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PRIMING TREATMENTS FOR IMPROVEMENT OF GERMINATION AND EMERGENCE OF COTTON SEEDS AT LOW TEMPERATURE

ABSTRACT

Early sowing in cotton is important for obtaining a high and qualiy yield. Since cotton prefers high temperatures to have a good stand establisment, this work is important to determine better performing genotypes or to promote germination and emergence at low temperatures (<20°C). This research aimed to increase seed germination rate and seed vigour under cold stress conditions. Two cotton genotypes belonging to *G. hirsutum* L. species (Aydin-110 and Paymaster-2379) were primed with PEG-6000 (200, 300, 400 g/L), KNO_3 (2, 3, 4%), KH_2PO_4 (0.3, 0.4, 0.5 M), NaCl (2, 3, 4%) or Mannitol (2, 4, 6%) to determine the best priming medium and its best amount. After priming, seeds were germinated in germination cabin at cold (18°C) and warm (30° C) temperatures and some characteristics (germination rates, radicle length, hypocotyl length and seedling length) were analyzed. Priming with KNO_3 (4% or 2%) was found to result in the highest germination rates in both cotton genotypes. The seeds of the genotypes were also primed with 5-aminolevulinic acid (ALA) (1, 10, 25 mg/L), gibberellic acid (GA_3) (100, 250, 500 µM), methyl jasmonate (MeJA) (1, 3, 5 µM), acetylsalicylic acid (ASA) (1, 10, 100 µM) or kinetin (1, 5, 10 mg/L) added to KNO_3 (4%) medium to choose the most effective plant growth regulator (PGR) application and its dose. PGRs were observed to have reverse effects on germination rate. Finally, emergence test was conducted with KNO_3 (4%) and KNO_3 (2%) using metabolic chill test, and some traits (radicle length, radicle weight, hypocotyl length, hypocotyl weight, seedling length, seedling fresh weight and seedling dry weight) were examined. Our results suggested that priming with KNO_3 (2%) might be an effecient way to have vigourous cotton plants under the cold stress.

Key words: cotton, emergence, germination, low temperature, PGR, priming

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INTRODUCTION

Cotton is the most important source of natural fiber used in textile industry. Increasing the yield and the fiber quality is the main goal of most breeding programs (Mert *et al.*, 2003). Many studies were conducted to produce cottons with high germination and emergence rates in various environmental conditions. Many researchers and producers aim at having seeds with a high quality. Germination and emergence duration of cotton seeds whose quality may change dependently on their germination rates, and climate and soil conditions (Shaheen *et al.*, 2012). To increase the stress tolerance in seeds, treatments, such as hormones, PGRs and various chemicals are commonly used. Many plant species, especially the ones with a tropical or sub-tropical origin, suffer injury when they are exposed to temperatures between the freezing point of tissue (0°C) and approximately 15°C. This injury is termed as chilling injury so as to distinguish it from freezing injury (Theocharis *et al.*, 2012). Many economically important crops, such as cotton, soybean, maize, rice and many tropical and sub-tropical fruits are classified as chilling-sensitive (Lyons, 1973; Azymi *et al.*, 2012).

When the amounts of oil, protein, carbonhydrate, ash, fiber and gossypol present in germinating cotton seeds were examined, it was found that most of the reduction in seed dry coat weight, oil, protein and carbohydrate consuption° Ccured eight days after germination. Total phospholipid percentage, on the other hand, was observed to increase during germination while phospholipid structures were changing (El-Nockrashy *et al.*, 2006). Since vegetative period in cotton takes a relatively long time, producers might prefer early (sub of optimum temperature for cotton growing) sowing; however, it needs to be considered that cotton radicle formation and growing stop when the soil temperature drops below 14.5°C (Jackson, 1986). Because temperature is so critical for cotton sowing, seedlings of the cotton seeds may be delayed, consequently growth rate may be decreased. In order to overcome this obstacle, a variety of methods were developed to promote rapid germination in different environments by considering that seed quality and germination potential are also crucial in cotton production. Generally, polyethylene glycol with varying molecular weight (PEG-6000/8000) and certain osmotic pressure of potassium and sodium composites (K_3PO_4, KH_2PO_4, KNO_3 or NaCl) are used in priming (Ghassemi-Golezani *et al.*, 2008; Ghassemi-Golezani *et al.*, 2013; Hameed *et al.*, 2013; Arif *et al.*, 2014). These priming techniques are chosen because they were shown to improve the seed vigor, to increase the germination rate and to trigger the seedling growth (Dastanpoor *et al.*, 2013; Hussian *et al.*, 2014). Thanks to these chemicals used in priming, the first stage of germination takes place in optimum conditions, therefore seeds become tolerant to stress conditions.

This study aimed to test the effects of different chemicals and PGRs as priming agents on the germination and emergence of cotton seeds using 18°C germination test developed for cold stress testing.

MATERIAL AND METHODS

Plant materials

Two genotypes, Aydın-110 and Paymaster-2379, having 72.50 and 85.63% germination rates at 18°C respectively, were selected from a preliminary work using the same seed lot. Aydın-110 was developed by Nazilli Cotton Research Center in Turkey and Paymaster-2379 was obtained from an institute located in USA. According to the definiton used by researchers (Smith and Varvil, 1984; Duesterhaus *et al.*, 2000) the former genotype is known as cold sensitive whilst the latter is described as cold tolerant.

Chemicals, PGRs and their applications

Priming was conducted at two levels: Firstly, the best chemical and its dose for priming medium were determined then the effect of PGRs on priming was tested. The chemicals and their doses (given inside the parentheses) investigated through this study were PEG-6000 (200, 300, 400 g/L), KNO_3 (2, 3, 4%), KH_2PO_4 (0.3, 0.4, 0.5 M), NaCl (2, 3, 4%), Mannitol (2, 4, 6%) and the PGRs examined here were 5-aminolevulinic acid (ALA) (1, 10, 25 mg/L), gibberellic acid (GA_3) (100, 250, 500 µM), methil jasmonat (MeJA) (1, 3, 5 µM), acetylsalicylic acid (ASA) (1, 10, 100 µM) and kinetin (1, 5, 10 mg/L). All cotton seeds (160 seeds with 4 replications) were treated with all amounts of the chemicals mentioned above and they were kept at 25^{o}C for 2 hours. Subsequently, the seeds were washed and dried in a germination cabin at 25°C for 20 hours until moisture content of the seeds decreased to ~7.5%±1. Treated seeds and their controls (both distilled water primed seeds and unprimed seeds) were germinated in a germination cabin with cold germination (18°C) as it was described below.

Primed and dried seeds were rolled with Whatman Paper. Then, the rolls were placed in a plastic container that was placed in a germination cabin at 18° C for 7 days. The rolls were opened and germinated seeds (hypocotyl + radicle > 1.5 inch) were counted. If germination percentages turned out to be higher than 60% of what was counted on the 7th day, then the seeds were indicated as cold tolerant (Smith and Varvil, 1984; Kerby *et al.*, 1989; Tolliver *et al.*, 1997; Savoy, 2005). After determining the best priming medium and its most effective dose, application of PGRs on the best priming medium was performed. Seeds belonging to Paymaster-2379 and Aydın-110 genotypes were treated with the best germination chemical dose (KNO_3 (4%)) and different PGR doses; 5-

aminolevulinic acid (ALA) (1, 10, 25 mg/L), gibberalik asit (GA_3) (100, 250, 500 μM), metil jasmonat (MeJA) (1, 3, 5 μM), asetil salisilik asit (ASA) (1, 10, 100 μM), kinetin (1, 5, 10 mg/L). After treatment, seeds were followed through the same procedure mentioned above. The same number of the treated seeds were used in both expriments. After the application of germination test, following charecters were measured:

- Cold germination percentages [%]: Percentage of hypocotyl and radicle length of seedlings> 1.5 inch.
- Germination rate [%]: Total percentage of germinated seeds.
- Radicle length [cm]: Radicle length of seedlings on the days 7.
- Hypocotyl length [cm]: Hypocotyl length of seedlings on the days 7.
- Seedling length (cm): Total length of seedlings from radicle tip to the top at days 7.

After selecting optimal dose for priming and investigating the effect of PGRs on germination, the best conditions were used to test the emergence of cotton seeds using metabolic chill test, which was applied as follows: Primed and control cotton seeds (distilled water primed seeds and unprimed seeds) were germinated in a germination cabin at 18°C for 21 days. Seeds were placed in sand that was wetted until it reached to the field capacity (3.8 cm) (7 kg of sand + 1 L of water) in a plastic container. The test was performed with six replicates (102 seeds for each treatment). Seeds were covered with dry sand (2.5 cm) (4.50 kg) then the containers were kept in a germination cabin at 18°C for 21 days. Emerging seedlings from the sand were counted on the 21st day and differences among the genotypes were determined (Duesterhaus *et al.*, 2000).

After the application of metabolic chill test, following traits were analyzed:

- Cold test [%]: Percentage of hypocotyl and radicle length of seedlings > 1.5 inch
- Germination rate [%]: Percentage of germinated seeds.
- Radicle length [cm]: Radicle length of seedlings on the 21st day.
- Radicle weight [g]: Radicle weight of seedlings on the 21st day.
- Hypocotyl length [cm]: Hypocotyl length of seedlings on the 21st. day.
- Hypocotyl weight [g]: Hypocotyl weight of seedlings on the 21st day.
- Seedling length [cm]: Total seedling length from radicle tip to the top on the 21st day.
- Seedling fresh weight [g]: Total weight of seedling cut from the sand surface on thet 21st day.
- Seedling dry weight [g]: Total weight of seedling cut from the sand surface on the 21st day and dried at 70°C for 2 days.

RESULTS

Priming effects of different chemicals on germination of cotton seeds

Cold germination (%)

Priming with KNO_3 showed the best vigour rates. Vigour rates were 84.17%. Paymaster-2379 had 89.33% vigour rate and this rate was higher than Aydın-110 (74.63%) (Figu. 1). When the results for treated and untreated samples were analyzed, the best vigour rate was observed in the conditions where the priming was made with KNO_3 (4%). Although priming with NaCl (2%) resulted in a higher germination rate than priming with KNO_3. Priming with PEG-6000 and KNO_3 seemed to trigger lateral roots production better in Aydın-110 compared to control, and this result was similar to what was observed for Paymaster-2379, except priming with NaCl. Mannitol (2%) (81.88%) was the best priming medium. However, KH_2PO_4 (0.3M) (50.63%) was the worst priming medium.

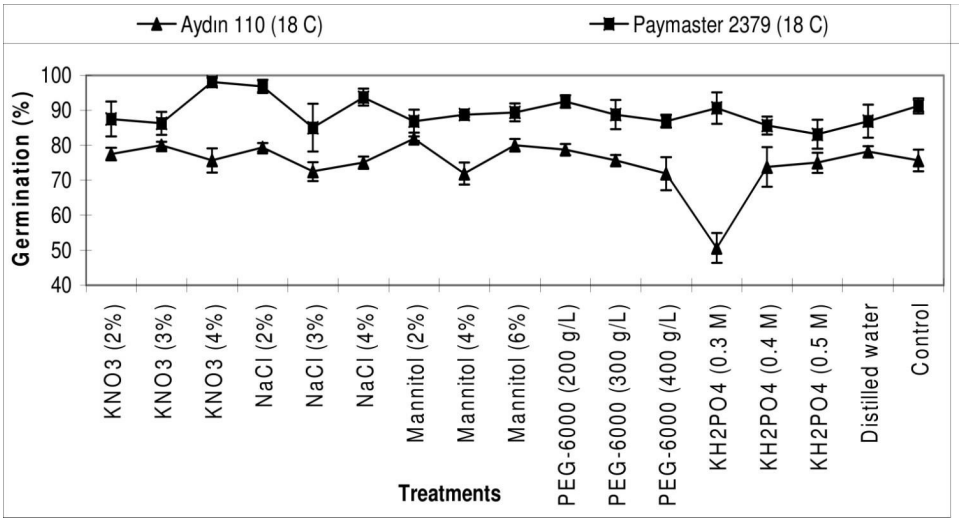

Fig. 1. Cold germination percentages for Aydın 110 and Paymaster 2379 after priming effects of chemicals

The highest vigour rates obtained from NaCl (91.88%) and KNO_3 (90.63%) and the lowest results belonged to KH_2PO_4 (86.46%). Compared to the control values, the highest vigour rate was measured when the samples were primed with KNO_3 (4%) (98.13%). The lowest vigour rate (83.13%), however, was seen when KH_2PO_4 (0.5M) was used as the priming agent. Comparison of the results of cold germination test is important for cotton cold tolerance (Kerby *et al.*, 1989; Tolliver *et al.*, 1997). It has been known that cold resistant seedlings are taller and heavier than the seedlings that are cold sensitived (Duan *et al.*, 2004; Guler *et al.*, 2007). Besides, seedling radicle and hypocotyl resistance are

also known to be important for cold tolerance. To estimate the effect of chemicals on seed germination, some other characters, such as seedling length and hypocotyl length were also measured.

Germination rate [%]

Priming with KNO_3 (97.40%) resulted in the highest germination rate while priming with KH_2PO_4 (%93.65) resulted in the lowest rate. However, the increase in germination rate of Aydın-110 was higher than of Paymaster-2379. Mannitol (96.77%) had the highest germination rate. On the contrary, KNO_3 (96.46%) and NaCl (94.90%) had the lowest germination rates. For Paymaster-2379, the highest germination rates were obtained with KNO_3 (4%) (98.75%). These results clearly indicated the effect of temperature in germination rate (Table 1).

Table 1

Parameters compared for two cultivars after priming effects of chemicals for cold tolerance

		18°C							
Chemical	Doses	Germination rate [%]		Radicle length [cm]		Hyrpocotyl length [cm]		Seedling length [cm]	
		A-110	P-2379	A-110	P-2379	A-110	P-2379	A-110	P-2379
KNO_3	2%	97.50^{ab}	96.25^{a}	8.84^{a-c}	7.51^{ab}	1.50^{a-e}	1.21^{a}	10.33^{a-c}	8.71ab
KNO_3	3%	98.75^{a}	96.25^{a}	9.01^{a-c}	7.72^{ab}	1.72^{a-d}	1.52^{a}	10.73^{ab}	9.24ab
KNO_3	4%	96.88^{ab}	98.75^{a}	7.68^{bc}	8.94^{a}	1.09^{b-e}	1.70^{a}	8.76^{bc}	10.64ab
NaCl	2%	98.13^{a}	98.13^{a}	9.05^{a-c}	8.77^{a}	1.92^{ab}	1.95^{a}	10.97^{ab}	10.71a
NaCl	3%	95.63^{ab}	93.75^{a}	8.28^{a-c}	7.22^{ab}	1.38^{a-e}	1.51^{a}	9.66^{a-c}	8.72ab
NaCl	4%	96.25^{ab}	96.25^{a}	8.15^{a-c}	8.85^{a}	1.05^{edc}	1.77^{a}	9.20^{bc}	10.62ab
Mannitol	2%	99.38^{a}	90.00^{a}	9.94^{a}	7.95^{ab}	1.87^{a-c}	2.28^{a}	11.81^{a}	10.23ab
Mannitol	4%	93.13^{ab}	94.38^{a}	8.69^{a-c}	8.68^{ab}	1.50^{a-e}	1.42^{a}	10.18^{a-c}	10.10ab
Mannitol	6%	100.00^{a}	92.50^{a}	9.89^{a}	8.46^{ab}	2.10^{a}	1.36^{a}	11.99^{a}	9.83ab
PEG-6000	200 g/L	98.75^{a}	95.63^{a}	8.59^{a-c}	8.59^{ab}	1.68^{a-d}	1.22^{a}	10.26^{a-c}	9.80ab
PEG-6000	300 g/L	97.50^{ab}	91.88^{a}	7.01^{cd}	8.66^{ab}	1.02^{de}	1.34^{a}	8.03^{cd}	10.00ab
PEG-6000	400 g/L	95.00^{ab}	93.13^{a}	8.29^{a-c}	8.39^{ab}	1.11^{b-e}	1.12^{a}	9.40^{bc}	9.51ab
KH_2PO_4	0.3 M	88.13^{b}	95.63^{a}	5.14^{d}	7.26^{ab}	0.82^{e}	1.51^{a}	5.96^{d}	8.76ab
KH_2PO_4	0.4 M	98.13^{a}	94.38^{a}	8.56^{a-c}	6.27^{b}	2.13^{a}	1.56^{a}	10.69^{ab}	7.83ab
KH_2PO_4	0.5 M	95.63^{ab}	90.00^{a}	8.27^{a-c}	6.64^{ab}	1.44^{a-e}	1.10^{a}	9.71^{a-c}	7.74b
Distilled water		96.25ab	90.63^{a}	9.66^{ab}	8.45^{ab}	1.39^{a-e}	1.16^{a}	11.05^{ab}	9.61^{ab}
Control		93.13ab	95.00^{a}	8.92^{a-c}	8.30^{ab}	1.13^{b-e}	1.73^{a}	10.05^{a-c}	10.03^{ab}
CV[%]		3.93	5.37	9.40	11.81	22.53	34.01	9.43	12.11
Mean		96.36	94.26	8.47	8.04	1.46	1.50	9.93	9.53

A - Aydın-110, P - Paymaster-2379

Radicle length [cm]

Radicle length of Aydın-110 genotype was changed from 5.14 to 9.94 cm and the mean radicle length was calculated as 8.47 cm. The difference in the mean radicle lengths measured at different temperatures was 1.20 cm, and this can be resulted to the fact enzymatic activity can be slowed by lower temperatures (Table 1). KNO_3, PEG-6000, Mannitol were found to increase the radicle length, which suggested that they could be used against the cold and water stress. Mannitol (2%) treatment resulted in the longest radicle (9.94 cm) for Aydın-110. Radicle length of Paymaster-2379 was ranged from 6.27 to 8.94 cm. The differences of the lengths between the tests were 1.40 cm. KNO_3 (4%) (8.94 cm) resulted in the highest increase in radicle length.

Hypocotyl length [cm]

Hypocotyl length of Aydın-110 changed between 0.82 and 2.13 cm and the mean value was determined as 1.46 cm. High chemical doses caused low hypocotyl length. Mannitol (2%) (2.28) had the longest hypocotyl length of Paymaster-2379 genotype. KH_2PO_4 (0.5M) (1.10 cm) resulted in the lowest hypocotyl length (Table 1.).

Seedling length [cm]

Mannitol (10.69 cm) provided fast growing seedlings for early harvesting and cold tolerant plants. Mannitol (%2) (11.81 cm) resulted in the longest seedlings while KH_2PO_4 (0.3M) (5.94) caused the production of the shortest seedlings. PEG-6000 (200g/L) (12.02 cm) had an increasing effect on seedling length. KNO_3 (4%) (10.64 cm) generated the longest seedlings. Germination results showed that KNO_3 gave the highest result and KNO_3 (4%) had the highest results thus KNO_3 (4%) was chosen as the best priming medium.

Priming effects of different PGRs on germination of cotton seeds

Cold germination [%]

The highest vigour rates belonged to the priming with GA_3 while MeJA (3 µM) (93.75%) was determined as the best treatment (Fig.2). ALA (84.69%) resulted in the lowest vigour rate. ASA (10 µM) had the best dose. GA_3 showed the highest result for Paymaster-2379. Since the highest vigour rate was obtained with MeJA (3 µM) (Fig. 2), MeJA was chosen as the best PGR for Aydın -110 and Paymaster-2379.

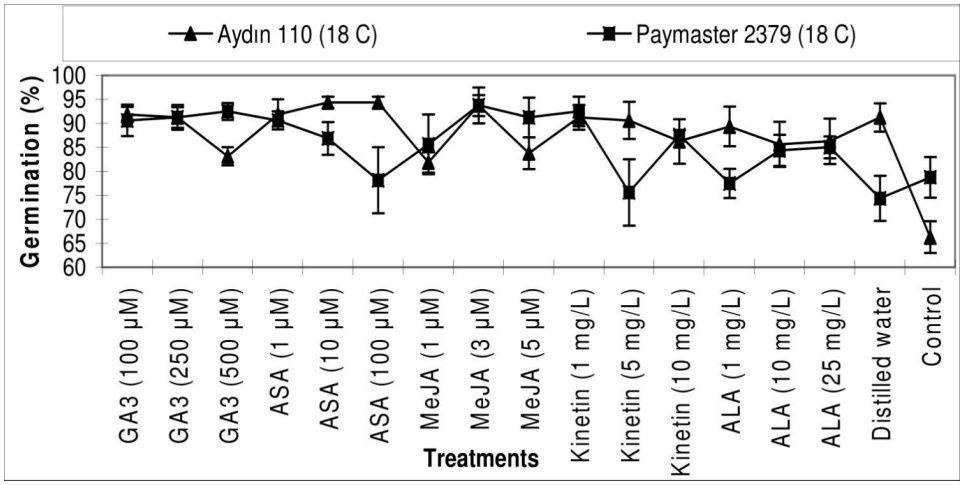

Fig. 2. Cold germination percentages for Aydın 110 and Paymaster 2379 after priming effects of PGRs

Germination rate [%]

The highest germination rate was obtained in GA_3 (97.50%). While GA_3 (250 µM) was determined as the best PGR in terms of its germination rate for Aydın-110, and GA_3 (100 µM) was picked as the best PGR for Paymaster-2379 were determined the best PGR for germination rate.

Radicle length [cm]

ASA (6.35 cm) promoted radicle elongation. While ASA (10 µM) generated the best results in Aydın-110, GA_3 (500 µM) was determined as the best PGR for Paymaster-2379. The longest radicle for Aydın-110 was obtained in distilled water primed seeds. The longest radicle for Paymaster-2379 was produced by MeJA (1 µM). Results showed that seeds primed with ASA (7.69 cm) were different from control (unprimed seeds) (4.15) 3.54 cm (Table 2).

Hypocotyl length [cm]

GA_3 provided the longest hypocotyl length. GA_3 (250 µM) was observed as the most effective PGR treatment for both Aydın-110 and Paymaster-2379. (Table 2).

Seedling length [cm]

GA_3 (7.65 cm) promoted seedling elongation. The longest seedlings were obtained by GA_3 (250 µM) in Aydın-110 and by GA_3 (500 µM) in Paymaster-2379 (Table 2). The highest results were obtained in MeJA. Germination results showed that PGRs in the best priming medium increased germination parame-

ters. But this increase was lower than using priming medium alone (Tiryaki and Buyukcingil, 2005). The results were lower when the chemicals (KNO_3 (4%)) was mixed with PGRs compared to when the chemical was used alone. According to the emergence test results, the best priming medium was KNO_3 (4%) and followed by KNO_3 (2%).

Table 2

Parameters compared for two cultivars after priming effects of PGRs for cold tolerance

		18 °C							
Chemical	Doses	Germination rate [%]		Radicle length [cm]		Hyrpocotyl length [cm]		Seedling length [cm]	
		A-110	P-2379	A-110	P-2379	A-110	P-2379	A-110	P-2379
GA_3	100 µM	97.50^{a}	98.75^{a}	7.03^{a-c}	5.78^{a-c}	1.57^{ab}	1.38^{ab}	8.60^{a-c}	7.16^{ab}
GA_3	250 µM	98.75^{a}	98.13	7.19^{ab}	5.54^{a-c}	1.99^{a}	1.77^{a}	9.17^{a}	7.30^{ab}
GA_3	500 µM	96.25^{a}	95.63^{ab}	4.63^{fg}	6.45^{a}	1.20^{b-d}	1.37^{ab}	5.83^{fg}	7.82^{a}
ASA	1 µM	96.88^{a}	98.13^{ab}	7.24^{ab}	6.30^{ab}	0.94^{cd}	0.94^{b-d}	8.18^{a-e}	7.24^{ab}
ASA	10 µM	96.25^{a}	96.88^{ab}	7.69^{a}	5.18^{a-c}	1.18^{b-d}	1.20^{bc}	8.87^{ab}	6.37^{a-d}
ASA	100 µM	98.13^{a}	91.88^{ab}	7.16^{ab}	4.51^{bc}	1.15^{b-d}	1.09^{b-d}	8.31^{a-d}	5.60^{b-d}
MeJA	1 µM	91.25^{a}	94.38^{ab}	5.10^{e-g}	5.33^{a-c}	1.12^{b-d}	1.06^{b-d}	6.22^{e-g}	6.38^{a-d}
MeJA	3 µM	97.50^{a}	96.88^{ab}	6.68^{a-e}	6.30^{ab}	1.44^{a-c}	1.18^{bc}	8.11^{a-e}	7.48^{ab}
MeJA	5 µM	93.13^{a}	94.38^{ab}	5.56^{b-g}	6.28^{ab}	1.48^{a-c}	1.01^{b-d}	7.04^{b-f}	7.28^{ab}
Kinetin	1 mg/L	96.88^{a}	96.25^{ab}	6.90^{a-d}	5.83^{a-c}	1.15^{a-c}	1.13^{bc}	8.06^{a-e}	6.96^{a-c}
Kinetin	5 mg/L	98.13^{a}	93.13^{ab}	6.46^{a-e}	4.21	0.94^{a-c}	0.87^{cd}	7.40^{a-f}	5.08^{cd}
Kinetin	10 mg/L	96.25^{a}	94.38^{ab}	5.69^{b-g}	4.78^{a-c}	0.94^{cd}	0.86^{cd}	6.62^{c-g}	5.63^{b-d}
ALA	1 mg/L	92.50^{a}	93.75^{ab}	6.13^{a-f}	4.55^{bc}	1.07^{b-d}	0.99^{b-d}	7.20^{a-f}	5.54^{b-d}
ALA	10 mg/L	93.75^{a}	93.13^{ab}	5.19^{d-g}	5.36^{a-c}	0.67^{d}	1.16^{bc}	5.86^{gf}	6.52^{a-d}
ALA	25 mg/L	91.25^{a}	93.13^{ab}	5.37^{c-g}	4.95^{a-c}	1.12^{b-d}	1.05^{b-d}	6.48^{d-g}	6.00^{a-d}
Distilled water			95.00^{a}	95.63^{ab}	6.24^{a-f}	4.18^{c}	0.74^{d}	0.65^{d}	6.97^{-g}
Control			93.75^{a}	88.13^{b}	4.15^{g}	4.74^{a-c}	0.79^{d}	0.84^{cd}	4.94^{g}
CV[%]			3.36	4.20	11.22	13.54	21.38	15.96	10.9
Mean			95.48	94.85	6.14	5.31	1.15	1.09	7.29

A - Aydın-110, P - Paymaster-2379

Effects of best priming agent and PGR on emergence of cotton seeds

Cold germination [%]

While KNO_3 (2%) showed 15.20% increase in vigour compared to the control, vigour rate in the cells treated with KNO_3 (4%) was found to be 9.80% higher than the control (Fig. 3). When KNO_3 (2%) was applied, 22.55% increase in germination rate was observed in Aydın-110. This rate was 7.85% in Paymaster-2379 compared to the control.

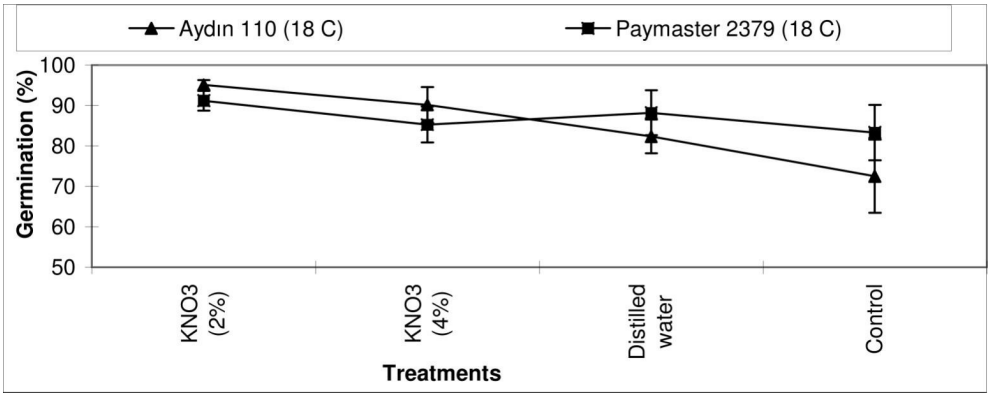

Fig. 3. Cold germination percentages for Aydın 110 and Paymaster 2379 after application of best priming agent and PGR

Germination rate [%]

The highest germination rate was obtained with KNO_3 (2%) (Table 3). KNO_3 (2%) showed 22.55% and 7.85% increase in germination rate in comparison with the control of Aydın-110 and Paymaster-2379, respectively.

Hypocotyl length [cm]

The longest hypocotyl lengths were measured in the seeds treated with KNO_3 (4%) (Table 3). KNO_3 (2%) showed 1.66 cm and 0.69 cm increase in hypocotyl length compared to the control in Aydın-110 and Paymaster-2379, respectively.

Radicle length [cm]

The highest result was obtained with KNO_3 (2%). Radicle length was increased 1.49 cm and 0.13 cm compared to the control (unprimed seeds) in Aydın-110 and Paymaster-2379, respectively (Table 3). However, radicle length of the seedlings generated from KNO_3 (4%) treated cells was longer than the ones treated with KNO_3 (2%). The difference in the radicle lengths of the seedlings of KNO_3 (4%) and KNO_3 (2%) treated seeds was 0.089 cm.

Table 3

Parameters compared for two cultivars after priming effects of PGRs for cold tolerance

Parameter à	CG	GR	HL	RL	SL	RW	HW	SFW	SDW
Chemical â					Aydın-110				
KNO_3(2%)	95.10^{a}	95.10^{a}	6.36^{a}	5.08^{a}	11.44^{a}	0.090^{a}	0.18^{a}	0.38^{a}	0.035^{a}
KNO_3(4%)	90.20^{ab}	90.20^{b}	6.00^{a}	4.83^{a}	10.83^{a}	0.091^{a}	0.18^{a}	0.34^{ab}	0.034^{a}
D.water	82.35^{ab}	82.35^{ab}	5.99^{a}	4.56^{a}	8.29^{b}	0.086^{a}	0.18^{a}	0.33^{ab}	0.034^{ab}
Control	72.55^{b}	72.55^{b}	4.70^{a}	3.59^{a}	10.55^{a}	0.077^{a}	0.14^{a}	0.27^{b}	0.028^{b}
CV(%)	12.89	12.89	21.86	20.72	10.89	18.87	22.72	16.64	11.58
Mean	85.05	85.05	5.76	4.51	10.28	0.09	0.17	0.33	0.03
Chemical â					Paymaster-2379				
KNO_3(2%)	91.18^{a}	91.18^{a}	5.39^{a}	4.61^{a}	10.00^{a}	0.087^{a}	0.16^{ab}	0.31^{a}	0.031^{a}
KNO_3(4%)	85.30^{a}	85.30^{a}	5.17^{a}	4.70^{a}	9.87^{a}	0.069^{a}	0.15^{ab}	0.27^{a}	0.029^{a}
D.water	88.24^{a}	88.24^{a}	6.15^{a}	3.77^{a}	9.93^{a}	0.079^{a}	0.18^{a}	0.32^{a}	0.030^{a}
Control	83.33^{a}	83.33^{a}	4.70^{a}	4.49^{a}	9.19^{a}	0.076^{a}	0.13^{b}	0.27^{a}	0.031^{a}
CV(%)	11.69	11.69	21.43	25.15	13.2	15.49	15.82	17.93	16.29
Mean	87.01	87.01	5.35	4.39	9.75	0.08	0.15	0.29	0.03

CG: Cold germination, GR: Germination rate, HL: Hypocotyl length, RL: Radicle length, SL: Seedling length, RW: Radicle weight, HW: Hypocotyl weight, SFW: Seedling fresh weight, SDW: Seedling dry weight

Seedling length [cm]

KNO_3 (4%) supported the seedling growth (10.83 cm). The longest seedlings were obtained with KNO_3 (2%) in all genotypes. KNO_3 (2%) showed 0.89 cm and 0.82 cm increase in seedling length compared to the controls of Aydın-110 and Paymaster-2379, respectively (Table 3).

Radicle weight [g]

Increase in radicle weight was 0.013 g and 0.011 g in the seedlings treated with KNO_3 (2%) in Aydın-110 and Paymaster-2379, respectively. KNO_3 (2%) was more effective for radicle weight increase than KNO_3 (4%) in Paymaster-2379 (Table 3).

Hypocotyl weight [g]

KNO_3 (2%) resulted in 0.04 g and 0.03 g increase in hypocotyl weight compared to the control in Aydın-110 and Paymaster-2379, respectively. KNO_3 (2%) and KNO_3 (4%) showed similar results with only an unremarkable amount of difference for Aydın-110 and Paymaster-2379 (Table 3).

Seedling fresh weight [g]

Seedling fresh weight 0.11 g and 0.04 g increased in KNO_3 (2%) treated seeds in Aydın-110 and Paymaster-2379, respectively. This result showed that KNO_3 (2%) was effective on cotton seedlings for cold tolerance. Cotton seed priming with KNO_3 (2%) can result vigorous seedlings in cold environmental conditions (Table 3).

Seedling dry weight [g]

KNO_3 (2%) treatment resulted in higher seedling dry weight than KNO_3 (4%) treatment (Table 3). Emergence test showed that KNO_3 (2%) was a more effective priming medium, which increased the values in all parameters except the hypocotyl weight of Aydın-110, compared to KNO_3 (4%) for cotton cold tolerance. When considered together, the results of this study indicates that KNO_3 (2%) is an effective priming chemical for cold stress conditions.

DISCUSSION

Cold stress tolerance in seed and different seedling parts increased with different chemicals and doses in priming. The amount of increase seemed to be dependent on germination parameters (germination rate, seedling lenght, hypocotyl length etc.), chemicals and doses. According to our results analyzed with control in comparison, the best vigour rate was measured when the priming was made with KNO_3 (4%). Although priming with NaCl (2%) resulted in higher values than the ones measured in KNO_3 (Figure 1).

Priming with Mannitol (96.77%) resulted in the highest germination rate and priming with KNO_3 (96.46%) and NaCl (94.90%) caused the germination of the seeds with the lowest germination rate. The highest germination rate of Aydın-110 was determined in Mannitol (6%) (100.00%) and the lowest germination rate of the same specimen was measured in KH_2PO_4 (0.3M) (88.13%). Saeidi *et al.* (2008) said that seed priming with KH_2PO_4 effective on canola roots. The highest germination rates were obtained with KNO_3 (4%) (98.75%). KH_2PO_4 (0.5M) produced the low results.

Mannitol is a good priming medium (Kaur *et al.*, 2002) and it showed the highest results in all parameters except hypocotyl length in Aydın-110. The highest results in all parameters of Paymaster-2379, except seedling and hypo-

cotyl length were obtained, were obtained by KNO_3. Germination results showed that KNO_3 (4%) yielded the highest result thus it was considered as the best priming medium, which can be supported by Dahiya *et al.*, 2007.

Priming effects of different chemicals on germination of cotton seeds showed that priming with KNO_3 increases germination features (Shim *et al.*, 2008; Farhoudi, 2012; Siadat *et al.*, 2012; Bian *et al.*, 2013). Seed priming for germination, emergence and vigorous seedling varies according to the parameters, genotypes, chemicals and doses, but cold tolerance effect of KNO_3 on seeds does not mostly remains unchanged (Dahiya *et al.*, 2007). Priming with KNO_3 (%2) increases germination rate and vigour in cold conditions. Results showed that after having sowed, priming with KNO_3 increased germination between 3.75 and 22.04%. When the priming effects of different PGRs on germination of cotton seeds were compared, the highest results were found to be in ASA, MeJA, GA_3. Previous studies reported that priming with this PGRs were effective on wheat, maize and herbage species (Afzal *et al.*, 2008; Jianhua *et al.*, 1999). Priming with KNO_3, Mannitol, KH_2PO_4 and NaCl are the best priming medium for all parameters.

Cotton seed priming and then germination at significantly increased germination percentage compared to control in Aydın 110 and Paymaster 2379. This results are important for cotton cold tolerance. As it was concluded by Rikin *et al.* (1979), primed seeds could be more resistant to cold environmental conditions.

PGRs mixed priming medium (second section) showed high germination results, but these results were lower than priming chemicals using alone (first section). Germination tests showed that is more effective to determine cotton germination rates.

This study showed that 2 hour priming with KNO_3 and then drying (moisture ~8%) provided the seeds with cold tolerance and vigorous seedlings. Priming with KNO_3 was an effective way to induce cold tolerance. Cotton seed priming with KNO_3 (2%) can be suggested for the best germination results and vigorous seedlings in cold conditions.

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