towels and left to dry back to initial seed weight at room conditions (about 22-24 ℃). The seed moisture of both primed and control was equilibrated at room temperature for 2 days.

Germination test

Four replicates with fifty seeds from each treatment were counted for the germination test. Fifty seeds were placed into three layers of filter paper with 21 mL of distilled water. After the papers were rolled, they were inserted into a sealed plastic bag to avoid moisture loss. The packages were transferred into an incubator at 25 ℃ for optimum and 18 ℃ for low temperature under darkness. Radicle protrusion of 2 mm was a germination criterion. The germinated seeds were counted every day for 14 days and germination speed was evaluated in terms of mean germination time (MGT) according to ISTA (2003) rules.

$MGT = \Sigma Dn / \Sigma n$

where D is the number of newly germinated seeds on each day and n is the day number on which the count took place.

Germination index (GI) was calculated according to Salehzade *et al.* (2009) with the following equation:

GI = Number of germinated seeds/days of first count +. . .+ Number of germinated seeds/ days of the final count.

Seedling fresh weight was measured from randomly selected ten seedlings in each replicate after the 14^{th} day. Vigor index (VI) is calculated by multiplying germination percentage and seedling length. The seeds with higher vigor index are more vigorous (Jahangir *et al.*, 2009).

Statistical analysis

The experiment was designed as two factors factorial (2 ×8) arranged in a completely randomized design; with 4 replicates and a total of 200 seeds for each treatment. Analysis of variance was performed by using the JMP 13.0 statistical package program. The differences between the means were compared using Tukey's test ($P < 0.05$).

Results

The mean values of germination characteristics and seedling parameters of high and low viable pepper seeds treated with different doses of GA_3 and KNO_3 under optimum germination temperature are shown in Table 1.

Table 1. Mean values of germination and seedling growth of high and low viable pepper seeds primed with different levels of GA_3 and KNO_3 at 25 ℃.

Factors	GP (%)	MGT (day)	Germination index	Seedling length (cm)	SFW (mg plant^{-1})	Vigor index
Viability (A)						
High	95.5^{a}	3.07^{b}	16.2^{a}	7.93^{a}	45.9^{a}	753^{a}†
Low	58.8^{b}	5.40^{a}	6.51^{b}	7.88^{b}	26.8^{b}	457^{b}
Priming (B)						
Control	73.0^{d}	4.35^{a}	10.4^{e}	8.12^{b}	34.1^{d}	606^{bc}
Hydration	75.1^{cd}	4.31^{a}	10.6^{de}	6.87^{d}	35.9^{bc}	511^{d}
100 ppm GA_3	77.1^{bc}	4.35^{a}	10.6^{de}	9.23^{a}	39.3^{a}	716^{a}
250 ppm GA_3	77.0^{bc}	4.19^{a}	11.1^{cd}	7.63^{c}	37.8^{ab}	552^{d}
500 ppm GA_3	82.5^{a}	3.82^{b}	13.1^{a}	8.85^{a}	37.0^{bc}	712^{a}
100 ppm KNO_3	76.5^{bc}	4.28^{a}	11.4^{bc}	6.83^{d}	35.2^{cd}	539^{d}
250 ppm KNO_3	79.0^{b}	4.26^{a}	12.0^{b}	8.27^{b}	36.9^{bc}	649^{b}
500 ppm KNO_3	76.8^{bc}	4.32^{a}	11.5^{bc}	7.45^{c}	34.9^{d}	558^{cd}
Analysis of Variance						
Viability (A)	**	**	**	**	**	**
Priming (B)	**	**	**	**	**	**
A×B	**	*	**	**	**	**

†: Levels not connected by the same letter(s) are significantly different. *, ** significant at 5% and 1% respectively. GP: germination percentage, MGT: mean germination time, SFW: seedling fresh weight

A significant two-way interaction was calculated for all the investigated traits. As expected, higher germination percentage and index, and faster germination time were observed in the seeds with high viability. All the seed treatments increased germination percentage of pepper seeds and the highest mean germination rate (82.5%) was determined in 500 ppm GA_3. However, primed seeds produced shorter mean germination time, and higher germination index, seedling length, fresh weight, and vigor index.

Analysis of variance and mean values of the investigated characteristics of pepper seeds at 18 ℃ were given in Table 2. Two-way interaction between seed viability and priming was found significant for all traits. Lower germination percentage and higher mean germination time, seedling length, seedling fresh weight, and vigor index were determined in pepper seeds with low viability. Among the seed primings, the most effective treatment for germination and seedling growth was 500 ppm GA_3. All the primed seeds germinated better than control seeds.

Table 2. Mean values of germination and seedling growth of high and low viable pepper seeds primed with different levels of GA_3 and KNO_3 at 18 ℃.

Factors	GP (%)	MGT (day)	Germination index	Seedling length (cm)	SFW (mg plant^{-1})	Vigor index
Viability (A)						
High	94.0^{a}	5.84^{b}	8.97	2.30^{a}	19.8^{a}	217^{a}†
Low	47.9^{b}	10.68^{a}	8.50	1.88^{b}	10.0^{b}	93^{b}
Priming (B)						
Control	63.5^{d}	8.62^{a}	6.98^{e}	1.54^{c}	11.6^{e}	120^{c}
Hydration	68.5^{c}	8.09^{abc}	9.11^{b}	1.74^{bc}	16.7^{ab}	122^{c}
100 ppm GA_3	69.5^{bc}	8.42^{a}	8.93^{bc}	2.42^{ab}	15.3^{bc}	192^{a}
250 ppm GA_3	73.3^{a}	8.28^{ab}	8.74^{bc}	2.15^{abc}	13.4^{d}	159^{abc}
500 ppm GA_3	74.3^{a}	7.56^{c}	12.17^{a}	2.22^{abc}	14.6^{cd}	166^{ab}
100 ppm KNO_3	72.0^{abc}	8.42^{a}	8.25^{bcd}	2.00^{bc}	16.8^{a}	155^{abc}
250 ppm KNO_3	69.5^{bc}	8.78^{a}	7.65^{de}	1.87^{bc}	14.7^{c}	136^{bc}
500 ppm KNO_3	72.8^{ab}	7.70^{bc}	8.04^{cd}	2.79^{a}	16.2^{ab}	187^{a}
Analysis of Variance						
Viability (A)	**	**	ns	*	**	**
Priming (B)	**	**	**	*	*	**
A×B	**	**	**	*	**	**

†: Levels not connected by the same letter are significantly different. *, ** significant at 5% and 1% respectively. ns: nonsignificant. GP: germination percentage, MGT: mean germination time, SFW: seedling fresh weight

Table 3. Changes in germination performance of high and low viable pepper seeds primed with different concentrations of GA_3 and KNO_3 under optimum and cool temperatures.

Priming	Germination percentage (%)		Mean germination time (day)		Germination index	
	Low	High	Low	High	Low	High
			Optimum temperature (25 ℃)			
Control	51.5^{f}	94.5^{ab}	5.62^{a}	3.09^{c}	5.39^{e}	15.5^{b}*
Hydration	53.3^{ef}	97.0^{ab}	5.50^{a}	3.11^{c}	5.37^{e}	15.9^{ab}
100 ppm GA_3	60.3^{d}	94.0^{ab}	5.57^{a}	3.13^{c}	5.40^{e}	15.9^{ab}
250 ppm GA_3	62.0^{cd}	92.0^{b}	5.38^{a}	3.00^{c}	6.37^{de}	15.9^{ab}
500 ppm GA_3	67.5^{c}	97.5^{ab}	4.64^{b}	3.01^{c}	8.99^{c}	17.2^{a}
100 ppm KNO_3	57.0^{def}	96.0^{ab}	5.49^{a}	3.07^{c}	6.54^{de}	16.3^{ab}
250 ppm KNO_3	59.5^{de}	98.5^{a}	5.41^{a}	3.12^{c}	7.45^{cd}	16.5^{ab}
500 ppm KNO_3	59.0^{de}	94.5^{ab}	5.60^{a}	3.03^{c}	6.58^{de}	16.3^{ab}
			Cool temperature (18 ℃)			
Control	33.0^{d}	94.0^{a}	11.1^{abc}	6.2^{d}	6.30^{d}	7.65^{bcd}
Hydration	44.0^{c}	93.0^{a}	11.2^{abc}	6.0^{d}	9.73^{b}	8.47^{bcd}
100 ppm GA_3	52.0^{bc}	96.0^{a}	11.3^{ab}	5.5^{d}	7.79^{bcd}	10.06^{b}
250 ppm GA_3	52.0^{bc}	94.5^{a}	11.0^{abc}	5.5^{d}	8.49^{bcd}	8.99^{bc}
500 ppm GA_3	55.0^{b}	93.5^{a}	9.67^{bc}	5.5^{d}	14.97^{a}	9.35^{b}
100 ppm KNO_3	49.0^{bc}	95.0^{a}	11.5^{a}	5.8^{d}	6.52^{cd}	9.97^{b}
250 ppm KNO_3	46.5^{bc}	92.5^{a}	11.3^{ab}	6.2^{d}	6.48^{cd}	8.81^{bcd}
500 ppm KNO_3	52.0^{bc}	93.5^{a}	9.3^{c}	6.1^{d}	7.67^{bcd}	8.40^{bcd}

*: Means followed by the same letter(s) in each column are significantly different at 5%.

The initial germination percentage of low viable pepper seeds was 51.5% and it reached up to 67.5% in seeds treated with 500 ppm GA_3 (Table 3). It means a 31.0% increase in germination percentage. Under cool temperature, germination of low viable pepper seeds was enhanced from 33.0% in control to 55.0% in seeds treated with 500 ppm GA_3; suggesting a 66.7% increase with this treatment. Mean germination time was shortened by seed treatments in the seeds with low viability at 18 °C and 25 °C; however, no significant improvement was observed in the high viable pepper seeds. The minimum germination time was obtained from 500 ppm GA_3 with 4.64 d at 25 °C and 500 ppm KNO_3 with 9.3 d at 18 °C. The germination index is a valuable indicator of higher and faster germination, and the higher index shows the higher vigorous seeds. At optimum temperature, all the seed treatments gave a higher germination index than the control, and the maximum germination indexes in low (8.99) and high (17.2) viable seeds were calculated in seeds treated with 500 ppm GA_3. At 18 °C, the highest germination index was obtained from 500 ppm GA_3 for low viable seeds, while it was determined in 100 ppm GA_3 for high viable seeds.

Table 4. Changes in seedling growth parameters of high and low viable pepper seeds primed with different concentrations of GA_3 and KNO_3 under optimum and cool temperatures

Priming	**Seedling length (cm)**		**Seedling fresh weight (mg plant^{-1})**		**Vigor index**	
	Low	**High**	**Low**	**High**	**Low**	**High**
			Optimum temperature (25 °C)			
Control	8.60^{abc}	7.65^{c-g}	23.7^{d}	44.6^{a}	489^{gh}	723^{cde}*
Hydration	6.56^{gh}	7.18^{d-g}	26.0^{bcd}	45.8^{a}	326^{j}	697de
100 ppm GA_3	8.74^{abc}	9.73^{a}	30.8^{b}	47.8^{a}	517^{gh}	914^{a}
250 ppm GA_3	8.69^{abc}	6.58^{fgh}	27.9^{bcd}	47.6^{a}	498^{gh}	605^{efg}
500 ppm GA_3	8.80^{abc}	8.80^{abc}	24.5^{cd}	48.3^{a}	565^{fgh}	858^{ab}
100 ppm KNO_3	5.89^{h}	7.78^{b-f}	27.1^{bcd}	43.3^{a}	332^{ij}	746^{bcd}
250 ppm KNO_3	8.18^{b-e}	8.35^{bcd}	29.0^{bc}	44.9^{a}	475^{h}	822^{abc}
500 ppm KNO_3	7.90^{b-e}	7.00^{e-h}	24.5^{cd}	45.3^{a}	453^{hi}	662^{def}
			Cool temperature (18 °C)			
Control	0.80^{d}	2.27^{bc}	7.9^{f}	15.3^{d}	27^{f}	214^{bc}
Hydration	1.62^{cd}	1.85^{c}	11.9^{e}	21.4^{a}	72^{ef}	172^{cd}
100 ppm GA_3	1.80^{cd}	3.02^{ab}	10.6^{e}	20.1^{ab}	94^{e}	290^{a}
250 ppm GA_3	2.07^{bc}	2.22^{bc}	7.9^{f}	18.9^{bc}	108^{e}	210^{bc}
500 ppm GA_3	2.16^{bc}	2.27^{bc}	11.2^{e}	17.9^{c}	119de	212^{bc}
100 ppm KNO_3	1.53^{cd}	2.47^{abc}	12.1^{e}	21.6^{a}	75^{ef}	236^{ab}
250 ppm KNO_3	1.59^{cd}	2.15^{bc}	7.6^{f}	21.9^{a}	74^{ef}	199^{bc}
500 ppm KNO_3	3.43^{a}	2.15^{bc}	11.1^{e}	21.4^{a}	172^{cd}	201^{bc}

*: Means followed by the same letter(s) in each column are significantly different at 5%.

Seedling growth was promoted by seed primings in low and high-viable pepper seeds. At 25 ℃, the longest seedling produced from low viable seeds was measured in 500 ppm GA_3, while it was obtained from 100 ppm GA_3 in high viable seeds (Table 4). However, seedling length was enhanced by all the primings at 18 ℃ and the highest seedling length was detected at 500 ppm KNO_3 in low-viable seeds and 100 ppm GA_3 in high-viable seeds. Similarly, seedling fresh weight was stimulated in seeds with low viability under optimum and cool conditions. Any significant difference among seed primings was not determined in high viable seeds of the pepper. Under cool stress, lower doses of GA_3 and KNO_3 were found to be more effective for increasing seedling fresh weight rather than higher doses. Increased doses of GA_3 and KNO_3 caused a significant increase in the vigor index of pepper. 500 ppm GA_3 in low viable seeds and 100 ppm KNO_3 in high viable seeds produced the highest vigor index at 25 ℃, but 500 ppm in low and 100 ppm GA_3 in high viable seeds exhibited the highest vigor index at 18 ℃.

Discussion

The application of GA_3 and KNO_3 enhanced germination and seedling growth performance of low and high-viable pepper seeds under both control and low-temperature condition. Increased germination percentage and shortened mean germination time were obtained from primed seeds, but low temperature retarded it considerably. Similar findings were demonstrated by Tombegavani *et al.* (2020) who reported the beneficial effects of GA_3 on germination percentage and seed vigor index in pepper. The minimum germination time was obtained from 500 ppm GA_3 at 25 °C and 500 ppm KNO_3 at 18 °C. Similar findings were also reported by Debbarma *et al.* (2018) and Hagroo and Johal (2019) in pepper. Our findings were also confirmed by the results of Tombegavani *et al.* (2020) who indicated the favorable effects of GA_3, especially under low temperatures. Jyoti *et al.* (2016) determined the highest germination in GA_3 concentrations between 25 ppm and 100 ppm. In addition, the advantages of KNO_3 treatments with various concentrations on germination at low temperatures were reported by Ali *et al.* (2020) in tomato with 0.75% KNO_3, Peyvast *et al.* (2010) in cucumber with 5% KNO_3, and Arin and Kiyak (2003) in tomato with 2% KNO_3 on emergence and seedling growth at low temperature. Moreover, Jahangir *et al.* (2009) indicated the favorable effects of 0.5% KNO_3 treatment on germination of lettuce seeds under supra optimal temperature. In this study, GA_3 was more effective in improving germination than KNO_3. The primed seeds gave higher germination index and this increase was more apparent in low viable seeds under low-temperature conditions. Similarly, Debbarma *et al.* (2018) reported an improvement in germination index in chilli pepper by applying a 50 ppm GA_3.

On the other hand, seed treatments did not show significant advantages in high viable seeds under both temperatures. That is why the initial seed viability was already high, and no significant enhancement was observed. Hagroo and Johal (2019) indicated that GA_3 and KNO_3 treatments increased the germination performance of artificially and naturally aged pepper seeds rather than control seeds. External applications of GA_3 enhance seed germination because GA_3 is released from the embryo and stimulates specific genes for mRNA transcription by a-amylase (Taiz and Zeiger, 2002).

Besides increased germination performance by GA_3 and KNO_3, post-germination growth of pepper was also induced. Jyoti *et al.* (2016) reported that the seedling length of tomato was promoted by GA_3 and KNO_3 treatments, but the highest seedling length was obtained from 2% KNO_3. Tombegavani *et al.* (2020) and Debbarma *et al.* (2018) indicated that GA_3 increased seedling growth in pepper. These results are in agreement with the findings of Obaidul-Islam *et al.* (2006) in tomato, Debbarma *et al.* (2018), and Hagroo and Johal (2019) in pepper, they found that a clear induction was observed in seedling growth when GA_3 was applied. The finding was confirmed by the observations in tomato by Farooq *et al.* (2005) with the highest seedling vigor in KNO_3, and Jyoti *et al.* (2016) determined the highest vigor index in 25 ppm GA_3. Furthermore, the vigor index of pepper seeds was enhanced by GA_3 treatments in line with earlier observations made for pepper by Debbarma *et al.* (2018), Hagroo and Johal (2019), and Tombegavani *et al.* (2020). Also, the beneficial effects of alternative agents such as SNP (Amooaghaie and Nikzad, 2013), 5-aminolevulenic acid (Korkmaz and Korkmaz, 2009), and NaCl (Khan *et al.*, 2009) were demonstrated.

It was concluded that GA_3 and KNO_3 resulted in enhancement in germination and seedling growth of pepper. Hydration and all the levels of GA_3 and KNO_3 showed superiority over unprimed seeds. Also, a higher germination percentage and seedling growth were observed in pepper seeds treated with GA_3 as compared with hydration. Therefore, the use of gibberellic acid can be a more effective method for improving germination and seedling growth of pepper seeds with low viability. The results revealed that the pepper seeds with low viability or vigor may be used for seedling production after they were treated with 500 ppm GA_3 instead of discarding the seeds. Moreover, 100 ppm GA_3 treatment should be suggested for high-viable or vigorous seeds of pepper under low temperatures.

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