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Evaluation of Water Stress Memory in Compensation Response of Cotton (*Gossypium hirsutum* L.) during Subsequent Water Deficiency

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ABSTRACT

This research was carried out to provide suitable cotton seed for seed propagation in dryland. In this study, the potential of cotton seeds that have been stressed for the third-consecutive year was investigated to evaluate water stress memory responses. The experiment was arranged in split-plot factorial design with four irrigation levels of W_0 (No- irrigation), W_1 (33% FC), W_2 (66% FC), and W_3 (100% FC), as the main factor, and five seed treatments (four third-stressed seeds, i.e. S_{21} to S_{24}, and registered seed), as a sub-plot. Seeds of cotton were grown under different levels of water-stress exposure for three crop-seasons. As results showed, S_{32} received water stress signal in both W_0 and W_3 conditions through physiological mechanisms change. Seeds of S_{32} accumulated the lowest ABA and the highest calcium in exposure to W_0 and W_3. Enhancement to superoxide dismutase and Aspartate peroxidase activity in leaves of S_{32} in exposure to W_0 and W_1 is another memorial stress mechanism for scarce water acclimation. The highest-potential thirty-boll weight, thirty-fiber weight, and first-harvesting yield were obtained from S_{32} against W_0, W_1, and W_2. Also, the seeds of S_{32} had the most seedling vigor and germination percentage in exposure to W_0, W_1, and W_2. It can be concluded that stress memory, via modification of physiology and morphology of plant behavior, helps plants to tolerate water deficiency when subjected to recurrent drought.

Keywords: Compensation mechanism, Drought tolerant, Seed, Yield.

INTRODUCTION

Drought is a common abiotic stress that severely limits production of cotton (*Gossypium hirsutum* L.) grown in rain-fed farms of Iran (Zare *et al*., 2014). Severe water stress may result in plant death, whereas mild water stress, in a short period of time, may improve drought tolerance by inducing stress memory that is introduced as drought imprinting, drought priming (as training and conditioning), hardening, and drought adaptation (Wojtyla *et al*., 2020). In fact, plant stress “memory” occurring in subsequent exposures to plants makes them more resistant to future exposure (Tombesi *et al*., 2018). The main habitat of cotton cultivation was the moderate climate in the north of Iran, but these days, the cotton planted area has been reduced (Kolahi *et al*., 2021). Indeed, irrigated cotton farmers have moved to dryland (Faghani *et al*., 2018). On the other hand, episodic water shortage influences cotton physiological traits and crop production. Plants often remember environmental stress that they have encountered during growth to harvesting time (Wojtyla *et al*., 2020). This ability of plants

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reactions refers to plant memory. Sometimes these physiological changes to manage environmental stress are temporary. However, when stress is continued, physiological modifications are stable and plants memorize them, and they transfer this information to the following generations as a stress memory (Sun *et al*., 2021). Moreover, modern plant physiologists emphasize "memorial plant" and their behavior (Wojtyla *et al*., 2020). There is limited information about stress memory and plant behavior against drought stress (Crisp *et al*., 2016). Therefore, recognizing cotton metabolic reactions to reproduce seed for dry field requires management practices in order to achieve potential yield and drought tolerant seed. Assimilation, which is important to growing bolls and subtending leaves, comes from "main stem leaves" (Schubert *et al*., 1986). Severity drought stress was declined by the accumulation of such compounds as abscisic acid (ABA), as a fundamental hormonal signal and enzymatic antioxidants adjustment in double-stressed reaction was declined. In exposure to water-stress consecutively, ABA can induce starch mobilization, which is an essential signal for metabolic adjustment against stress (Kempa *et al*., 2008). Wang *et al*. (2019) results suggest that ABA mainly acts in cotton seed after ripening. Therefore, ABA plays an important role in seed germination and dormancy. Then, it reduces the damage effects to seed germination against abiotic stress by changing ABA regulation (Zhang *et al*., 2014). The most common special role of Calcium (Ca) is known as adaptation against water stress. Under drought-stress condition, an increase in ABA induced the accumulation of intracellular Ca, which is known as a secondary messenger (Nayyar and Kushal, 2002). ABA and Ca increase the activities of Superoxide Dismutase (SOD) and Ascorbate Peroxidase (APX) against drought stress. The fundamental scavenging enzymatic defense antioxidants, in exposure to water deficiency stress, are SOD and APX (Ighodaro and Akinloye, 2018). ROS scavenging enzymes, such as SOD and APX, play an important role in cellular parts and act as a detoxifier for cellular survival (Wahid *et al*., 2007). Prolonged water stress exposure during subsequent drought stress leads to further acclimation by activating ABA, Ca, antioxidant enzyme, and morphological trait changes (Kolahi *et al*., 2020). Antioxidant enzyme and the accumulation of ABA and Ca compound influence seed germination and seedling vigor. Seed vigor is important to evaluate the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions (Alvarenga and Marcos-Filho, 2014). Therefore, yield components, such as thirty boll weight and fiber weight, were affected in exposure to subsequent drought stress. In cotton, water deficit at the filling stage influences crop production (Foulkesa *et al*., 2007). Due to unlimited growth, cotton is often known as a drought-resistant crop and compensates for the loss of yield by activating the drought tolerance mechanism, including physiological and biochemical changes (Niu *et al*., 2018). Evidence has indicated that plants adapt through changing their physiology and morphology characteristics in reaction to the previous occurrence of water stress (Kolahi *et al*., 2021). Then, some physiological mechanisms store this information from previous water stress exposure by activating memory on plant behavior during subsequent water deficiency stress.

On the basis of the aforementioned points, the aim of this research was to investigate the role of three season's exposures to water stress in activating water stress memorial reaction in cotton seed production in order to improve acclimatization and adaptive capacity to water deficiency condition, and also its transference to the next generations.

MATERIALS AND METHODS

Plant Material

Field experiments were carried out at Hashemabad Cotton Research Station. This station site is at the southeast corner of the Caspian Sea (36° 51' N latitude, 54° 16' E

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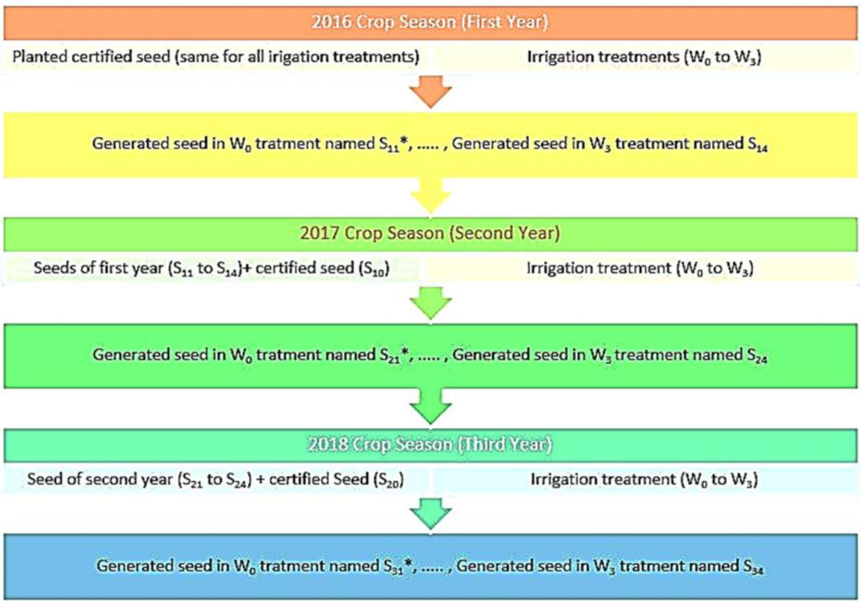
longitude, and 13.3 m above mean sea level). The soil texture was sandy clay silt (6, 6.8, 3) throughout the 0.5 m soil profile. Water content at field capacity and wilting point were 28.1 and 14.1% by soil volume.

Figure 1 shows the diagram that summarizes three years of research. In the first year, once water stress exposure was done, (2016), registered seeds were prepared from the market for the purpose of the study. Water treatments were no-irrigation [rain-fed], 33, 66, and 100% FC (W_0, W_1, W_2, and W_3, respectively). The seeds, i.e., Golestan cultivar, were named S_{10}, S_{11}, S_{12}, S_{13}, and S_{14} and were grown under W_0, W_1, W_2, and W_3 treatments.

In the second year (2017), the second water stress exposure was done. Seed treatments were chosen from the ones exposed to stress once (S_{11}, S_{12}, S_{13}, and S_{14}) and the certified seeds were obtained from the market (S_{10}). These cultivated seeds were subjected to irrigation treatments similar to the first year. Then, the harvested seeds were named S_{20} (from the market), S_{21}, S_{22}, S_{23}, and S_{24}' respectively, and were used for the following crop season.

The third water-stress exposure was done in the third year of the experiment (2018). Five seed treatments, i.e., S_{20} (purchased from the market), S_{21}, S_{22}, S_{23}, and S_{24}, were cultivated. Then, the registered seeds, i.e., (S_{21} andW_0), (S_{22} andW_1), (S_{23} andW_2), and (S_{24} andW_3), were labeled as S_{30}, S_{31}, S_{32}, S_{33}, and S_{34} according to exposure to four irrigation treatments (Non-irrigation [rain-fed] and 33, 66, and 100% FC) (Figure 1). This experiment was done in split-plot factorial design with three replications. Different irrigation treatments were started from the first square formation to the boll opening (in average 50 days) (Figure 2). Different irrigation treatments were set in the main plots, and seeds with two water stress exposures were cultivated in the sub-plots. In this experiment, seeds spacing were 20 cm (inter row) and 80 cm (intra rows) (Figures 3-a and -b). The amount of irrigation was measured by a water flow meter, and the time of irrigation was



* S_{11} means S (Seeds), the first number (1) used for first generation, and the second number (1) Used for the plot in which seed generated (first irrigation treatment or W_1)

Figure 1. Diagram of conducting three years of research.

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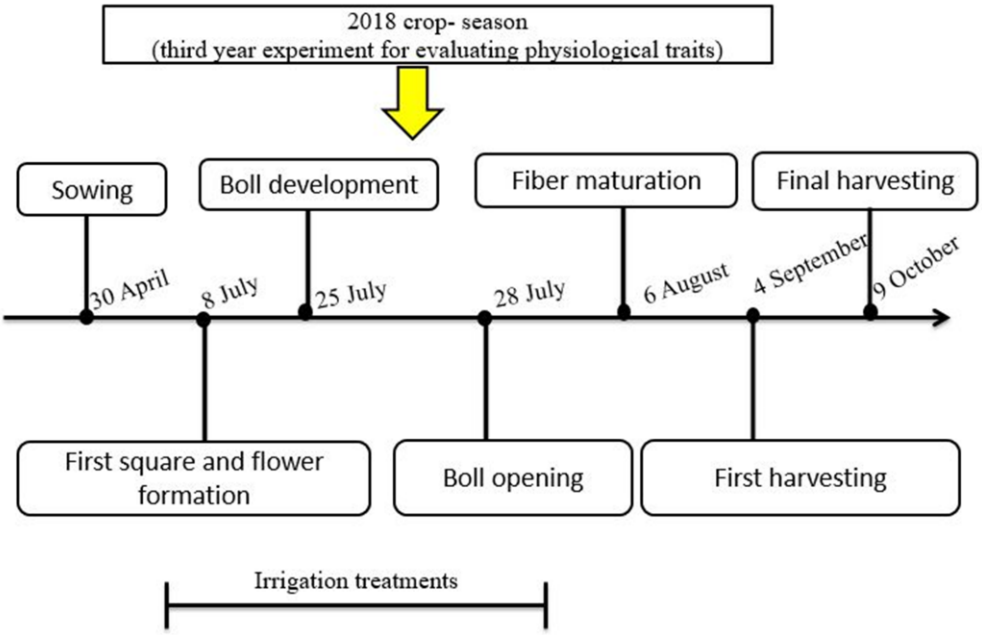

Figure 2. Sowing to harvest schedule and irrigation treatments for seed productions from double water stress exposure to the third water stress exposure experiments (2018 crop-season).

(a)

(b)

Figure 3. (a) Cotton seed cultivation and different irrigation treatments in farm. (**b)** Sampling cotton leaves of different seeds from third water stress exposure under different irrigations.

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determined by gravimetric determination of soil moisture.

Biochemical Studies

The seed and leaf samples were collected for biochemical analyses and were kept in liquid nitrogen to be carried out at the plant physiology laboratory. The following biochemical traits were evaluated:

Calcium and ABA Assay

Ca was extracted using EDTA method based on Motsara and Roy (2008). After burning 5 g of powdered seed samples in the muffle furnace at 550°C for about 12 hours, ashes were dissolved in HCl.

ABA content of seeds were estimated using HPLC (Unicam-Crystal-200, UK) according to Kelen *et al*. (2004) method. In brief, 100 mg seed dry weight was homogenized with 10 mL, 80% methanol that contained PVP. The pH of extraction was adjusted to 2.8 with H_3PO_4. The acidic solution was passed through a Diamonsic, C_{18}, 5 µm cartridge to trap and concentrate ABA**.** The acidic solution was passed through HPLC 184 Eurospher-100 C18 column (250×4 mm ID; 5 µm particle size, 185 Knauer, Germany) by filtering a 0.45 µm membrane 183 syringe filter (Whatman, England). The isocratic mobile phase consisted of 100% methanol and 0.2% acetic acid (50:50 v/v) at a flow rate of 0.7 mL/min. ABA peak was estimated using a standard solution and the signal was monitored at 257 nm at 40°C.

Antioxidant Enzyme

SOD Activity Enzyme

SOD was analyzed using Beyer and Fridovich (1987) method. In doing so, about 1 g fresh weight was grounded with a mixture of 1 mL of 50 mM potassium phosphate and 1 mM EDTA (pH 7.5) buffer. The homogenates solutions were centrifuged at 4ºC for 15 minutes at 43,000×g. The supernatants were used for the estimation of enzymatic activities and protein concentrations. One enzyme unit of SOD was determined by spectrophotometer at 560 nm (Hitachi model U- 2000) based on the amount of enzyme reduction required to cause 50% inhibition of the rate of NBT (Nitroblue tetrazolium) was measured.

APX Enzyme Activity

APX assay was carried out following Nakano and Asada (1981). One g seed fresh weight was homogenized in the extracted solution [50 mM Na_2HPO_4/KH_2PO_4 (pH 7.0), 1 mM EDTA, 5% polyvinylpyrrolidone (PVP-40) and 1 mM ASA]. The homogenate was centrifuged at 105,000×g for 10 minutes at 4°C. Oxidation of ASA was read at 300 nm (extinction coefficient 0.74 $mM^{-1}cm^{-1}$). APX was figured as the amount of enzyme oxidizing 1 µmol of ASA per minute.

Germination Percentage Test (Gmax)

Fifty Fuzzy seeds were placed on a filter paper (25×38 cm) and between double layered rolled germination papers in three replications. Each roll had been soaked with deionized water for 24 h. Paper rolls with seeds were put in a germination chamber (Paradise, Iran) at a constant temperature of 30°C with 250 mol $m^{-2}s^{-1}$ light intensity (diurnal cycle was 8 hours light and 16 hours darkness). After nine days, the germination percentage (%) was assessed using the following equation:

Gmax= 100×Germinated seed number at 9th day/50

Cool Warm Vigor Index (CWVI)

The testing procedure consisted of the following steps:

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The cool germination test was estimated at 18°C. After seven days, the number of normal seedlings greater than 1.5 inches long was counted (AOSA, 1993). The standard warm germination test was studied (alternating 20°C for 16 hours and 30°C for 8 hours, respectively). Then, the number of normal seedlings that were greater than 1.5-inch-long were counted after four days. Finally, the results of the two tests were added.

The following rating scale is currently being used based on AOSA (1993):

CWVI	Rating
160+	Excellent
140-159	Good
120-139	Fair
<120	poor

Statistical Analysis

The collected data were analyzed based on the average of three plants as one replicate for each treatment. SAS package (9; SAS Institute, Cary, NC, USA) was utilized to analyze data. Specifically, analysis of variance was estimated by Proc-Mixed model using Fisher's Least Significant Difference (LSD) test at the 5% level (SAS, 2001).

RESULTS

Biochemical Studies

ABA and Calcium Concentration

As the results demonstrated, the highest and the lowest ABA levels were obtained in S_{34} and S_{32} against W_0 water stress exposure with 46.4 and 19.4 ng g^{-1} FW, respectively. Also, in W_2 treatment, ABA content in seeds of S_{34} was the highest. In W_1 treatment, ABA accumulation in the seeds of S_{32} increased to 41.8 ng g^{-1} FW compared to other seed treatments (Figure 4). As Figure 3 displays, ABA concentration seeds of S_{31} in the third

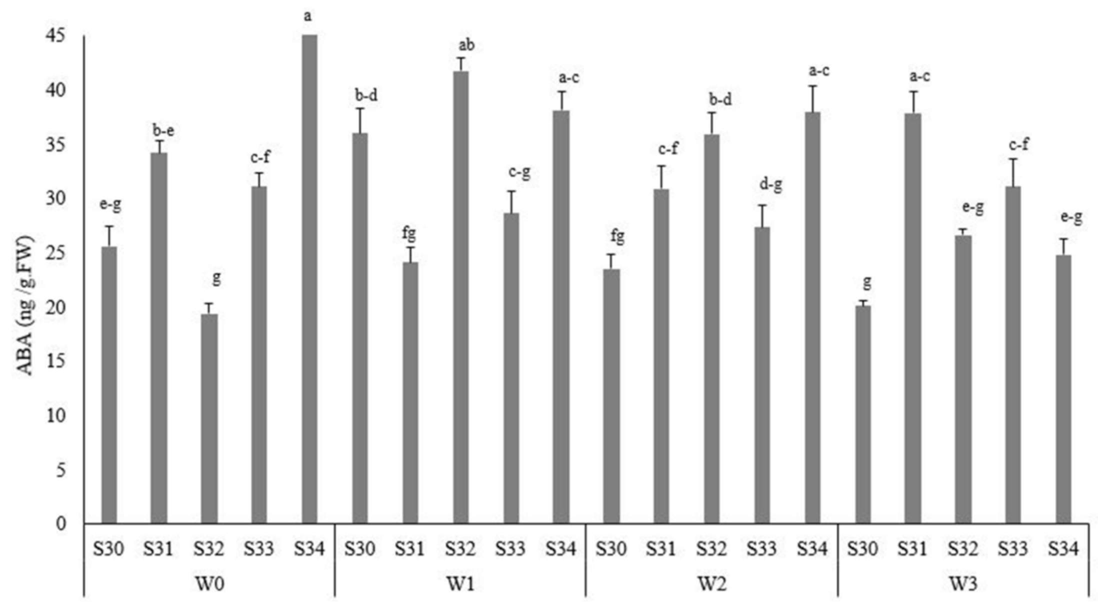

Figure 4. ABA content trend in cotton seed under different irrigation treatments [W_0; Rain-fed (without irrigation); W_1 (33% FC); W_2 (66% FC) and W_3 (100% FC)] and seed sources [(registered seeds); (S_{21}andW_0); (S_{22} and W_1); (S_{23} and W_2); and (S_{24} and W_3) were labeled as S_{30}, S_{31}, S_{32}, S_{33}, and S_{34} in the third water stress exposure. (Different letters represent significantly different).

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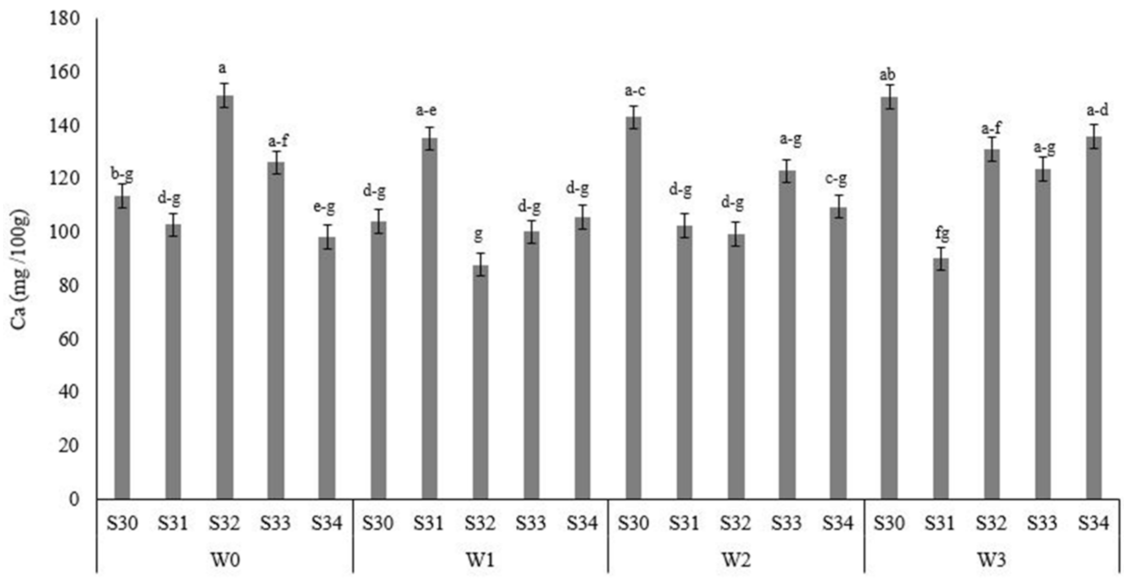

Figure 5. Calcium content trend in cotton seed under different irrigation treatments [W_0; Rain-fed (without irrigation); W_1 (33% FC); W_2 (66% FC) and W_3 (100% FC)] and seed sources [(registered seeds); (S_{21} and W_0); (S_{22} and W_1); (S_{23} and W_2); and (S_{24} and W_3) were labeled as S_{30}, S_{31}, S_{32}, S_{33}, and S_{34} in the third water stress exposure. (Different letters represent significantly different).

exposure to W_3 irrigation was the most compared to other seed treatments. As the result of Ca analysis showed, S_{32} against W_0 and W_3 had the highest (151.35 mg 100 g^{-1}) and the lowest (87.85 mg 100 g^{-1}) Ca content in matured seed, respectively. Being exposed to W_0 for the third time, Ca content in seeds of S_{34} decreased compared to other seed treatments (Figure 5). As the data showed, there was a reverse relationship between the accumulation of ABA and Ca in cotton seed. Also, S_{31} received drought-signal in treatment W1 and W3 irrigations due to having more ABA and low Ca content compared to other seed treatments, which might have weak germination for the next crop season. Generally, in W_0, S_{32} seeds stored the most Ca and the least ABA, but in W_1, seeds of S_{32} had the greatest ABA and the lowest Ca (Figures 4 and 5).

Antioxidant Enzyme

The effect of drought stress on the activities of antioxidant enzymes participating in the scavenging of ROS is shown by evaluating SOD and APX activities.

SOD Activity

SOD activity, as a radical scavenger enzyme, plays an important role against water shortage. However, on the basis of the results of the present study, SOD activity in seeds was more than its activity in cotton leaves (Figure 6).

In water stress exposure, SOD activity in cotton-seeds showed a significant variation among seed source and irrigation treatments. The values of SOD in cotton seed varied from 32.7 to 47.4 Ug^{-1} total protein (Figure 6). Among these treatments, the highest SOD activity was found in S30 (47.7 Ug^{-1} total protein) seeds in W_0 treatment, but the lowest SOD activity was related to S_{33} in W3 and W0 treatments (Figure 6). In W_0 to W_2, SOD activity in the seeds of S_{32} was the least (Figure 6).

Different seed irrigating results were observed in a wide range of variations of SOD activity of leaves (Figure 6). The most

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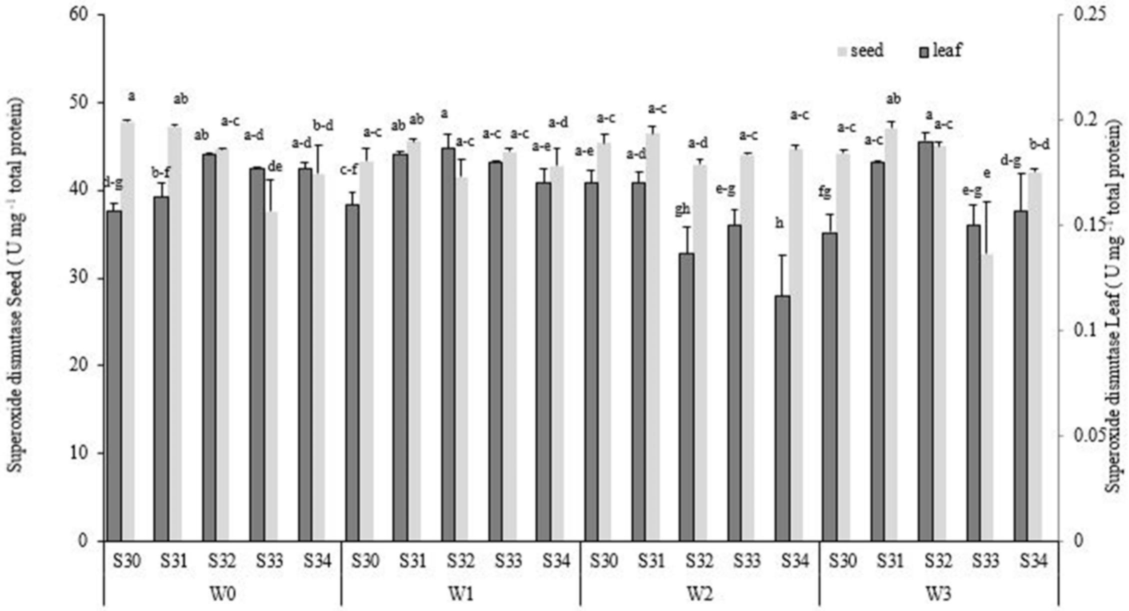

Figure 6. SOD activity content trend in cotton seed and different irrigation treatments [W_0; Rain-fed (without irrigation); W_1 (33% FC); W_2 (66% FC) and W_3 (100% FC)] and seed sources [(registered seeds); (S_{21} and W_0); (S_{22} and W_1); (S_{23} and W_2); and (S_{24} and W_3) were labeled as S_{30}, S_{31}, S_{32}, S_{33}, and S_{34} in the third water stress exposure. (Different letters represent significantly different).

SOD activity in the leaves of S_{32} was obtained in W_3 with 0.19 Ug^{-1} total protein. Nevertheless, in W_0, W_1, and W_3 irrigation treatments, S_{32} leaves had the most SOD activity (Figure 6). Moreover, SOD activity in the leaves of S_{32} under W_0 and W_1 irrigation were 17 and 16.6%, respectively, more than S_{30} (control) (Figure 6). In W_2 and W_3, S_{32} leaves had, respectively, 25.5% lower and 29.5% more SOD activity than S_{30} (Figure 6). Also, SOD activity in the leaves of S_{32} under W_0 and W_1 irrigations were 7 and 4.4% lower than that in S_{30}.

APX Activity

As the data showed, APX activity was lower than SOD activity in seeds and leaves. The present study indicated that the most significant APX activity was observed in the seeds of S_{31} W_3 (Figure 7). Also, APX activity in the seeds of S_{32} W0 was the lowest in comparison to other treatments in this condition. In the third W_2 irrigation, APX activity in the seeds of S_{32} was the most while APX activity in S_{33} seeds was the lowest, compared to others. The assessment of the APX activity in W_3 revealed that the S_{31} and S_{32} seeds had the highest while the seeds of S_{33} had the lowest APX activity (Figure 7). Evaluation of APX activity of leaves against several irrigation treatments and seeds gathered from the third water stress exposure indicated that the highest APX activity was related to the seeds of S_{31} against W_2 irrigation. (Figure7). APX activity in the leaves of S_{32} was about 84% lower than the leaves of S_{31} in W_2 exposure (Figure 7).

Seed Gmax and Seedling Vigor

Gmax and the vigor of seeds, which were harvested after the third water stress exposure, were estimated in each irrigation plot. The data showed that the maximum Gmax (100%) was related to S_{32} and S_{33} in W_2, and S_{33} in W_3 irrigation. To state it differently, the least Gmax, which was 80%, was obtained from S_{30} against W_2 and S_{31} in W_3. Seedling vigor resulting in W_0 stress appeared to reverse the

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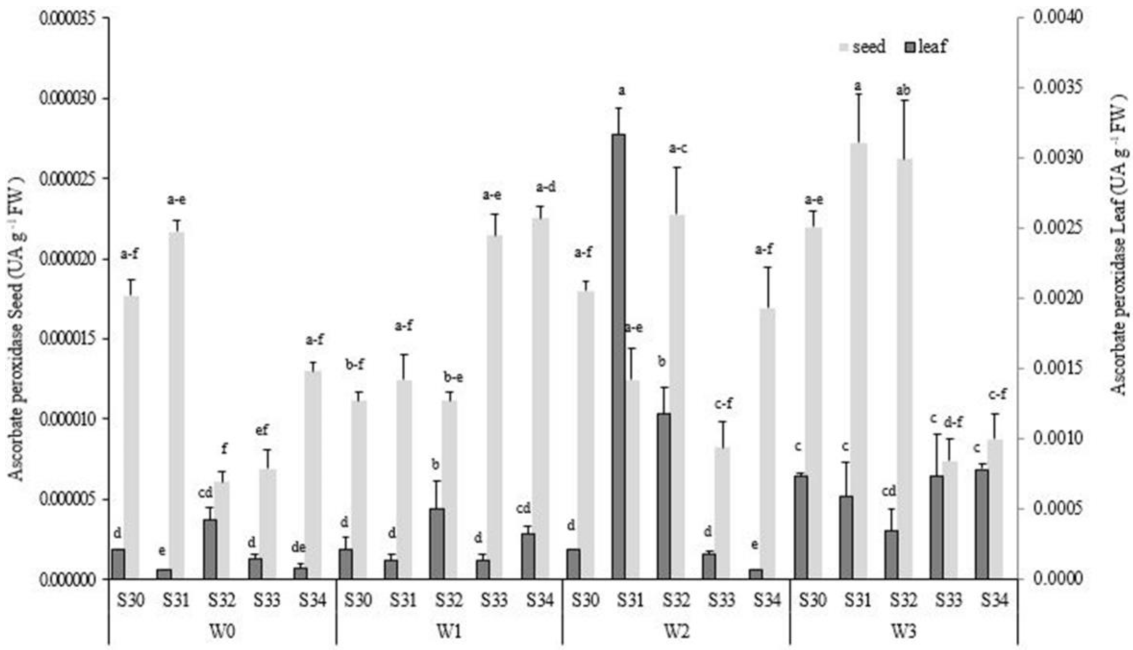

Figure 7. APX activity content trend in cotton seed and leaves under different irrigation treatments [W_0; Rain-fed (without irrigation); W_1 (33% FC); W_2 (66% FC) and W_3 (100% FC)] and seed sources [(registered seeds); (S_{21} and W_0); (S_{22} and W_1); (S_{23} and W_2); and (S_{24} and W_3) were labeled as S_{30}, S_{31}, S_{32}, S_{33}, and S_{34} in the third water stress exposure. (Different letters represent significantly different).

G max result (Table 1). It means that, in exposure to W_0 water stress, seeds with high potential G max had weak seedling vigor and vice versa. Also, in exposure to W_1, G max of S_{32} with 100% had the maximum seedling vigor index, i.e. 176, and S_{34} with 86% Gmax showed the lowest seedling vigor index, i.e. 138. In addition, W_2 water exposure, seed of S_{32} and S_{33} in subjected to W_3 irrigation represented 100% Gmax (Table 1). S_{32} and S_{33} in exposed to W_2 irrigation were classified in the 'excellent' class. Seeds of S_{32} and S_{33} in exposure to W_1 (with 176 and 152 seed vigor indexes, respectively) were set in the 'excellent' and 'good' classes, and seeds of S_{32} and S_{33} in exposure to W_0 (with seedling vigor index 154 and 158, respectively) were classified in the 'good' group.

Yield

Water stress significantly affected the weight and fiber of thirty-boll. S_{32} in W_1 and W_2 had the highest thirty-boll weight with 164 g and fiber of thirty boll weight with 61.66 g per plot in the first harvest. However, the minimum thirty-boll weight and fiber at the first harvest were obtained from S_{31} against W_3 and S_{33} in W_0 with 99.7 g and 31.53 g per plot, respectively.

Boll weight trait was affected differently among deficit irrigated conditions and seed treatments. The most significant boll weight at the first harvest was observed in S_{32} W_1 (Table 1). Additionally, thirty-boll weight and first harvest yield in S_{32} were increased in all irrigation treatments, i.e., W_0, W_1, and W_2 and were more than other seed treatments (Table 1). The highest yield in the first harvest was gained from S_{32} W_2 with 1581.3 g per plot, while in 6.2-fold was more in comparison to S_{32} under W_0 treatment. As the results showed, in W_0 , the first harvest yield of S_{32} produced 2.9- and 4.1-fold of S_{30} and S_{31} (Table 1).

DISCUSSION

Recently, dryland areas have been extended. Although facing long-term water shortage in cotton causes loss in yield, the impact of

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Table 1. Irrigation treatments [W_0; Rain-fed (without irrigation); W_1 (33% FC); W_2 (66% FC) and W_3 (100% FC)] and seed sources [(registered seeds); (S_{21} and W_0); (S_{22} and W_1); (S_{23} and W_2); and (S_{24} and W_3) were labeled as S_{30}, S_{31}, S_{32}, S_{33}, and S_{34} effects on first harvest, thirty- Boll- and thirty-fiber weight, germination (Gmax) and seedling vigor in exposed to third water stress condition. (Different letters represent significantly different).

WS Treatment	Thirty-Boll Fiber Weight (First harvest) (g 3 m^{-2})	Thirty-Boll Weight (First harvest) (g 3 m^{-2})	Yield (First harvest) (g 3 m^{-2})	Germination (percentage)	Seedling Vigor (Index)
W_0S_{30}	38.90 ±7.60 g-i	121.00 ±17.05 c-h	55.3 ± 6.00 h	88 ±3.25 a-c	150 ±1.62 i-l
W_0S_{31}	42.80 ±1.94 d-i	134.33 ±5.78 a-f	42.0 ± 21.35 h	96 ±3.25 ab	164 ±3.25 e-g
W_0S_{32}	46.33 ±0.88 c-h	136.33 ±1.85 a-e	217.3 ± 78.28 gh	94 ±1.62 ab	154 ±1.62 g-j
W_0S_{33}	33.53 ±8.13 i	104.10 ±26.70 f-h	126.0 ± 66.74 gh	92 ±3.25 a-c	158 ±1.62 f-i
W_0S_{34}	39.73 ±6.36 f-i	122.00 ±22.32 c-h	198.3 ± 75.98 gh	98 ±1.62 ab	146 ±1.62 j-m
W_1S_{30}	49.70 ±2.40 b-f	144.47 ±7.29 a-d	526.0 ± 86.53 d-g	90 ±4.87 a-c	160 ±3.25 e-i
W_1S_{31}	50.83 ±1.75 b-e	151.00 ±4.04 a-c	260.3 ± 172.34 gh	96 ±3.25 ab	162 ±4.87 e-h
W_1S_{32}	58.86 ±2.25 ab	164.00 ±6.25 a	1297.3 ± 33.94 ab	96 ±3.25 ab	176 ±3.25 b-d
W_1S_{33}	47.13 ±8.23 c-h	131.33 ±19.00 b-f	717.0 ± 119.65 d-f	94 ±1.62 ab	152 ±0.0 k-j
W_1S_{34}	56.13 ±1.27 a-c	154.77 ±2.42 ab	788.7 ± 104.18 de	86 ±4.87 bc	138 ±1.62 m
W_2S_{30}	55.80 ±1.74 a-c	143.33 ±4.05 a-d	1219.3 ± 192.04 a-c	80 ±6.50 c	138 ± 4.87 m
W_2S_{31}	51.66 ±1.76 a-d	129.00 ±2.00 b-h	1308.7 ± 81.71 ab	94 ±1.62 ab	178 ±1.62 bc
W_2S_{32}	61.66 ±1.20 a	149.67 ±3.28 a-c	1581.3 ± 95.69 a	100 ±0.0 a	162 ±4.87 e-h
W_2S_{33}	51.00 ±2.00 a-d	126.67 ±5.84 b-h	912.7 ± 82.28 b-d	100 ±0.0 a	192 ±3.25 a
W_2S_{34}	53.73 ±3.41 a-c	127.80 ±5.77 b-h	1496.0 ± 72.53 a	98 ±1.62 ab	170 ±4.87 c-e
W_3S_{30}	47.00 ±1.52 c-h	115.43 ±4.16 d- h	846.0 ± 136.72 cd	98 ±1.62 ab	166 ±1.62 d-f
W_3S_{31}	36.83 ±0.84 hi	99.77 ±2.47 h	399.3 ± 41.95 d-h	80 ±0.0 c	142 ±1.62 k-l
W_3S_{32}	40.06 ±0.96 e-i	101.13 ±0.94 gh	260.3 ± 34.72 f-h	90 ±8.12 a-v	140 ±0.0 ml
W_3S_{33}	47.73 ±1.82 c-g	117.67 ±2.60 d-h	698.7 ± 109.09 d-f	100 ±0.0 a	182 ±1.62 ab
W_3S_{34}	47.33 ±1.20 c-h	112.77 ±10.63 e-h	872.7± 43.63 cd	94 ±1.62 ab	166 ±1.62 df

drought-stress memory is induced by using some morphological parameters and physiological mechanisms to compensate for the effects of damage. Moreover, the heritable memory, which was known as “plant stress memory”, helps plants to react efficiently in exposure to stresses, not only for the current crop season but also for the next generations. Developing stress tolerance in plants for achieving high yield potential and stability affects modification of physiological mechanism.

ABA and Calcium Concentration

According to the results, seeds that had different irrigation treatments during water stress exposure displayed different drought stress signals using ABA and Ca changes. ABA data showed that the selection of rain-fed and 100% FC plots for seed production was not appropriate since the seeds had high ABA concentration and showed drought signal. As Richardson *et al.* (2019) believed, high ABA stored in seeds would affect seed viability. It is evident that ABA accumulation in these seeds may induce dormancy and inhibition of germination (Papenfus *et al*., 2013). High Ca plays an important role in adaptation to drought by improving seedling vigor and leaf water potential protection (Kaczmarek *et al*., 2017). Thus, the existence of high Ca and low ABA in matured cotton seed makes the stress injury sign better and different

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treatments have various characters (Nayyar and Kaushal, 2002). Therefore, the seeds that were cultivated in exposure to one-third and full-irrigation needs, were reproduced under rain-fed conditions. This production occurred because these seeds had more ABA and low Ca content compared to other seed treatments, which may have weak germination for the next crop season. It can be concluded that, ABA storage in seed reproduction of S_{32} in 33% FC water need exposure was not enough to prevent seed germination in the next crop season. Li *et al*. (2019) believed that ABA storage as a memory sign induced after the initial stress but was not stable for memory reactions to subsequent water stress. However, it should be noted that the most Ca content, as a secondary messenger in response to stress (Vishwakarma *et al*., 2017), and the least ABA content of S_{32} seeds under rain-fed illustrated drought tolerant sign. Therefore, on the basis of ABA and Ca content of seed, seed production with 33% FC can be recommended for rain-fed farms.

Antioxidant Enzyme Activity

SOD Activity

According to our results, the increase in SOD activity in S_{30} seeds under rain-fed conditions was representative of the water deficiency sign. Studies of SOD activity of leaves showed that with the aim of seed reproduction, S_{32} in comparison to S_{30} (control), revealed a high potential to eliminate O2 under water shortage and high irrigation conditions. Also, the most SOD activity in the seeds of S_{32} in 33% and 66% FC treatments revealed that these water deficiencies were sufficient to activate antioxidant metabolism. Moreover, the activity of SOD in seeds was more than SOD activity in leaves. Also, the seeds that were grown in this treatment would be more tolerant than rain-fed and 100% FC water stress exposure. The induction of SOD activity is recognized as plant ability to overcome oxidative stress by up-regulation of antioxidant enzymes (Alscher *et al*., 2002). According to this fact, S_{33} seeds under W_3 and W_0 and S_{32} seeds in W_2 and W_3 had low active oxygen species against substrate; therefore, SOD activity would decrease. Moreover, the lowest SOD activity in S_{33} seeds in exposure to W_1 and W_2 irrigation proved that these seeds do not show any water shortage signal. It seems that in spite of increasing SOD activity in the leaves of S_{32}, the activity of SOD was reduced in cotton seeds under W_1 irrigation. This result indicated that seeds of S_{32} could activate memory of drought-tolerant stress for planting against W_1. Many reports have shown that abiotic stress could provoke activation of SOD by overproduction of ROS, but other information displayed the opposite trend. These contradictions were related to plant species and long- period or short-period exposure to stress (Szollosi, 2014).

APX Activity

As the results show, S_{32} would be able to decrease APX activity in seeds and increase APX activity in leaves under W_0 and W_1 irrigation. Higher APX activity in the seeds of S_{32} in W_2 water exposure enhances tolerance to low water shortage more than in W_1 treatment. APX plays an important role in the removal of H_2O_2, like its substrate, i.e., ascorbic acid. According to our data, APX activity in leaves was higher than that in seed. The activity of changes in antioxidant enzymes was also highlighted as one of the mechanisms of drought stress memory. The report of Gunes *et al*. (2008) in sunflower is in line with our result. One of the highest important signals of drought-tolerant mechanism is overexpression of SOD with increasing H_2O_2 scavenging enzyme, such as APX activities (McKersie *et al*., 1999). It is clear that S_{33} produced seeds with low APX activity in exposure to W_0, W_2, and W_3 and that it did not show any drought signal through serving memorial

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stress. However, S_{32} leaves reacted to drought stress by increasing APX and SOD activation in exposure to W_0 and W_1.

Gmax and Seedling Vigor Index

The potential attributes of seed vigor index as a crucial physiological seed feature was evaluated for seedling establishment in farm. Gmax and seedling vigor traits were studied. The increase in ABA concentration in seed may be related to seed dormancy and low seed vigor (Yao *et al*., 2019). Metabolic compound seed affects seedling vigor directly or indirectly. Therefore, seed tolerance and seed quality are assessed by seed vigor (Marcos Filho, 2015). As it was revealed in this research, seeds of S_{33} against 100% FC irrigation, had high potential seed vigor index, while in this irrigation conditions, seeds of S_{32} had fair seedling vigor index. It was found that seed reproduction, after the third 33 and 66% FC water stress exposures, were irrigated with 66% FC and had high seed vigor index. Based on Hopper and Hinton (1980) researches, S_{32} and S_{33} exposed to W_0, W_1, and W_2 were able to have potential establishment in these irrigation conditions; therefore, using a low planting rate is needed.

Yield

The cotton yield will suffer serious irreversible damage when seeds are exposed to a range of intolerable water shortages (Niu *et al*., 2018). Oour data and Wang *et al*. (2013) report also showed that the thirty-boll weight and yield decreased under drought. Nevertheless, at the first harvesting period, thirty-boll weight and thirty-fiber weight enhanced in S_{32} when exposed to W_0, W_1, and W_2 compared to W_3. Accordingly, the severity of the compensation aspect varied in different seed treatments against different irrigations. On the other hand, being exposed to W_1 and W_2, S_{32} showed the influence of low water use in potential yield of rain-fed fields by producing 29.7 and 150% more yield in comparison to S_{30}. It is in line with Papastylianou and Argyrokastritis (2014) studies that showed the enhancement of cotton yield and fiber weight of cotton cultivars when subjected to moderate drought stress. The greatest thirty-boll weight and the first harvest yield of S_{32}, which was exposed to W_2 water stress, can be a sign of stress memory. This finding is also in line with Kim *et al*. (2020).

CONCLUSIONS

Decrease in yield under drought stress has been a major challenge for staple crops. Therefore, the identified drought-induced changes are primarily required in intergenerational stress memory. The long-term physiological memory in response to drought stress exposure showed a decrease in ABA level of seed, Ca content of seed, and activity of SOD and APX activity. These changes might be due to the stress memory mechanism. In summary, S_{32} was able to overcome the applied stresses and produced the highest yield in the first-harvest. Memory of drought stress through activating antioxidant enzyme in leaves prevented transport and storage of free radicals in seed. Based on the basis of role regulation of memorial water stress, that is formed or forgotten in cotton during period of stress, S_{32} could balance resources allocated and improved acclimation physiological mechanisms under W_0, W_1, and W_2 irrigation. Generally, for seed propagation in *Gossypium hirsutum*, 33% FC treatment activated plant stress memory by adjusting physiological metabolism for the third generation.

Abbreviations

Abscisic Acid (ABA), Aspartate Peroxidase (APX), Calcium (Ca), Leaf Area

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(LA); Field Capacity (FC); Super Oxide Dismutase (SOD).

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ارزیابی حافظه تنش آبی در پاسخ جبرانی گیاه پنبه (*Gossypium hirsutum* L.) در کم آبی

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چکیده

این تحقیق به منظور تامین بذر پنبه مناسب برای تکثیر بذر در مزارع دیم انجام شد، در این مطالعه، توانایی بذرهای پنبه در مواجه با تنش به مدت سه سال متوالی جهت ارزیابی پاسخ های حافظه تنشی بررسی شدند. آزمایش در طرح اسپلیت پلات فاکتوریل با چهار سطح آبیاری، W_0 (بدون آبیاری)، W_1 (۳۳درصد ظرفیت زراعی)، W_2 (۶۶ درصد ظرفیت زراعی) و W_3 (۱۰۰درصد ظرفیت زراعی)، به عنوان فاکتور اصلی و پنج تیمار بذری (چهار تیمار بذر سه بار تنش دیده، S_{21} تا S_{24} و بذر گواهی شده) به عنوان فاکتور فرعی که در معرض سطوح مختلف تنش آبی برای سه سال زراعی پرورش داده شدند. نتایج نشان داد که، S_{32} پیام تنش آبی را در هر دو شرایط W_0 و W_3 از طریق تغییر مکانیسم‌های فیزیولوژیکی دریافت نمود. بذرهای S_{32} ، کمترین مقدار اسیدآبسزیک و بیشترین مقدار کلسیم را در شرایط W_0 و W_3 انباشته کردند. افزایش فعالیت سوپراکسید دیسموتاز و آسپارتات پراکسیداز در برگ‌های S_{32} ، در معرض W_0 و W_3، مکانیسم دیگری از حافظه تنشی برای خوگیری به کم‌آبی بود. بیشترین وزن ۳۰ غوزه، وزن الیاف ۳۰ غوزه و عملکرد چین اول از S_{32} در معرض تیمارهای W_0، W_1 و W_2 بدست آمد. همچنین بذرهای S_{32} که در معرض W_0، W_1 و W_2 قرارگرفتند، بیشترین بنیه بذر و درصد جوانه‌زنی را داشتند. می‌توان نتیجه گرفت که حافظه تنشی از طریق تغییر فیزیولوژی و مورفولوژی رفتار گیاه می‌تواند در تحمل به کم آبی به گیاهانی که به طور متوالی در معرض خشکی قرار دارند، کمک نماید.

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