

RESEARCH ARTICLE

Stimulate drought stress tolerance by polyethylene glycol in wheat seedling by various concentrations of ascorbic acid

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Abstract

A lab experiment was carried out to investigate the role of ascorbic acid on wheat seedling drought stress induced by polyethylene glycol (PEG-6000) on some physiological traits and enzyme activity. The Wheat seeds were activated using ascorbic acid in four concentrations: 0, 25, 50 and 100 mg L⁻¹. The germination medium was treated with PEG-6000 compound to achieve 0, -0.4, -0.8 and -1.2 bar tensions. The study's findings showed that the 50 mg L⁻¹ ascorbic acid concentration provided the highest rates of wheat seed germination percentage; 99.2 %, root length; 12.30 cm, shoot length; 11.64 cm and relative water accounting for 84.75 %. Additionally, catalase and peroxidase enzymes increased most at the same ascorbic acid concentration. Also, the same concentration (50 mg L⁻¹) improved the electrical conductivity of wheat seedling cells by recording the lowest rate of 3.53 dS/m. On the other hand, showed the lowest rates at high stress of PEG-6000 -1.2 bar when recording a germination percentage of 52.58 %, root length; 5.24 cm, shoot length; 5.79 cm and relative water content reached 56.32 %. In addition to causing the highest rise in enzymatic antioxidants for catalase and peroxidase, the same stress also had the highest rate of electrical conductivity, reaching 11.49 dS/m.

Keywords

ascorbic acid; drought stress tolerance; polyethylene glycol; wheat

Introduction

More than half of all farmed land is used for the strategic crop wheat (*Triticum aestivum* L.), which ranks first globally. An estimated 260 million hectares of land are planted for wheat, producing 761.6 million mega grams (1). On the other hand, the Central Statistical Organization (2) estimates that the cultivated area in Iraq is around 1.583 million hectares with a projected yield rate of 2.744 mega grams' ha⁻¹ and a production rate of 4.343 million mega grams. Iraq is one of the first places where this crop originated, but it produces very little. In addition, the country faces drought and a lack of freshwater resources due to climate change-related factors like global warming, desertification and the shrinkage of agricultural lands from the destruction of forests, soil erosion and loss of fertility. Iraq, which lies in arid and semi-arid regions of the world, experiences drought waves because of high temperatures, precipitation loss and a drop in the Tigris and Euphrates River levels. Water is the key factor determining agricultural production, especially in arid and semi-dry regions due to the prevalent dry weather conditions. Since water is a solvent and a medium that transports different nutrients, it plays a significant role in enhancing the absorption and availability of nutrients, regulating the process of cell elongation and

division and controlling the metabolism of carbon. A lack of water in the soil and the resulting decrease in the water potential of plant tissues cause physiological harm to the plant, which inhibits development and production (3, 4). One of the primary abiotic variables influencing plant growth in arid and semi-arid settings is water stress. Globally, it inhibits growth and production leading to large losses in agriculture.

Ascorbic acid is one of the non-enzymatic antioxidants that control biological processes like growth and photosynthesis. It has also been shown that ascorbic acid plays a significant role in plant life because it is linked with the defense mechanisms against various stresses, particularly drought stress. Research suggests that it may be used to decrease the adverse effects of drought on crops that are either already experiencing it or are expected to experience it at a specific stage of their growth. This is because it plays a physiological role in plant growth and development including the absorption of ions through the inhibition and prevention of ethylene metabolism accelerating the germination of seeds, root meristematic cell division and enzyme activity preventing free radicals from destroying chloroplasts. Stimulating antioxidants that mitigate free radicals' damaging impact on cells improving the plant's resistance to environmental challenges such as drought stress (5).

Plant exposure to varying degrees of humidity is a key factor in studying the effects of drought on plants. This can be achieved by exposing seeds or roots to low-water potential environments, varying irrigation frequency or quantity, or reducing water potential by using organic materials. These components include polyethylene glycol (PEG), which creates an osmotic potential difference between the germination media and the interior of the cells to hold water molecules in the medium (6, 7) which functions to block the availability of water, which is required for cell growth and elongation. This limits the plant's ability to grow by lowering the medium's water potential, creating an environment that is like the conditions of a field drought because the plant is unable to continue absorbing water. Because of its high molecular weight, polyethylene glycol functions differently from other materials like mannitol in that it does not enter plant cells and does not have any harmful effects on them. This allows it to get past the holes in plant cells. Due to the significance of the previously stated, the study aimed to determine how ascorbic acid functions in mitigating the impact of drought-induced polyethylene glycol (PEG-6000) on various physiological traits of wheat seedlings.

Materials and Methods

A study was carried out in the Plant Physiology Laboratory of the Field Crops Department, College of Agriculture, University of Anbar, Iraq which is located at 33°25'33"N 43°17'57"E to investigate the role of ascorbic acid in mitigating the effects of drought-induced stress on certain physiological traits of wheat seedlings through the use of polyethylene glycol (PEG-6000). Ascorbic acid and (PEG-6000) were purchased from Perrigo Drug Company USA. Ten healthy Wheat seeds (Var.

Ibaa99) were hand sorted, soaked in water for 5 hr and surface sterilized with 2.5 % sodium hypochlorite to prevent mycosis and bedded in 15 cm diameter whatman petri dishes, which were used to serve the seedbed in two layers of filter paper tissues arranged by Complete Randomized Design (CRD) replicated four times. Two variables were present in the experiment. Soaking wheat seeds with four ascorbic acid concentrations (0, 25, 50 and 100 mg/L⁻¹) for 12 hours was the first factor in the process. The other proceeding factor was adding polyethylene glycol - 6000 at four different intensities (0, -0.4, -0.8 and -1.2) bar as a moisture solution beginning by adding 10 ml from the first day of the experiment and the moisturizing from these concentrations continued depending on the seedling situation until the end of the experiment.

Data collection

1- Germination percentage (%): Determined using the following formula:

Germination percentage (%) = Number of germinated seedlings / Total number of seeds × 100

2- Root length (cm): A final average for this characteristic was extracted after five naturally occurring seedlings were selected at random and the root length was measured.

3- Shoot Length (cm): Five naturally occurring plumule were selected at random and the plumule's length was measured from the point where it touched the root to the tip.

4- Relative Water Content (RWC) %: Five seedlings were used for each treatment to determine the RWC, which was calculated using the following formula:

$$RWC = (FW - DW) / (TW - DW) \times 100$$

FW = Fresh weight of seedlings (g)

TW = Turgor weight of seedlings (g). After taking the fresh weight, the seedlings are placed directly in distilled water for two hours and then weighed to obtain TW.

DW = Dry weight of seedlings at 70 °C temperature for 72 hours.

5- Electrical conductivity (EC %): To determine the EC₁, five seedlings from each experimental unit were put in test tubes with equal volumes of distilled water for a whole day. They were then autoclaved for 15 minutes at 100 °C, then left for 24 hours. The Tube EC measured by the Hanna Ec meter and the EC % measured by the following equation:

$$REC \% = (EC_1 / EC_2) \times 100 \text{ (8).}$$

6 - Enzyme Activity: The activity of Catalase Enzyme (CAT) and Peroxidase Enzyme (POD) were measured by the modified method (7, 9). Briefly, the wheat seedling sample was washed with distilled water three times. Five grams of the sample were chopped quickly into thin layers and added with 50 mL sodium phosphate buffer (pH 7) containing 1 mM ascorbic acid and 0.5 % (w/v) polyvinylpyrrolidone for 5 min at 4 °C. The homogenate was filtered through three layers of fine filter paper and then the filtrate was centrifuged at 5000 × g for 20 min and the supernatant was collected. CAT activity was measured using a spectrophotometer at room temperature at 240 nm. While POD measured at 420 nm using 4-methyl catechol as

substrate.

Statistical analysis

The data were statistically assessed using the computer and the Genstat program and the results were compared using the Least Significant Difference (LSD) at the 5 % probability level.

Results

Germination percentage (%)

The germination percentage trait is significantly impacted by ascorbic acid concentrations, as demonstrated by Table 1, where the concentration of 50 mg L⁻¹ recorded the highest rate of the trait, reaching 99.2 %, while the comparison treatment recorded the lowest rate, reaching 72.08 %. This could be because, in addition to its function in activating enzymes to produce energy, ascorbic acid gives the seed nutrients to represent protein during germination by stimulating latent genes that are crucial for the representation of nucleic acids and increasing the efficiency of enzymes (5, 10).

The results of the same table also demonstrate that the germination percentage trait was significantly impacted by the levels of polyethylene glycol water stress, with the comparison treatment resulting in the highest rate of 99.33 % and the -1.2-bar tension treatment recording the lowest average for this trait at 52.58 %. The reason for this could be that the seeds have less water available to them because of the increased high tension brought on by the higher concentration of polyethylene glycol, which acts to limit water molecules and harm plant growth. The osmotic stress that results from this will cause a decrease in water absorption even though the water is available in sufficient amounts (11).

All concentrations provided the maximum rate for the trait, but the comparison treatment at the stress level

of -1.2 bar provided the lowest rate for the trait, reaching 4 %. This indicates that the interaction between ascorbic acid and water stress levels was also significant.

Root length (cm)

The results of Table 2 indicate evidence that ascorbic acid had a significant impact on the root length trait. The highest average of 12.30 cm was obtained at a concentration of 50 mg L⁻¹, followed by an average of 9.91 cm at a concentration of 25 mg L⁻¹, which did not differ significantly from it and the lowest average of 6.18 cm at a concentration of 100 mg L⁻¹. This rise might result from ascorbic acid's ability to promote root cell growth, which in turn allows the root to grow longer (12).

According to the same table's results, the root length was significantly impacted by the levels of polyethylene glycol water stress. The -0.4 bar tension treatment produced the highest average for this trait, measuring 11.42 cm, while the -1.2-bar tension treatment recorded the lowest average, measuring 5.24 cm. In addition to a lack of free water available to the seedling roots, which lowers the filling potential required for cell elongation and adversely impacts plant root growth, this could be caused by the production of free radicals, which interfere with the function of enzymes involved in metabolic processes and result in a reduction in root length (6).

The concentration of 50 mg L⁻¹ at a stress level of -0.4 bar produced the highest average root length of 15.03 cm, surpassing all other treatments; in contrast, the concentration of 0 mg L⁻¹ at a stress level of -1.2 bar produced the lowest average root length of 2.20 cm. This indicates that the interaction between ascorbic acid and water stress levels was also very significant.

Shoot length (cm)

The findings in Table 3 demonstrate that ascorbic acid has a significant impact on shoot length. The highest average for this trait was recorded at 50 mg L⁻¹ of ascorbic acid, which reached 11.64 cm, while the lowest average was recorded at 100 mg L⁻¹ of ascorbic acid, which reached 5

Table 1. Effect of ascorbic acid, polyethylene glycol and their interaction on germination percentage (%) of wheat seeds

Ascorbic acid concentrations (mg L ⁻¹)	Water potential				Mean
	0	0.4-	0.8-	1.2-	
0	7.14±1.02	9.60±1.17	6.76±0.65	2.20±0.65	6.42±2.10
25	11.18±2.12	12.17±1.30	9.70±1.02	6.62±0.55	9.91±2.45
50	13.44±0.92	15.03±2.02	11.50±2.00	9.24±1.16	12.30±2.11
100	7.37±1.06	8.88±0.88	5.50±0.51	2.92±0.15	6.18±1.35
Mean	9.78±1.10	11.42±0.93	8.38±1.44	5.24±0.21	
L.S.D	Water potential		Ascorbic acid concentrations		Interaction
P ≤ 0.05	0.4575		0.4575		0.9151

Table 2. Effect of ascorbic acid, polyethylene glycol and their interaction on root length (cm) of wheat seedling

Ascorbic acid concentrations (mg L ⁻¹)	Water potential				Mean
	0	0.4-	0.8-	1.2-	
0	97.33±2.19	92.33±1.02	94.66±0.89	4.00±0.23	72.08±4.11
25	100.00±0.0	100.00±0.0	97.66±1.04	95.33±1.21	98.25±1.07
50	100.00±0.0	100.00±0.0	99.33±0.11	97.66±0.51	99.2±0.76
100	100.00±0.0	100.00±0.0	95.3±0.29	13.33±2.09	77.17±3.01
Mean	99.33±0.21	98.08±0.33	96.75±0.71	52.58±2.57	
L.S.D	Water potential		Ascorbic acid concentrations		Interaction
P ≤ 0.05	0.413		0.413		0.826

Table 3. Effect of ascorbic acid, polyethylene glycol and their interaction on plumule length (cm) of wheat seedling

Ascorbic acid concentrations (mg L ⁻¹)	Water potential				Mean
	0	0.4-	0.8-	1.2-	
0	6.41±0.34	7.16±0.53	5.12±0.65	2.79±0.35	5.37±0.95
25	9.56±0.52	9.56±1.15	8.50±0.77	7.48±0.40	8.78±1.02
50	12.02±1.05	12.88±1.21	11.38±1.05	10.26±1.08	11.64±1.31
100	6.14±0.45	5.91±0.45	5.30±0.65	2.64±0.35	5.00±0.55
Mean	8.53±0.33	8.88±0.26	7.58±0.61	5.79±0.40	
L.S.D	Water potential		Ascorbic acid concentrations		Interaction
P ≤ 0.05	0.364		0.364		0.728

cm. This could be because ascorbic acid increases cell division and elongation, which lengthens the shoot (12).

The same table's results also demonstrate that the length of the shoot was significantly impacted by the polyethylene glycol water stress levels, with the -0.4-bar tension treatment achieving the highest average of 8.88 cm and the -1.2-bar tension treatment recording the lowest average of 5.79 cm. This could be because of the production of free radicals, which interfere with the function of enzymes involved in metabolic processes, causing the shoot to shorten. Additionally, under high stress, the relative water content (Table 4) decreases, which harms the process of cell division, which in turn harms enzymes and the synthesis of nucleic acids (6, 13).

The concentration of 50 mg L⁻¹ at a tension level of -0.4 bar produced the highest average shoot length of 12.88 cm, surpassing all other treatments, while the tension concentration at the comparison treatment of 0 mg L⁻¹ at a tension level of -1.2 bar produced the lowest average shoot length of 2.64 cm, indicating a significant interaction between ascorbic acid and water stress levels (Table 3).

Relative Water Content (RWC) %

As demonstrated by Table 4, ascorbic acid concentrations have a substantial impact on the RWC trait. The concentration of 50 mg L⁻¹ registered the highest rate of the trait, reaching 84.75 %, while the comparison treatment recorded the lowest rate reaching 56.16 %.

The results of the same table also demonstrate that the RWC characteristic was significantly impacted by the polyethylene glycol water stress levels, with the comparison

treatment resulting in the highest rate of 80.78 % and the -1.2-bar tension treatment recording the lowest average for this characteristic at 56.32 %. It could be because the comparator treatment helped the wheat seedling maintain its water balance, which increased its absorption of water (12).

The interaction between ascorbic acid and water stress levels was also significant, as the concentration of 50 mg L⁻¹ at the stress level of -0.4 bar gave the highest rate of water content, reaching 92.11 % cm, thus surpassing all other treatments, while the stress concentration at the comparison treatment of 0 mg L⁻¹ at the stress level of -1.2 bar gave the lowest rate of water content, reaching 22.64 %.

Electrical Conductivity (EC)

Table 5 indicates evident that ascorbic acid has a significant impact on this trait because the treatment with the highest ascorbic acid concentration, 100 mg L⁻¹, recorded the trait's highest average, 14.02 dS/m, while the treatment with the lowest ascorbic acid concentration, 50 mg L⁻¹, recorded the trait's lowest average, 3.53 dS/m. The explanation could be because the 50 mg L⁻¹ concentration enhanced the plasma membranes' permeability and characteristics while raising their RWC (Table 4), which reduced electron leakage (13).

The results of the same table also demonstrate that the electrical conductivity property was significantly impacted by the polyethylene glycol water stress levels, with the comparison treatment recording the lowest average for this property at 6.07 dS/m and the -1.2-bar tension treatment achieving the highest rate at 11.49 dS/m. This might be because high pressures cause the membranes to deteriorate more quickly, impair their permeability, increase electron leakage and decrease their RWC (14).

Table 4. Effect of ascorbic acid, polyethylene glycol and their interaction on the RWC % of wheat seedling

Ascorbic acid concentrations (mg L ⁻¹)	Water potential				Mean
	0	0.4-	0.8-	1.2-	
0	72.76±3.65	66.78±1.12	62.44±2.65	22.64±0.50	56.16±2.90
25	76.41±2.33	73.11±2.10	70.40±2.33	74.53±2.65	73.61±3.00
50	90.09±3.66	92.11±3.23	80.51±3.10	76.28±3.05	84.75±2.36
100	83.88±4.11	81.37±1.90	78.19±2.66	51.84±1.95	73.82±2.81
Mean	80.78±2.11	78.34±2.54	72.89±2.22	56.32±1.35	
L.S.D	Water potential		Ascorbic acid concentrations		Interaction
P ≤ 0.05	3.65		3.065		6.131

Table 5. Effect of ascorbic acid, polyethylene glycol and their interaction on Electrical Conductivity (EC) dS/m of wheat seedling

Ascorbic acid concentrations (mg L ⁻¹)	Water potential				Mean
	0	0.4-	0.8-	1.2-	
0	12±1.00	7.85±1.10	12.10±2.00	16.30±1.21	10.77±1.59
25	3.81±0.24	4.36±0.63	5.56±0.47	6.54±0.85	5.07±0.39
50	2.84±0.30	2.86±0.43	4.02±0.51	4.38±0.37	3.53±0.73
100	10.83±1.10	11.39±0.62	15.12±1.16	18.75±1.75	14.02±1.11
Mean	6.07±0.55	6.62±0.43	9.20±1.22	11.49±1.13	
L.S.D	Water potential		Ascorbic acid concentrations		Interaction
P ≤ 0.05	0.686		0.686		1.373

Ascorbic acid and water stress levels also interacted significantly; at a stress level of -1.2 bar, a concentration of 100 mg L⁻¹ produced the highest electrical conductivity rate of 18.75 dS/m, surpassing all other treatments, while at a concentration of 50 mg L⁻¹, with a comparison treatment of 0 mg L⁻¹, the lowest electrical conductivity rate was 2.84 dS/m.

Catalase enzyme CAT unit mL⁻¹

Given that the ascorbic acid concentration of 50 mg L⁻¹ produced the highest average was 20.64-unit mL⁻¹ and the control treatment produced the lowest 8.56-unit mL⁻¹, Fig. 1 demonstrates the variations in ascorbic acid concentrations. The reason for the increased enzyme activity at high ascorbic acid concentrations may be that ascorbic acid assists in the plant's biosynthesis and is regarded as a crucial defense mechanism. This could lead to an increase in the activity of enzymes, such as the catalase enzyme as well as the formation of certain enzyme companions and cell organelles that help the plant become more resistant to heat and drought by improving the activity of the catalase enzyme. This was reflected in the increase in the percentage of germination, root length and shoot length, as shown in Tables (1-3) which supports previous results (15, 16).

Given that significant water stress produced the highest average was 19.77-unit mL⁻¹ at a high-tension level -1.2 bar, while the comparison treatment produced the lowest was 8.94-unit mL⁻¹, the same graphic also demonstrates the notable variations in drought stress. As evidenced by the rise in the values of high oxidative stress indicators in the electrical conductivity trait (Table 5), the increase in the catalase enzyme activity at high water stress may be caused by an increase in free radicals under these stresses. This would harm all seedling traits including germination percentage, root length, shoot length and relative water content (Tables 1- 4). The study concurs with the wheat crop study (15).

Peroxidase Enzyme (POD)

The differences in ascorbic acid concentrations are evident in Fig. 2, where the control treatment produced the lowest average was 10.86-unit mL⁻¹ and the ascorbic acid concentration of 50 mg L⁻¹ produced the greatest 25.42 unit mL⁻¹. Since ascorbic acid aids in the plant's biosynthesis and is thought to be an essential defensive mechanism, it is plausible that the elevated enzyme activity at high ascorbic

acid concentrations is the cause. Along with the development of specific enzyme buddies and cell organelles that aid in the plant's increased resistance to heat and drought by enhancing the activity of the peroxidase enzyme, this may result in a rise in the activity of other enzymes, including the POD. Tables (1- 3) demonstrate that this was mirrored in the growth in the percentage of germination, root length and shoot length agrees with the results on the study of wheat crops (10, 15).

The same figure also shows enormous variations in drought stress, with the comparison treatment producing the lowest average was 14.57-unit mL⁻¹ and significant water stress producing the highest was 21.68-unit mL⁻¹ at a high tension level of -1.2 bar. The rise in high oxidative stress markers' values in the electrical conductivity trait (Table 5) suggests that an increase in free radicals under high water stress may be the reason for the peroxidase enzyme's increased activity. All the seedling characteristics, such as the percentage of germination, the length of the roots and shoots and the relative water content, would suffer as a result (Tables 1- 4). The results supports the previous wheat crop study (16, 17) .

Conclusion

We conclude from this study that ascorbic acid prevented the plasma membranes from degrading under high stress, particularly at a concentration of 50 mg L⁻¹ from polyethylene glycol, for all the traits examined in this experiment. However, a concentration of 100 mg L⁻¹ did not act for all the traits examined, possibly because it reached a point of toxicity that put the wheat seedlings under more stress. Further studies, including field assays, will be done to examine the role of ascorbic acid on the various stresses experienced by the wheat crop.

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Authors' contributions

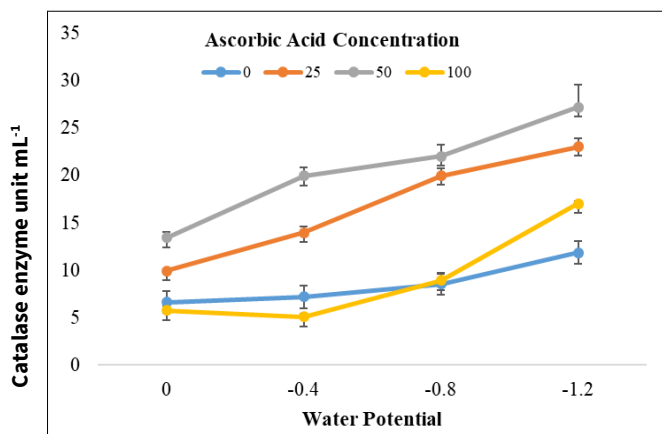


Fig. 1. Effect of ascorbic acid on catalase enzyme (CAT) activity unit mL⁻¹ in wheat seedling induced drought stress by polyethylene glycol.

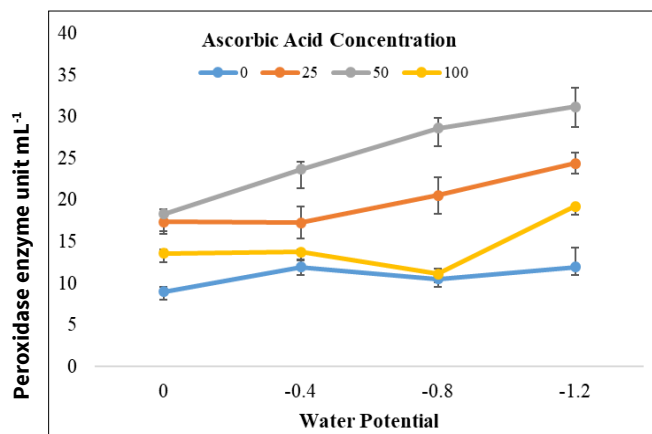


Fig. 2. Effect of ascorbic acid on peroxidase enzyme (POD) activity unit mL⁻¹ in wheat seedling induced drought stress by polyethylene glycol.

IM forth ideas and designed experiments. NA performed the experiments. AH drafted the manuscript. AA conceived and coordinated the overall study. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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