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Hydropriming and hot water-induced heat shock increase cotton seed germination and seedling emergence at low temperature

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Abstract: Increasing seed germination ability in cold soil conditions is necessary for effective cotton production. A major aim of cotton producers is to have good stand establishment and healthy seedlings. To determine the effects of hydropriming and heat shock treatment on seed germination and seedling emergence, seeds of 3 cotton cultivars, i.e. Stoneville-468, Maraş-92, and Sayar-314, were primed in distilled water at 5 °C or 25 °C for 2, 4, 6, 8, or 10 h or subjected to a hot water bath (96 °C) for 10, 30, 60, 90, 120, or 240 s. Germination and emergence percentage, and seedling fresh weight were determined at 18 ± 0.5 °C. Hydropriming at 25 °C increased germination rates by 54%, 17%, and 6% for Maraş-92, Sayar-314, and Stoneville-468, respectively. Longer durations of hydropriming at 5 °C decreased germination percentage. Hot water treatment for 10 s increased seed germination rates for Maraş-92 and Sayar-314 by 27% and 71%, respectively. Hot water treatment for 60 s increased germination of Stoneville-468 by 7%. Moreover, emergence percentages of seeds subjected to heat shock for 10 s were higher than those of the control seeds. Seedling fresh weights of hydroprimed seeds and seeds subjected to heat shock for 10 s were higher than those of the control seeds. The results indicate that hydropriming cotton seeds at 25 °C for 4–6 h or heat shock for 10 s increased seed germination and seedling emergence at low temperature. The results could have practical uses in cotton breeding programs.

Key words: *Gossypium hirsutum* L., cultivar, heat shock, hydropriming, low temperature, germination, emergence

1. Introduction

Low temperature is an important stress factor for germination and emergence of cultivated plants, especially tropical and subtropical plants such as cotton, soybeans, maize, and rice, which are known as cold-sensitive plants (Lyons 1973). These plants can exhibit significant physiological dysfunction when they are exposed to low temperatures.

Cotton development and production depend on many environmental factors affecting the plant itself, and its boll and fiber development. Germination of cotton seeds is sensitive to low temperatures (Ahmad 1999; Baloch et al. 1999). Cotton producers face a problem in their planting schedule. If they plant seeds late in the season (e.g., mid to late May) when soil temperature is ideal for seedling emergence, they face reduced fiber and seed quality resulting from maturation under the cool fall temperatures (Gipson et al. 1969). If, however, producers plant seeds early in the season (e.g., mid to late April) so that crop maturation occurs under warmer fall conditions, seedling emergence and stand establishment are compromised due to the low early spring soil temperature (Christiansen 1964; Christiansen and Thomas 1969). Cool temperatures, below 20 °C, may cause chilling injury to seedlings and reduce stand establishment (Cole and Wheeler 1974). Initial injury starting from the imbibition of cold water and water imbibition at 5 °C for 12 h can kill cotton seeds (Christiansen 1971). Secondary injury can occur 18 to 24 h after the initiation of germination when temperatures remain below 18 °C (Christiansen 1967). Therefore, cotton cultivars with enhanced cold tolerance are always desired (Duesterhaus et al. 2000).

Seed priming is a method that prevents radicle emergence promoting seed drying in osmotic solutions (McDonald 2000). This method is used for increased germination, vigorous seedlings, earlier flowering and maturity, higher grain yield, and reduced germination time (Khan 1992; Demir and Ellis 1994; Harris et al. 1999, 2001, 2002; Basra et al. 2005b; Farooq et al. 2005; Nouman et al. 2012). Seed priming has been used previously to improve seed germination of various species (Pill and Necker 2001; Korkmaz et al. 2004). Pretreatment of cotton seeds with abscisic acid almost completely prevented chilling injury when the seeds were exposed to 4 °C for less than 5 days

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(Rikin et al. 1979). Under the stress of low temperature, the initiation of lateral roots was also significantly promoted by mepiquat chloride (Duan et al. 2004). The levels of endogenous auxin, zeatin, and zeatin riboside in the middle segment of primary roots were all increased by mepiquat chloride, which might be the key reason for lateral root induction (Duan et al. 2004). When different cotton genotypes were primed with 3% sodium chloride (NaCl), germination rates of some of these genotypes increased at low temperature (Bölek et al. 2008).

Hot water treatment with various temperatures is a method for improving the seed germination rate (Msanga and Maghembe 1986). Hot water treatments were used to improve plant germination (Passam and Polyzou 1997; Güneş and Gübbük 2006) and for disease control (Bridges and Youtsey 1966). The hot water treatment greatly reduced seed chilling injury and fungal development and improved the visual fruit quality of *Opuntia ficus indica* clones (Rodriguez et al. 2005).

The cool germination test (Texas Cool Test) is the most widely used measure of seed/seedling vigor to evaluate cotton (*Gossypium hirsutum* L.) planting seed quality (Drummond and Savoy 1996). The most critical elements of this test are maintenance of constant 18 ± 0.5 °C in a germination chamber for the duration of the test and moisture content of germination substrata (Tolliver et al. 1997). On the other hand, a metabolic chill test (Duesterhaus et al. 2000), seedling fresh weight, seedling shoot fresh weight, seedling dry weight (Watanabe et al. 2000), seedling fresh weight (Sawan et al. 2000), seedling length (Halloin 1976), and emergence rate (Zhang et al. 1990; Murungu et al. 2003) have been proposed to measure seedling performance.

The effects of hydropriming and heat shock on cotton seed germination and seedling emergence percentages, and on seedling fresh weight under cold stress have not been documented so far. In the present study, cotton seeds were hydroprimed for various durations at different temperatures, or were subjected to hot water treatment to determine the effects of these methods on cotton seed germination and seedling emergence under cold conditions.

2. Materials and methods

2.1. Plant materials

Seeds of 3 different sets of upland cotton cultivars of *G. hirsutum* L. were used in this experiment. Of these cultivars, Maraş-92 and Sayar-314 were improved and registered in Turkey, while Stoneville-468 originated in the USA. Maraş-92 and Sayar-314 were cold intolerant (Kartal and Bölek 2006) while Sayar-314 was more sensitive to verticillium wilt than was Maraş-92. Stoneville-468 was more cold and verticillium tolerant than were Maraş-92 and Sayar-314. Cultivars were selected according to low (Maraş-92), medium (Sayar-314), and high (Stoneville-468) germination percentages (Bölek et al. unpublished data) to evaluate the effect of priming on different genotypic structures. Seeds were obtained from the same seed lot but their genetic backgrounds were different. After a pregermination test at 18 °C, Maraş-92, Sayar-314, and Stoneville-468 had 40%, 60%, and 80% germination rates, respectively.

For hydropriming, pure sulfuric acid delinted seeds were primed in distilled water at 5 °C or 25 °C for 2, 4, 6, 8, or 10 h and then dried in a germination cabin at 25 °C for 20 h. Dried seeds contained about 7.5% moisture according to an AND MX-50 moisture analyzer. Drying temperature and duration were selected based on a preliminary study that yielded the highest germination percentages after priming (Bölek et al. unpublished data). Heat shock was applied using a hot water (96 ± 1 °C) bath for 10, 30, 60, 90, 120, and 240 s. Nonprimed seeds in both experiments were used as controls.

2.2. Cool germination test (18 °C, paper towels)

Primed and dried seeds with nonprimed control seeds were placed on distilled water saturated paper towels (13 × 40 cm in size) and rolled. Four replications, 40 seeds each (to obtain sufficient distance between 2 seeds), were used. Rolled paper towels were placed in plastic containers (27 × 39 × 15 cm size), which were put into a germination cabin at 18 ± 0.5 °C (AOSA 1983) for 7 days. After 7 days, one count of normal seedlings that had a combined hypocotyl and root length of 3.8 cm or longer was made for cold tolerance rating (AOSA 1983). The root–hypocotyl measurement was made from the point of cotyledon attachment to the tip of the radicle. Seed lots having germination percentages over 60% were considered cold tolerant (Smith and Varvil 1984).

2.3. Metabolic chill test (18 °C, sand)

A modified metabolic chill test was applied to evaluate seedling performance of hydroprimed and heat shock treated seeds under cold stress. Four sets of 25 seeds for each cultivar were planted on wet sand (3.8 cm) in plastic boxes (32 × 49 × 11 cm size). The seeds were then covered with 2.5 cm of dry sand and placed in a germination cabin at 18 ± 0.5 °C for 21 days. After 21 days, the emergence percentage (Duesterhaus et al. 2000) and seedling fresh weight were measured. Cultivars having metabolic emergence percentages above 80%, 65%–80%, and 50%–65% were ranked as excellent, good, and fair cold tolerant as described by Duesterhaus et al. (2000). Seedlings were cut off just above the sand level (planting medium) 21 days after sowing and weighed using an analytical balance to determine seedling fresh weights.

2.4. Statistical analysis

Statistical analysis of the data was done by analysis of variance (ANOVA) using SAS (v8) computer software. Means were separated by Tukey's test when the F test was significant.

3. Results

Statistical analysis showed significant differences among the germination percentages of the cultivars primed with distilled water for different temperatures and different priming durations (Table 1). Compared to the control, treatments had some positive effects on germination percentages of the cultivars (Figure 1). Mean germination percentages over temperatures ranged from 59.37% (10 h) to 75.83% (2 h) for the 5 different durations, while they ranged from 41.75% (Maraş-92) to 94.50% (Stoneville-468) for the cultivars over the time periods (Figure 1). Among the cotton cultivars, Maraş-92 (having a low germination percentage, 51.25%) reached the highest germination percentage (78.75%) after hydropriming for 8 h at 25 °C, while Stoneville-468 (having a high germination percentage, 92.50%) still had the highest germination percentage (98.12%) after hydropriming for 2 h at 25 °C. Sayar-314 (having a medium germination percentage, 76.25%) possessed the best germination percentage (89.38%) after hydropriming for 6 h at 25 °C (Figure 1).

Hydropriming increased the germination percentages of the cultivars compared to the controls by 53.66%, 17.21%, and 6.08% for Maraş-92, Sayar-314, and Stoneville-468, respectively (Figure 1). The highest germination percentages were obtained after priming for 6 and 8 h at 25 °C (84.79%) and the lowest germination percentage was obtained after priming for 10 h at 5 °C (41.66%) (Figure 1). The highest increase in germination

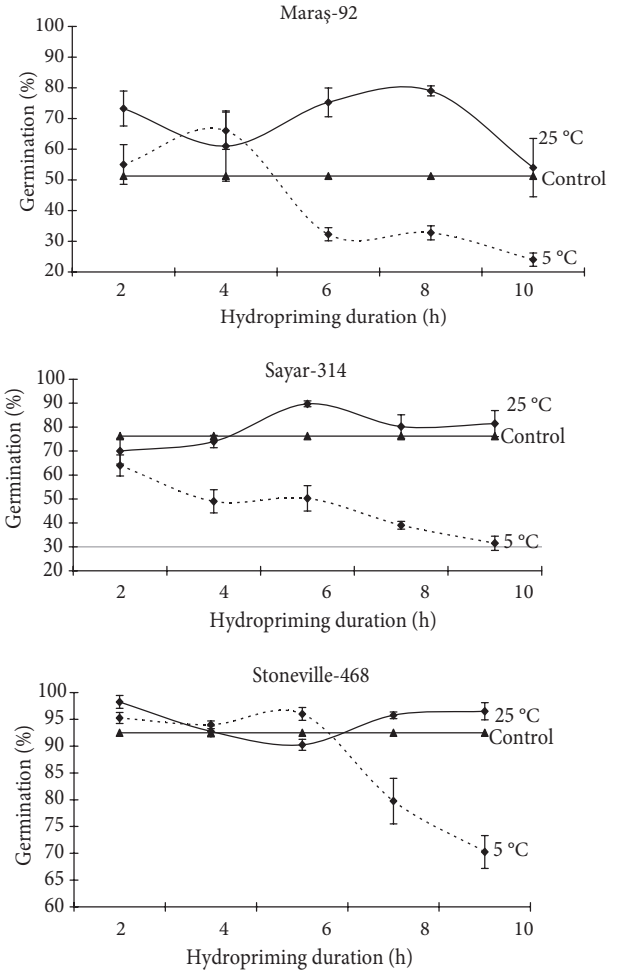

Figure 1. Mean germination percentages of 3 cultivars primed at 5 °C or 25 °C for varying time periods (2, 4, 6, 8, and 10 h) with their control (nonprimed seed germination).

Table 1. Analysis of variance for germination percentages of the cultivars primed at different temperatures and for different durations.

Variation source	DF	SS	MS
Cultivar (C)	2	28,116	14,058**
Time (TI)	4	3882	970**
Temperature (TE)	1	14,796	14,796**
C × TI	8	1304	163*
C × TE	2	3308	1654**
TI × TE	4	4584	1146**
C × TI × TE	8	2640	330**
CV (%)	12.67		
Mean (%)	69.43		

**, * Significant at 0.01 and 0.05, respectively.

percentage occurred in the seeds hydroprimed at 25 °C. Germination percentages at 5 °C were higher for Maraş-92 for 2 and 4 h priming, and for Stoneville-468 for 2, 4, and 6 h priming compared to the controls. However, compared to the controls, Sayar-314 sustained lower germination percentages for all hydropriming times at 5 °C. Analysis of variance for the emergence percentages showed significant differences among cultivars and hydropriming durations, while cultivars were only significantly different for the seedling fresh weight (data not shown).

Averaging the time over the cultivars, hydroprimed seeds for 6 h gave the highest germination percentage in the emergence test (Figure 2). The best hydropriming duration to have higher emergence percentages was 4 h for Sayar-314 and 6 h for Maraş-92 and Stoneville-468. In all cultivars, treatments had higher numerical values than the control (Figure 2). The increase in the emergence percentages of hydroprimed seeds was highest in Sayar-314 (41.34%), followed by Maraş-92 (37.33%) and Stoneville-468 (5.33%) compared to nontreated seeds. Emergence percentages of Maraş-92 and Sayar-314 were significantly higher while that of Stoneville-468 was not significantly different than the control treatment (Figure 2).

The highest seedling fresh weights for Maraş-92, Sayar-314, and Stoneville-468 were 7.9 g (6 h priming), 5.0 g (10 h priming), and 8.2 g (4 h priming), respectively. Fresh weight of seedlings obtained from hydroprimed seeds of Sayar-314 was significantly different, while for the other 2 cultivars it was not compared to the controls. Difference in seedling fresh weight was the highest in Sayar-314 (3.65 g), followed by Maraş-92 (3.62 g) and Stoneville-468 (2.14 g) (Table 2).

In the hot water treatment, the highest germination rates were obtained by 10-s application for Maraş-92 (76.87%) and Sayar-314 (82.50%), but 60 s was observed to be the best for Stoneville-468 (93.12%) (Figure 3). All cultivars showed low germination rates when they were subjected to hot water treatment for 240 s. The 10-s application was significantly better than the other application durations and the control for Maraş-92 and Sayar-314. On the other hand, 10–60-s application duration and control for Sayar-314 were in the same LSD group and significantly different than the other application durations (Figure 3). Hot water treatment increased the germination percentages of the cultivars by 26.80%, 71.44%, and 7.19%

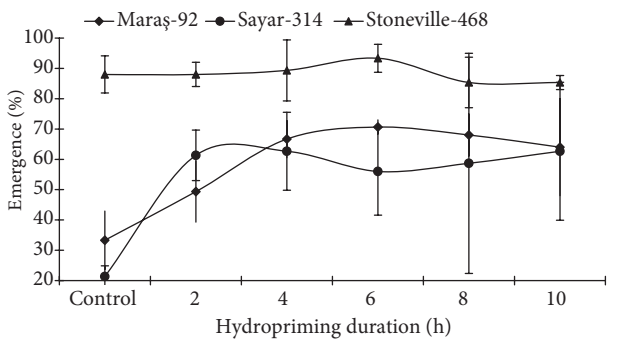

Figure 2. Emergence percentages of cultivars primed at 25 °C and for different time periods (2, 4, 6, 8, and 10 h) after metabolic chill test.

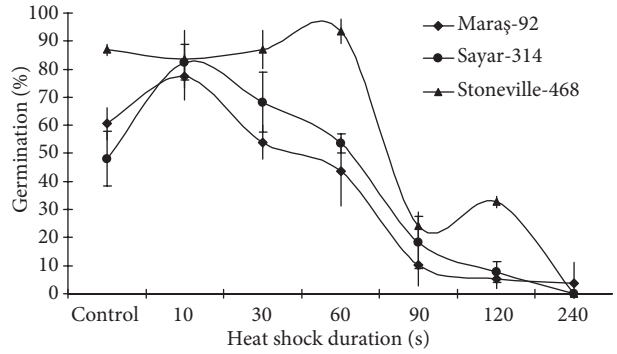

Figure 3. Mean germination percentages of 3 cultivars treated with hot water (96 ± 1 °C) for different time periods.

Table 2. Effect of hydropriming durations on seedling fresh weight after metabolic chill test at 18 °C.

Time (h)	Seedling fresh weight (g)		
	Maraş-92	Sayar-314	Stoneville-468
2	6.06 ± 2.07 a	4.83 ± 1.02 a	6.51 ± 1.26 a
4	7.80 ± 0.96 a	3.93 ± 0.59 ab	8.18 ± 2.60 a
6	7.89 ± 1.04 a	2.94 ± 0.50 ab	7.77 ± 1.51 a
8	7.81 ± 1.07 a	3.24 ± 1.67 ab	6.09 ± 1.50 a
10	7.42 ± 1.66 a	5.03 ± 1.05 a	6.67 ± 1.31 a
Control	4.27	1.38	6.04

for Maraş-92, Sayar-314, and Stoneville-468 compared to their controls, respectively. Heat shock applications for 60 s or longer durations decreased the germination rates of the cultivars (Figure 3).

The best priming heat shock durations (10–60 s) were used to test emergence percentages in cold conditions. Hot water treatment for 10 s yielded the highest emergence percentages for all cultivars tested. Although application for 10 s was not significantly different than the control for Maraş-92 and Sayar-314, it was significant for Sayar-314. The increase in emergence after heat treatment was 15%, 10.25%, and 33.33% for Maraş-92, Sayar-314, and Stoneville-468, respectively (Figure 4). Seedling fresh weight was the highest for seeds that were subjected to heat shock for 10 s and it was significantly higher than the controls for all 3 cultivars (Figure 4).

4. Discussion

In the initial work performed with Stoneville-468, 2 h of drying after priming (moisture >10%) resulted in a lower germination percentage than the control treatment. The study showed that 20 h of drying (moisture about 7.5%) yielded the highest germination percentages (Bölek et al., unpublished data). Since seed will not be damaged during storage because of the appropriate moisture content, this temperature was chosen in this experiment.

Hydropriming resulted in a positive effect on germination of cotton seed. This beneficial effect was the highest in Maraş-92, followed by Sayar-314 and Stoneville-468. Cultivars maintained different germination percentages depending on the time period for priming. Stoneville-468, Sayar-314, and Maraş-92 had the highest germination percentages following hydropriming for 2, 6, and 8 h, respectively. Averaging the cultivars and temperatures, 2 h was the best priming duration to obtain higher germination percentages at 25 °C. Higher initial germination percentage or viability of the seed (based on the germination percentages of the untreated seed) resulted in a shorter priming duration. The longer priming duration

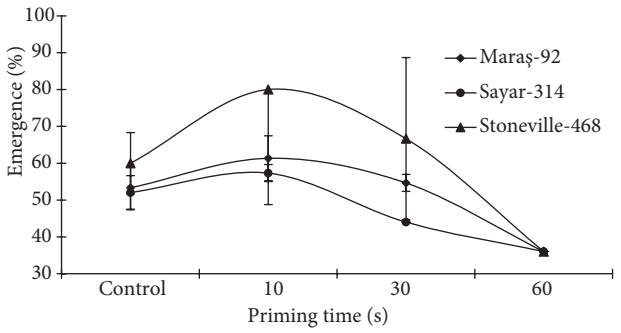

Figure 4. Effects of 3 different hot water durations on emergence percentages of the seedlings on the metabolic chill test.

in cold conditions (5 °C) decreased the germination percentages of the cultivars, while in warm conditions (25 °C) germination percentages were mainly affected by the cultivars rather than the priming duration. Hydropriming at 5 °C for 10 h resulted in the lowest germination percentages for all the cultivars. Seeds hydroprimed at 25 °C gave higher germination percentages than at 5 °C, and 2-h hydropriming was also better than longer priming durations. Keeping the seed longer at cold temperature during priming might have caused cell damage, thus affecting the germination. Germination percentages of the cultivars were higher than those of the controls, but the difference for Stoneville-468 was not statistically significant. This probably was due to the high germination potential of Stoneville-468. Even at the control level, Stoneville-468 had a high germination percentage and the increase in germination with hydropriming remained insignificant. The best hydropriming durations to have the highest emergence percentages were 4 and 6 h for the cultivars. On average 6-h priming seems better than the other treatment times for higher emergence percentages. The increase in emergence percentage after priming was lower for Stoneville-468, while it was higher for Sayar-314 and Maraş-92.

Seedling fresh weight was affected by genotypic effects. Averaging the cultivars, 4-h priming provided the highest seedling fresh weight. All the time applications resulted in higher seedling fresh weight than the control treatment. Germination and emergence percentages as well as seedling fresh weight were the highest for Stoneville-468, followed by Maraş-92 and Sayar-314. On the other hand, hydropriming treatment showed a significant increase in germination percentage, especially in Maraş-92 and Sayar-314. Our results of increasing germination percentages after priming are supported by the study by Toselli and Casenave (2007), who stated that hydropriming was effective in improving seed germination rate and seedling fresh weight compared with the control seeds. In addition, hydropriming was effective in increasing vigor in wheat (Basra et al. 2005a; Afzal et al. 2006). Thus, hydropriming is an effective way to increase vigor in stress conditions.

Hot water treatment increased the germination percentages of the cultivars by 26.8%, 71.4%, and 7.2% for Maraş-92, Sayar-314, and Stoneville-468, respectively. All cultivars showed low germination rates after hot water treatment for 60–240 s. The metabolic chill test was used only for seeds that had the highest germination rates following hot water treatment for 10, 30, and 60 s. In the metabolic chill test, all cultivars showed high germination rates if hot water treated for 10 s. Hot water treatment increased the germination percentages of the cultivars by 15%, 10.3%, and 33.3% for Maraş-92, Sayar-314, and

Stoneville-468, respectively. The results showed that treatment of seeds with hot water (96 ± 1 °C) for 10 s and then sowing under diverse conditions (such as cold stress) will help cotton seeds to germinate better than nontreated seeds. The cultivars showed different responses to hot water applications.

Hydropriming had positive effects on germination and emergence percentages as well as seedling fresh weight of the cultivars, which responded differently to the priming duration and temperature. Thus, after determining the best priming duration for the cultivar, it can be suggested that cotton be planted earlier.

Seeds hydroprimed at 25 °C for 2–6 h increased germination and emergence percentages of the cultivars at low temperatures but keeping the seeds in the water for longer periods or not enough drying decreased the germination percentages of the cultivars.

Hot water treatment of seeds followed by drying seeds to 7%–8% moisture content could help to increase the chance of having good stands under stress conditions. There are genotypic differences in response to hot water application and further work needs to be done to test different genotypes for cooling and drying time after priming with hot water.

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