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THE EFFECT OF ASCORBIC ACID TREATMENT ON WHEAT (*TRITICUM AESTIVUM* L.) SEEDLINGS UNDER DROUGHT STRESS

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Key Words: Ascorbic acid, drought stress, chlorophyll, enzymes activity

ABSTRACT

Three Egyptian wheat (Shanadaweel 1, Giza168, and Masr 1) varieties were evaluated under drought stress using different concentrations of polyethylene glycol (PEG) (0, 1, 2, and 3 bars) and investigate the effects of the application of ascorbic acid (0, 150, and 200 mg/L) in the alleviation of drought stress at the seedling stage. The results revealed that drought stress caused a decrease in germination percentage, growth parameters (shoot length and root length) and photosynthetic pigments (chlorophyll A, chlorophyll B, and total carotenoids) with increasing PEG concentrations in all wheat varieties. Moreover, Catalase (CAT) activity increased in Giza168 with increasing drought stress at 3 bar, whereas CAT activity increased in Masr 1 and a nonsignificant increase in Shandaweel 1 at 1 bar and then decreased at 2 bar. Also, proline accumulation under drought stress in all wheat varieties. On the other hand, pretreatment of seeds treated with ascorbic acid (0, 150, 200 mg/l) enhanced all parameters studied under drought stress. 200 mg/L of ASA is the most effective in mitigating the effects of drought stress on the morphological and chemical characteristics of wheat varieties at the seedling stage.

INTRODUCTION

Drought, one of the environmental stresses, is the most significant factor restricting plant growth and crop productivity in the majority of agricultural fields of the world **(Tas and Tas 2007).** Drought decreased germination and seedling growth, and this is one important case to produce crops **(Gamze et al., 2005).** Drought stress causes reduced stomatal conductance resulting in decreased net photosynthetic rate. Chlorophyll degradation due to drought stress also inhibits the photosynthetic rate in wheat **(Moaveni, 2011).** In addition, it inhibits the photochemical activities and decreases the activities of enzymes in the Calvin Cycle in photosynthesis **(Monakhova and Chernyadev 2002).** PEG as a factor causing drought stress by reducing water potential results in reducing growth in seed germinated and stopping seedling growth so that this effect has been observed more in the shoot than primary roots **(Zhu, 2006). Dodd and Donovan (1999)** also suggested that PEG prevents water absorption by seeds, but penetrable ions by reducing

potential inside cell results in water absorption and starting to germinate. Proline accumulation under salinity/ drought stress (**Kavi Kishor *et al.*, 2005)**, which protect the proteins against denaturation and act as osmotic balancing agents **(Sivakumar *et al.*, 2000)**. The generation of ROS is limited or scavenged by an antioxidant system, including antioxidant compounds (ascorbate, salicylate, glutathione, tocopherol, etc.) and antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) **(Foyer and Noctor, 2003).** Catalase (CAT) and peroxidase (POD) are enzymes that catalyze the conversion of H_2O_2 to water and O_2 (**Gratao *et al.*, 2005**).

Wheat (Triticum aestivum L.) is one of the most important crops in Egypt, which plays a special role in people's nutrition. Ninety-five percent of the cultivated wheat is used for the preparation of bread and other baked products, while the remaining 5% is durum wheat, which is used essentially for making pasta and macaroni (**Bushuk, 1998**). External application of ascorbic acid activates antioxidant mechanisms, and this improves resistance to drought stress **(Shalata and Neumann, 2001).** Pre-sowing treatment with ascorbic acid is widely used. It improves performance and stand establishment at different external factors such as high salinity (**Shaddad *et al.* 1990 and Afzal *et al.* 2005).** The objectives of this investigation are to study the effects of ascorbic acid applications as an antioxidant on seeds germination, growth parameters, Photosynthetic pigments, proline, and activity of enzymes in three wheat seedlings grown under drought stress.

MATERIALS AND METHODS

Plant material and treatments: -

The present study was carried out in Biochemistry Department, Faculty of Agriculture, Al-Azhar University, three varieties of wheat (Triticum aestivum L.) included Shandaweel 1, Giza168 and Masr 1 were used in this study. wheat seeds were obtained from the Department of Seed Technology Research, Field Crops Research Institute, Agriculture Research Center (ARC), Ministry of Agriculture, Giza, Egypt. Homogeneous seeds were sterilized using 70% ethanol for 2 min. followed by 0.2% sodium hypochlorite (NaOCl) for 3 min. then rinsed for 3 times with distilled water.

Application of Ascorbic acid and growth conditions

After washing the seeds, they were soaked for 24 h at room temperature in distilled water or 150 and 200 mg/L Ascorbic acid before sowing in Petri dishes. Effect of drought stress induced by different osmotic potential level (0, 1, 2 and 3 bars) of polyethylene glycol 8000 (PEG 8000) **(Michel, 1983).** Germination trials were carried out in 15 cm Petri dishes containing a layer of two filter paper whatman's two filter

paper sterilized with distilled water or PEG solution. Three replications were sown in Petri dishes on two filter paper beds and each treatment contained 50 pure seeds, then irrigated with 10 ml solution of different concentration of PEG solution (0, 1, 2 and 3 bars) and incubated in growth chamber at 20±2 °C for 10 days. Seed germination was observed daily with fresh PEG solution added to the Petri dishes as necessary to maintain moisture levels. Germination percentage was calculated using the formula outlined by **Krishnasamy and Seshu (1990)**. Seedling shoots and roots lengths of ten randomly selected seedlings were measured after 10 days of germination (**ISTA, 1993**).

Biochemical analysis: -

Determination of of photosynthetic pigments:-

To extract photosynthetic pigment from wheat shoots, 0.2 g of fresh shoot was homogenized with 10 ml of 100% acetone in a porcelain mortar and centrifuged at 2500 rpm for 10 min. The supernatant was separated and the absorbances were read at 662 and 645 nm for chlorophyll a and b and 470 nm for carotene. Were calculated using following equations of **Lichtentaler and Wellburn (1985).**

Chlorophyll a (µg/ml) = 11.75 A662 – 2.350 A645

Chlorophyll b (µg/ml) = 18.61 A645 – 3.960 A662

Carotenoids (µg/ml) = 1000 A470 – 2.270 Chl a – 81.4 Chl b/227.

Extraction of antioxidant enzymes:-

Enzymes were extracted from 0.5 g leaf samples homogenized in a pre-chilled pestle and mortar containing ice cold 0.1 M phosphate buffer (pH 7.5) and 0.5 mM EDTA. Each homogenate was transferred to centrifuge tubes and centrifuged at 4°C in a Sorval model T21 (Thermo Scientific, Waltham, MA) refrigerated centrifuge for 15 min at 15000 x g. The supernatant was decanted and used for measuring enzyme activity assays (**Esfandiari *et al.*, 2007**).

Enzymes activity assay:-

Catalase activity:-

Catalase activity was determined according to the method used by **Aebi (1984)** in which the disappearance of H_2O_2 in a reaction mixture containing 0.3 mL 3% H_2O_2, 2.5 mL of 0.05 M phosphate buffer (pH 7), and 2.5 mL of plant extract is monitored by the decrease in absorbance at 240 nm.

Peroxidase activity:-

Peroxidase was assayed spectrophotochemically according to (**Amako *et al.*, 1994**) the assay was carried out at 25 °C in 1.0 cm light

path cuvette and the reaction mixture consisted of 1500 µL phosphate buffer, 1000 µL pyrogallol and 480 µL H_2O_2 solution. After mixing, the reaction was initiated by adding the enzyme extract (20 µL) and the increase in optical density at 430 nm against blank (without extract) was continuously recorded every minute (for 1 min).

Determination of proline:-

Proline content of shoot was determined according to a modification of the method **of Bates et al (1973)**. Samples of shoots (0.5 g) were homogenized in a mortar and pestle with 10 ml sulfosalicylic acid (3% w/v), and then centrifuged at 18,000 g for 15 min. Two ml of the supernatant was then added to a test tube, to which 2 ml glacial acetic acid and 2 ml freshly prepared acid ninhydrin solution (1.25 g ninhydrin dissolved in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid) were added. The test tubes were incubated in a water bath for 1 h at 100°C and then allowed to cool to room temperature. Four ml of toluene were then added to the tubes and then mixed on a vortex mixer for 20 s. The test tubes were allowed to stand for at least 10 min to allow separation of the toluene and aqueous phases. The toluene phase was carefully pipetted out into a glass test tube and its absorbance was measured at 520 nm in a spectrophotometer. The content of proline was calculated from a standard curve and calculated on a fresh weight basis as follows: {(µg proline/ml × ml toluene) / 115.5 µg/µmole}/ {(g sample)/5} = µmoles proline/g of fresh weight material.

Statistical analysis:

Randomized Complete block design analysis for all data obtained was carried out and differences between means were calculated using L.S.D test according to **Steel and Torrie (1980).**

RESULTS AND DISCUSSION

The effect of ascorbic acid on germination and growth parameters in three wheat varieties under drought stress by PEG

The effect of ascorbic acid on germination percentage and growth the parameters under drought stress are presented in Table (1). Under control, the highest values of germination percentage were observed in Giza168 and Shandweel 1 (98%). Germination percentage decreased by increasing of drought stress by PEG level. Germination percentage in all wheat varieties were significantly decreased at 1 and 2 bars but Giza168 has a higher germination percentage (86%) at 2, while the lowest value of germination percentage was found in Masr 1 variety (56%) at the same level of drought stress. At 3 bar drought stress germination percentage was (0%) in all wheat varieties. From the results in Table (1) indicated that Giza168 more drought tolerant than Shandaweel 1 and Masr 1.

Table (1): The effect of ascorbic acid on germination and growth parameters in three wheat varieties under drought stress by PEG.

ASA	PEG	Germination %			Shoot length (cm)			Root length (cm)		
mg/l	bars	Shandaweel 1	Giza168	Masr 1	Shandaweel 1	Giza168	Masr 1	Shandaweel 1	Giza168	Masr 1
0	0	98.000	98.000	92.000	11.000	11.333	10.333	10.333	10.667	8.333
	1	93.000	94.000	75.000	6.000	6.333	4.667	7.667	8.000	6.000
	2	76.000	86.000	56.000	1.833	4.000	2.333	2.333	6.000	5.667
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
150	0	96.000	98.000	96.000	11.333	12.000	10.333	10.667	11.333	9.000
	1	96.000	97.000	81.000	6.667	6.667	4.667	8.000	8.333	7.333
	2	81.000	91.000	53.000	2.667	4.667	3.333	5.333	7.000	5.333
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
200	0	98.000	99.000	95.000	12.667	11.333	11.333	12.667	11.000	8.667
	1	95.000	98.000	83.000	10.667	8.333	10.000	8.000	8.667	7.333
	2	85.000	95.000	65.000	9.000	6.667	7.333	5.667	7.333	5.667
	3	65.000	85.000	60.000	6.333	5.333	7.667	4.333	6.333	4.667
L.S.D 0.05			2.10			0.32			0.88	

Application of ascorbic acid (ASA) as soaking led to increasing of germination percentage in all wheat varieties (Table 1). 150 mg/L of ASA caused significantly increase in Shandaweel 1 and Giza168 from (76 and 86%) to (81 and 92%), respectively at 2 bar , while led to decrease in Masr 1 from 56% to 53% at 2 bar, 150 mg/L of ASA was not effective on germination in all wheat varieties at 3 bar. Application of 200 mg/L of ASA caused increasing of germination in all wheat varieties. Germination percentage increased from 0% to 65, 85 and 60 % at 3 bar in Shandaweel 1, Giza168 and Masr 1, respectively with 200 mg/l of ASA. On the other hand, data in Table (1) showed that shoot, root length decreased in all wheat varieties with increasing of drought stress by PEG. The highest values of shoot and root length were found in Giza168 (11.33 cm) and (10.66 cm), respectively under normal

conditions. The lowest values of shoot and root length were observed in Shandaweel 1 (1.83 cm) and (2.33 cm) respectively at 2 bar drought stress. Application of ASA as soaking significantly increased shoot, root length under drought and non-drought. Shoot length in shandaweel1 significantly increased from 6.0 cm to 6.66 cm at 1 bar and increased from 1.83 cm to 2.66 cm at 2 bar with 150 mg/L of ASA. Also, 200 mg/L of ASA significantly increase of shoot length in Giza 168 and Masr 1 from 4.0 and 2.33 cm to 6.66 and 7.33 cm, respectively at 2 bar. From the results, it is obvious that ascorbic acid pretreatment alleviated the inhibitory effect of drought stress on germination and growth parameters. Also, 200 mg/L of ASA had higher effect compared to 150 mg/l of ASA. Increased shoots and root length by ascorbic acid might be due to the cell division and differentiation of meristem cells **(Liso et al., 1998).** These results agreed with **DolatAbadian and Sanavy (2008)** reported that priming with ascorbic increased germination percentage, Length of shoot and root, their dry weight, and seedling total dry weight in sunflower.

The effect of ascorbic acid on photosynthetic pigments in shoots of three wheat varieties under drought by PEG

Data presented in Table (2) showed that photosynthetic pigments i.e. chlorophyll a, b and carotenoids were significantly decreased with increasing of drought stress by PEG. chlorophyll a, b and carotenoids of Giza 168 higher than Masr 1 and Shandaweel 1 under drought and control. under control, the maximum values of chlorophyll a, b and carotenoids were 0.470,0.394 and 0.628 mg/g, respectively were found in Giza 168. Drought stress led to significantly decreased chlorophyll a, b and carotenoids in all wheat varieties. The lowest values of chlorophyll a was 0.293 mg/g in shandaweel 1, while, the lowest values of chlorophyll b and carotenoids were 0.193 and 0.187 mg/g, respectively in Masr 1 at 2 bar. Application of ASA as soaking led to increase chlorophyll a, b and carotenoids contents under drought stress. Chlorophyll a, b and carotenoids of Masr 1 significantly increased from (0.212, 0.194 and 0.298 mg/g) to (0.309, 0.255 and 0.389 mg/g), respectively with 150 mg/l of ASA and significantly increased to (0.388, 0.323 and 0.562 mg/g), respectively with 200 mg/l of ASA at 1 bar. The highest values of chlorophyll a, b and carotenoids (1.217, 0.842 and 1.079 mg/g) were found in Giza 168 with 200 mg/l of ASA under normal conditions. At 3 bar, the chlorophyll a, b and carotenoids of Giza 168 were higher than Shandaweel 1 and Masr 1 with 200 mg/L of ASA. From these results showed that Giza 168 had higher chlorophyll a, b and carotenoids under drought stress and 200 mg/L is the most effective concentration of ASA in reducing the effect of drought stress on total pigment. The decrease of chlorophyll content under water limited condition is reported to take place because of its photo-oxidation and degradation under drought **(Anjum et al., 2011).** Exogenous application of ascorbic and

mitigated the adverse effects of drought on photosynthesis in all wheat varieties by increasing stomatal conductance. This could have also been due to the fact that ascorbic acid as an antioxidant has the ability to mitigate the negative effects of stress on plants by neutralizing harmful oxidants which have been reported to damage plant membranes such as the thylakoid membranes of chloroplasts **(Dolatabadian et al., 2009).** These results agree with **Khalil et al. (2010)** who found that the application of ascorbic acid in different concentrations showed significant increase in all photosynthetic pigments in *Ocimun basilicum* plant under water stress. Also, **Malik and Ashraf, (2012)** found that Ascorbic acid treated seeding of both genotypes maintained higher chlorophyll contents, net- photosynthesis and growth- compared to the non-treated plants.

Table (2): The effect of ascorbic acid on photosynthetic pigments in seedling of three wheat varieties under drought stress by PEG.

ASA	PEG	Chlorophyll a (mg/g F. Wt)			Chlorophyll b (mg/g F.Wt)			Carotenoids (mg/g F.Wt)		
mg/l	bars	shandaweel 1	Giza168	Masr 1	shandaweel 1	Giza168	Masr 1	shandaweel 1	Giza168	Masr 1
0	0	0.413	0.470	0.408	0.370	0.394	0.359	0.592	0.628	0.515
	1	0.358	0.366	0.212	0.199	0.244	0.194	0.348	0.386	0.298
	2	0.293	0.352	0.317	0.243	0.251	0.193	0.218	0.228	0.187
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
150	0	0.646	0.825	0.591	0.577	0.621	0.530	0.657	0.733	0.548
	1	0.261	0.380	0.309	0.392	0.416	0.255	0.475	0.583	0.389
	2	0.188	0.268	0.163	0.193	0.224	0.102	0.217	0.271	0.176
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
200	0	0.937	1.217	0.829	0.566	0.842	0.309	0.862	1.079	0.650
	1	0.642	0.869	0.388	0.417	0.942	0.323	0.622	0.647	0.562
	2	0.376	0.479	0.443	0.265	0.307	0.252	0.373	0.430	0.273
	3	0.226	0.232	0.192	0.195	0.204	0.148	0.106	0.128	0.094
L.S.D 0.05			0.002			0.30			0.01	

The effect of ascorbic acid on Catalase, Peroxidase and Proline in seedling of three wheat varieties under drought stress by PEG

Results in Table (3) showed that catalase (CAT) activity significant increase in Giza168 with increasing of drought stress at 3 bar, whereas CAT activity significant increase in Masr 1 and nonsignificant increase in Shandaweel 1 at 1 bar and then decreased at 2 bar. Under control without ASA, the maximum CAT activity was observed in Giza168 (40.33 U/mg F.W) at 2 bar and the minimum CAT activity was found in Masr 1 (29.400 U/mg F.W) under normal conditions. Pretreatment of with 150 mg/L of ASA caused significant increasing of CAT activity in Masr 1 at all level of drought stress while not effective in Giza168. 200 mg/L of ASA is the most effective in increase CAT activity as compared to 150 mg/L (Table 3). CAT activity significant increase in Masr 1 from 32.380 to 35.433 U/mg F.W with 150 mg/L of ASA and significantly increased to 45.63 U/mg F.W with 200 mg/L of ASA at 2 bar. CAT activity in Shandaweel 1 decreased to 25.53 U/mg F.W with 150 mg/L of ASA, while significantly increased to 35.56 U/mg F.W with 200 mg/L of ASA at 2 bar.

Also, the results in Table (3) showed that peroxidase (POX) activity significantly increased by increasing of drought stress at 3 bar in all wheat varieties. Under control without ASA, the highest value of POX activity was found in Shandaweel 1 (40.33 U/mg F.W) at 2 bar and the lowest value of POX activity was found in Shandaweel 1 (23.36 U/mg F.W) under normal conditions. Application of ASA as soaking caused significantly increasing of POX activity in all wheat varieties (Table 3). POX activity significantly increased in Giza168 from 30.40 U/mg F.W at 2 bar to 55.33 U/mg F.W with 150 mg/L of ASA and increased to 80.63 U/mg F.W with 200 mg/L of ASA at the same level of drought stress. The maximum POX activity was observed in Shandaweel 1 (93.36 U/mg F.W) and Masr 1 (92.40 U/mg F.W) at 3 bar with 200 mg/L of ASA. The increased activities of CAT and POX led to limit cellular damage and enhance the plants oxidative capacity to defend stress. CAT and POX activities play a central protective role in the O_2 and H_2O_2 scavenging process **(Hoque et al, 2007)**. The CAT and POX activity increased under drought stress when compared to control plants. Similar results reported under drought stress in wheat **(Shao et al, 2005).** These results are similar to that obtained by **Rezaei et al. (2013)** showed that ascorbic acid led to increasing of catalase, peroxidase activities *Dracocephalum moldavica* L. under drought stress.

Table (3): The effect of ascorbic acid on Catalase, Peroxidase and Proline in seedling of three wheat varieties under drought stress by PEG

ASA	PEG	Catalase (U/mg F.W)			Peroxidase (U/mg F.W)			Proline (µmoles/g F.W)		
mg/l	bars	Shandaweel 1	Giza168	Masr 1	Shandaweel 1	Giza168	Masr 1	Shandaweel 1	Giza168	Masr 1
0	0	30.590	36.433	29.400	23.367	27.317	32.367	8.250	10.220	9.330
	1	32.300	38.267	34.467	31.200	28.300	33.767	9.550	11.560	9.880
	2	29.300	40.333	32.380	40.333	30.400	36.533	10.360	13.960	12.200
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
150	0	32.667	38.400	30.467	40.367	44.500	35.533	10.850	12.570	10.290
	1	35.467	38.700	36.567	52.400	52.300	41.333	15.600	18.960	15.690
	2	25.533	40.667	35.433	66.633	55.333	65.667	17.740	21.500	19.560
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
200	0	38.400	45.533	40.367	68.800	67.733	70.700	16.850	23.580	20.110
	1	42.400	44.500	44.400	52.467	77.700	67.533	18.560	26.550	21.550
	2	35.567	46.667	45.633	87.667	80.633	77.500	19.710	25.890	21.630
	3	36.667	50.400	48.700	93.367	75.367	92.400	20.250	28.960	24.960
L.S.D 0.05			2.21			2.98		0.98		

On the other hand, drought stress increased the proline contents in all wheat varieties Table (3). Under control without ASA, a maximum increase of proline contents was observed in Giza168 (13.96) mmoles/g at 2 bar. Proline contents in Masr 1 was (12.20) mmoles/g higher than Shandaweel 1 (10.36) mmoles/g at 2 bar. Application of ASA as soaking significantly increased proline contents in all varieties (Table 3). The highest value of proline was found in Giza168 (28.96) mmoles/g at 3 bar with 200 mg/L of ASA. Application of 200 mg/L of ASA had higher

effect compared to 150 mg/L in increasing of proline contents in all wheat varieties. For example proline contents significantly increased in Shandaweel 1 from 10.36 mmoles/g at 2 bar to 17.74 mmoles/g with 150 mg/L of ASA while increased to 19.71 mmoles/g with 200 mg/L of ASA. The role of proline as a protective agent against reactive oxygen species is also very important **(Hare et al., 1999).** Drought tolerance is positively correlated with high accumulation of proline in many crops like wheat and barley **(Nayyar and Walia, 2003).** These results agreed with **Baghizadeh et al. (2009)** who found that proline contents increased with ascorbic acid under drought stress in Okra (*Hibiscus esculentus L.*).

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تأثير المعاملة بحمض الاسكوربيك على بادرات القمح تحت إجهاد الجفاف

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تم تقييم ثلاثة أصناف من القمح المصري (شندويل ١، وجيزة ١٦٨، ومصر ١) تحت اجهاد الجفاف باستخدام تركيزات مختلفة من البولي إيثيلين جلايكول (0، ١ ، ٢ ، ٣ بار) ودراسة تأثير تطبيق حمض الأسكوربيك بتركيزات (٠ و ١٥٠ و ٢٠٠ ملجم / لتر) في التخفيف من إجهاد الجفاف في مرحلة الانبات لأصناف القمح. أوضحت النتائج أن إجهاد الجفاف تسبب في انخفاض نسبة الإنبات ومقاييس النمو (طول النبات وطول الجذر) وصبغات البناء الضوئي (الكلوروفيل أ ، والكلوروفيل ب ، والكاروتينات) مع زيادة تركيزات البولى ايثلين جليكول(PEG) في جميع أصناف القمح. علاوة على ذلك، زاد نشاط الكتاليز (CAT) في صنف جيزة ١٦٨ مع زيادة إجهاد الجفاف عند ٣ بار ، بينما زاد نشاط CAT في صنف مصر ١ وزاد زيادة غير معنوية في صنف شندويل ١ عند ١ بار ثم انخفض عند ٢ بار. أيضا، تراكم البرولين تحت ضغط الجفاف في جميع أصناف القمح. من ناحية أخرى، أدت معاملة البذور بحمض الأسكوربيك قبل زراعتها إلى تحسين جميع العوامل المدروسة تحت إجهاد الجفاف. يعتبر ٢٠٠ مجم / لتر من حمض الاسكوربيك هو الأكثر فاعلية في تخفيف آثار إجهاد الجفاف على الخصائص المورفولوجية والكيميائية لأصناف القمح في مرحلة الأنبات.