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Effect of Various Environmental Factors and Treatment with Ascorbic Acid on Several Physiological Attributes in Wheat (*Triticum aestivum* L.)

Kamal Abdul Kader Mohammed#, Mohammed Said Faisal
Department of Biology, College of Education for Pure Sciences,University of Mousl, Mousl, Iraq.

THE CURRENT study was conducted at the University of Mosul/ College of Education for Pure Sciences / Department of Biology, where an experiment was carried out in the wired house by cultivating wheat variety (buhuth 22). The plants were exposed to two dry periods (6, 9) days and three levels of light intensity (100%, 75%, 35%), and the shoots were sprayed with three concentrations of ascorbic acid (0, 100, 200ppm) to see their effect on physiological characteristics and the results were analyzed statistically using a complete random design (C.R.D) with three replicates. The results showed that drought had a negative effect on plant height, leaf area, relative water contents, total chlorophyll and carotenoids, at the second dry period, with a percentage of 17.12, 36.02, 9.70, 28.57, and 30.26%, respectively, compared with their controls treatment, while plants growing under normal light intensity (100%) outperformed in the characteristic of plant height and total chlorophyll, while there was an increase in the leaf area and relative water content with reduced light intensity compared to normal lighting. On the other hand, the interaction of ascorbic acid, drought and the intensity of lighting had a positive effect on plant growth in most of the studied physiological characteristics.

Keywords: Ascorbic acid (AsA), Drought, Light intensity, Wheat .

Introduction

Wheat is considered one of the most important strategic crops locally and internationally because it is used as the main staple food, like bread in many countries. It was the most abundant source of calories and protein in the diet, providing approximately 20% of total dietary protein worldwide (Braun et al., 2010). Wheat is the first crop in the world, Iraq, in terms of nutritional and economic importance, and the area planted with wheat crop in Iraq reached 331116 tons for the 2019 season (Agricultural Statistics Directorate, 2019). Drought is defined as a climatic and environmental event, as it results from the absence of rain for a sufficient period accompanied by high temperatures to deplete soil water, damage plants, and disrupt some biological actions. The first signs of drought are a decrease in plant growth and a reduction in the size of leaves (Saab & Sharp, 2004). Light plays a pivotal role in regulating plant growth and development. The three characteristics are the quality of the light, its intensity, and the photoperiod are extremely important to the formation of a plant, the importance of plant photoreceptors as major regulatory proteins controlling metabolic events and developmental changes within plants has been documented (Gupta, 2017). Ascorbic acid (vitamin C) is one of the most common and essential vitamins due to its protective role, as it regulates various physiological processes in plants, as it performs a variety of functions such as being a major oxidation and reduction factor for enzymes involved in multiple processes including hormone biosynthesis and photosynthesis, respiration (Ortiz-Espín et al., 2017). Kasim

#Corresponding author email: kamal.esp45@student.uomosul.edu.iq
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et al. (2020) observed a significant decrease in the chlorophyll content of wheat varieties when subjected to waterstress, and this decrease increased with increasing the level of water stress. The study Hussein & Khursheed (2014) about the effect of spraying ascorbic acid on the growth characteristics and some components of wheat crops exposed to drought stress conditions was determined. The results showed the effective contribution of the ascorbic acid in reducing the deleterious effects of drought conditions on the plant by improving plant lengths, increasing water content and elongating leaves in addition to its effect on increasing chlorophyll pigments (a and b) contents thus, ascorbic acid is one of the growth regulators for plants under drought stress conditions. The study of Roman et al. (2013) indicated the effect of shading on wheat and barley plants, when exposed to a light intensity of 75% of normal lighting, as it was observed that shading increased the leaf area and decreased the rate of photosynthesis compared to the shaded plants. The aim of the study is to know the effects of environmental stress (drought and light stress) on some physiological processes of wheat and increase the resistance of plants to environmental stresses using AsA as environmentally safe materials.

Materials and Methods

The current study was conducted in the wired house and laboratories of the Department of Biology/ College of Education for Pure Sciences/ University of Mosul for the year 2020-2021, where soil was brought from agricultural fields in the waterfalls area at a depth of (0-30) cm, then it was air dried and cleaned through a sieve with a diameter of its holes (2mm) and the grains of wheat were obtained from the Seed Examination and Certification Center/ Nineveh. On 21/12/2020, (10) seeds of the wheat variety (buhuth 22) were planted in pots (capacity 7kg) and after two weeks of planting, the number of seedlings in each pot were reduced to (5) seedlings, after 40 days of planting, the plants were subjected to two drought periods: the first for 6 days and the second for 9 days with the presence of the control treatment (without drought). Plants were also exposed to three levels of light intensity; The first is natural lighting (100%), the second is light intensity (75%) by covering the plants with Tull cloth, and the third is light intensity (35%) by covering the plants with a Bored cloth, the light intensity levels were set using a luxmeter type (Digital Luxmetre Lx-1010 B). After 40 days of planting, the shoots were sprayed with three concentrations of ascorbic acid (0, 100, 200ppm) until completely wet. After 50 days from the date of spraying, three replications were used foreach treatment to study several physiological characteristics:

1- Plant height (cm), using a ruler.

2- Leaf area using the method (Kemp, 1960).

3- The relative water content according to the Turner method (Turner, 1981), used by many researchers such as Schon-Feld et al. (1988).

4- The total chlorophyll content in the leaves according to the method reported by Said (1990) and carotenoids using the method Jaleel et al. (2009).

Statistical analysis: A completely random design (C.R.D) was used in the implementation of the experiments and the results were analyzed using the Duncan test to determine the significance between treatments at the level (0.05). The percentage of increase or decrease relative to control in all characteristics was calculated according to the following equation.

Reduction or Stimulation%= [(100*A)/B]- 100

where, A= Measurement of the adjective in the transaction, B= Measurement of the adjective in the control

Results

Plant height

Table 1 showed that there was a significant decrease in plant height at a probability level of 5% as a result of exposing the plants to two drought periods compared with control (drought unstressed) treatment and at a rate of (17.1 and 11.1%) respectively. The results confirmed a significant increase in plant height with a decrease in the intensity of illumination, at a rate of (4.6 and 8.2%) respectively. Sprayings plants with AsA led to a significant increase in plant height, as compared with control (without spraying).

In terms of the interaction(Ascorbic acid ×

TABLE 1. Effects of foliar application with ascorbic acid (ppm) and the light intensity (%) on plant height (cm) of wheat plant grown under different periods of drought stress

Ascorbic acid	Light intensity	Periods of drought			Ascorbic acid x Light intensity	Effect of ascorbic acid	Effect of light intensity
		Control	First drought	Second drought			
0.0ppm	100%	57.60 d-i	52.50 g-l	47.50 l	52.50 e		
	75%	58-83 c-g	53.33 g-l	51.67 i-l	54.61 cde		
	35%	60.50 c-f	54.33 f-k	52.25 h-l	55.69 b-e		
100ppm	100%	58.67 c-g	52.33 g-l	48.67 kl	53.22 de		
	75%	62.83 a-d	55.92 e-j	50.50 jkl	56.42 a-d		
	35%	65.25 ab	57.75 d-i	53.58 g-l	58.86 ab		
200ppm	100%	61.50 a-e	53.58 g-l	50.58 jkl	55.22 cde		
	75%	64.25 abc	56.67 d-j	51.50 i-k	57.47 abc		
	35%	66.83 a	57.58 d-i	54.67 f-k	59.69 a		
Ascorbic acid x Periods of drought	0.0ppm	58.94 b	53.39 cde	50.47 e		54.27 b	
	100ppm	62.25 a	55.33 cd	50.92 e		56.17 a	
	200ppm	64.19 a	55.94 bc	52.25 de		57.46 a	
Light intensity x Periods of drought	100%	59.22 bc	52.81 ef	48.92 g			53.65 c
	75%	61.97 ab	55.31 de	51.22 fg			56.17 b
	35%	64.19 a	56.56 cd	53.50 def			58.08 a
Effect of drought		61.79 a	54.89 b	51.21 c			

Similar letters refer to non significant difference found between treatments at probability 5% according to Duncan test.

periods of drought), the significant superiority was obtained in drought unstressed plants the sprayed with AsA (200ppm). The interaction of three factors (drought x light intensity x AsA), plants were not exposed to drought, sprayed with AsA (200ppm), and exposed to light intensity (35%) had highest height value (66.83).

Leaf area (cm^2)

Plant leaves are the most affected members of drought stress. Table 2 showed the highest leaf area obtained in the control treatment and reached (37.86cm^2) while the lowest values observed in the second dry period (24.22cm^2) Decreasing light intensity was associated with an incensement in leaf area. As the light intensity exceeded 35%, at a rate it reached (30.0%) compared to the light intensity of 75% and normal light intensity, respectively. The results showed that treatment with (AsA) increases the leaf area, especially at a concentration of 200ppm, at a rate of (12.1%) compared to the control. The interaction (drought × light intensity) induced significant increase in leaf area, the lowest value (20.41cm^2) obtained in plants normally illuminated (100%) and secondly exposed to drought compared to the rest of the treatments.

(%) Relative water content

The results in Table 3 showed that exposing the plants to two drought periods, especially the second dry period, reduced the relative water content, reaching (74.84%), relative to the control to (82.88%). While the percentage of superiority for relative water content attained (2.5 and 5.2%) respectively in plants growing under light intensity of 100% compared to the other two light intensities. Treatment of plants with AsA stimulated a positive significant increasing effect on relative water content compared to their analogous untreated control plants with a ratio of (3.0 and 5.3%) respectively. In term of interaction (drought × AsA), acid spraying reduced the negative effect of drought on the relative water content compared to their controls (0.00ppm).

Photosynthetic pigments

Tables 4 and 5 showed that there was a significant decrease in the total chlorophyll and carotenoids contents with an increasing in plant exposure to droughts, especially the second dry period, and the percentage of decrease was (28.5 and 30.3%) respectively, compared with the control treatment. From the results of the two tables, it is evident that decreasing levels of light intensity (compared to normal light intensity), led to a significant decrease in total chlorophyll and carotenoids contents, especially the light intensity 35%, and the percentage of decrease was (13.56 and 15.82%) respectively, compared with their contents at the intensity of light 100% .

On the other hand, treatment of plants with AsA

TABLE 2. Effects of foliar application with ascorbic acid (ppm) and the light intensity (%) on leaf area (cm^2) of wheat plant grown under different periods of drought stress

Ascorbic acid	Light intensity	Periods of drought			Ascorbic acid x Light intensity	Effect of ascorbic acid	Effect of light intensity
		Control	First drought	Second drought			
0.0ppm	100%	30.89 f-i	24.82 j-n	20.07 n	25.26 d		
	75%	39.23 bc	29.71 g-j	23.78 k-n	30.91 b		
	35%	40.58 bc	30.22 f-h	25.56 i-m	32.12 b		
100ppm	100%	31.25 fgh	27.10 h-k	20.68 mn	26.35 cd		
	75%	37.53 b-e	32.95 efg	23.18 lmn	31.22 b		
	35%	42.32 ab	33.74 d-g	23.75 k-n	33.27 b		
200ppm	100%	35.40 c-f	28.55 g-k	20.47 mn	28.14 c		
	75%	37.85 b-e	30.60 f-h	29.32 g-j	32.59 b		
	35%	45.73 a	38.16 bcd	31.15 fgh	38.35 a		
Ascorbic acid x Periods of drought	0.0ppm	36.90 a	28.25 c	23.14 d		29.43 b	
	100ppm	37.03 a	31.27 b	22.54 d		30.28 b	
	200ppm	39.66 a	32.43 b	26.98 c		33.02 a	
Light intensity x Periods of drought	100%	32.51 cd	26.83 e	20.41 f			26.58 c
	75%	38.20 b	31.09 d	25.43 e			31.57 b
	35%	42.88 a	34.04 c	26.83 e			34.58 a
Effect of drought		37.86 a	30.65 b	24.22 c			

Similar letters refer to non- significant difference found between treatments at probability 5% according to Duncan test.

TABLE 3. Effects of foliar application with ascorbic acid (ppm) and the light intensity (%) on leaf relative water content (%) of wheat plant grown under different periods of drought stress

Ascorbic acid	Light intensity	Periods of drought			Ascorbic acid x Light intensity	Effect of ascorbic acid	Effect of light intensity
		Control	First drought	Second drought			
0.0ppm	100%	83.93 a-d	80.76 a-h	72.32 jkl	78.87 bc		
	75%	82.88 a-e	76.84 f-k	71.35 kl	77.02 cd		
	35%	80.61 a-h	71.31 kl	70.39 l	74.10 d		
100ppm	100%	84.93 ab	81.34 a-g	77.66 d-j	81.31 ab		
	75%	82.68 a-f	79.71 b-i	75.04 h-l	79.14 abc		
	35%	78.88 c-i	76.62 g-k	74.42 i-l	76.64 cd		
200ppm	100%	86.15 a	82.95 a-e	77.75 d-j	82.28 a		
	75%	83.81 abc	79.69 b-i	77.46 e-j	80.32 ab		
	35%	82.48 a-g	79.33 b-i	77.16 e-j	79.66 abc		
Ascorbic acid x Periods of drought	0.0ppm	82.34 ab	76.30 de	71.35 f		76.67 c	
	100ppm	82.16 abc	79.22 cd	75.71 e		79.03 b	
	200ppm	84.15 a	80.66 bc	77.46 de		80.75 a	
Light intensity x Periods of drought	100%	84.87 a	81.68 bc	75.91 de			80.82 a
	75%	83.12 ab	78.74 cd	74.62 e			78.83 b
	35%	80.66 bc	75.75 de	73.99 e			76.79 c
Effect of drought		82.88 a	78.73 b	74.84 c			

Similar letters refer to non significant difference found between treatments at probability 5% according to Duncan test.

TABLE 4. Effects of foliar application with ascorbic acid (ppm) and the light intensity (%) on total chlorophyll content (mg g-1 F.wt) of wheat plant grown under different periods of drought stress

Ascorbic acid	Light intensity	Periods of drought			Ascorbic acid x Light intensity	Effect of ascorbic acid	Effect of light intensity
		Control	First drought	Second drought			
0.0ppm	100%	1.951 b-h	1.603 e-i	1.440 ghi	1.665 bc		
	75%	1.611 e-i	1.520 e-i	1.400 hi	1.510 c		
	35%	1.561 e-i	1.497 f-i	1.112 i	1.390 c		
100ppm	100%	2.529 ab	2.123 a-g	1.889 c-h	2.181 a		
	75%	2.413 abc	2.062 a-g	1.732 c-h	2.069 a		
	35%	2.155 a-e	1.908 b-h	1.504 f-i	1.855 ab		
200ppm	100%	2.603 a	2.135 a-f	1.707 d-i	2.148 a		
	75%	2.454 abc	2.049 a-g	1.591 e-i	2.013 a		
	35%	2.280 a-d	1.939 b-h	1.542 e-i	1.920 ab		
Ascorbic acid x Periods of drought	0.0ppm	1.708 bc	1.540 cd	1.317 d		1.520 b	
	100ppm	2.366 a	2.030 b	1.708 bc		2.040 a	
	200ppm	2.446 a	2.041 b	1.613 cd		2.030 a	
Light intensity x Periods of drought	100%	2.361 a	1.954 bc	1.679 cde			1.990 a
	75%	2.159 ab	1.877 bcd	1.574 de			1.870 ab
	35%	1.999 bc	1.781 cd	1.386 e			1.720 b
Effect of drought		2.170 a	1.870 b	1.550 c			

Similar letters refer to non -significant difference found between treatments at probability 5% according to Duncan test.

TABLE 5. Effects of foliar application with ascorbic acid (ppm) and the light intensity (%) on carotenoids content (mg g-1 F.wt) of wheat plant grown under different periods of drought stress

Ascorbic acid	Light intensity	Periods of drought			Ascorbic acid x Light intensity	Effect of ascorbic acid	Effect of light intensity
		Control	First drought	Second drought			
0.0ppm	100%	0.810 abc	0.639 c-f	0.543 ef	0.664 abc		
	75%	0.709 cde	0.599 def	0.526 ef	0.612 bc		
	35%	0.656 c-f	0.556 ef	0.509 f	0.574 c		
100ppm	100%	0.891 ab	0.641 c-f	0.544 ef	0.692 ab		
	75%	0.718 b-e	0.615 def	0.527 ef	0.619 bc		
	35%	0.661 c-f	0.579 def	0.510 f	0.583 c		
200ppm	100%	0.978 a	0.650 c-f	0.555 ef	0.728 a		
	75%	0.763 bcd	0.627 c-f	0.540 ef	0.644 abc		
	35%	0.668 c-f	0.601 def	0.521 ef	0.596 bc		
Ascorbic acid x Periods of drought	0.0ppm	0.725 a	0.598 b	0.526 b		0.616 a	
	100ppm	0.756 a	0.612 b	0.527 b		0.632 a	
	200ppm	0.803 a	0.627 b	0.539 b		0.656 a	
Light intensity x Periods of drought	100%	0.893 a	0.643 bcd	0.547 de			0.695 a
	75%	0.703 b	0.614 cde	0.531 e			0.625 b
	35%	0.661 bc	0.579 cde	0.513 e			0.585 b
Effect of drought		0.762 a	0.612 b	0.531 c			

Similar letters refer to non significant difference found between treatments at probability 5% according to Duncan test.

caused a significant increase in the concentration of the chlorophyll pigment, while there was no significant increase in the carotenoids. The triple interference (AsA × drought × light intensity), the highest value of chlorophyll content (2.603mg g^{-1} F.wt) was recorded in unstressed plants, sprayed with 200ppm AsA and received full light intensity (100%) compared with the rest of the treatments.

Discussion

The growth process of any plant needs basic elements, foremost of which, is water. All physiological processes are affected, and we noted from the results that there was a significant decrease in all the studied traits in response to the effect of drought and that the decreasing values are directly proportional to the increasing in drought periods as reported in the plant height (Table 1). These results was in agreement with Moharram & Habib (2011) there was a significant decrease in plant height for ten varieties of bread wheat subjected to water stress. We also noted that there was a decrease in the relative water content (Table 3) with increasing in drought periods, which is due to the closing of the stomata to reduce the absorption of water, which is reflected in the elongation of the cells. Also, dehydration which, reduce the leaf area and thus reduces the area received light as well as affects synthesis of organic compounds. This data agreed with Al-Obaidy (2015) that the relative water content is affected by the moisture stress of the wheat plant, as well as Raziuddin et al. (2010) indicated that exposing ten varieties of bread wheat to four levels of water stress significantly reduced the relative water content. It is known that there is a correlation between the state of water and the chlorophyll content. Among the manifestations of the effect of drought on plants is the lack of growth, and in severe cases, the process of photosynthesis stops, thus the concentration of plant pigments decreases. In this regard, Moaveni (2011) explained the effect of water stress on wheat for two seasons, which, is the decrease in the total chlorophyll content compared to the control treatment for both seasons. Studying the effect of light, we noted that light is an important environmental factor in plant growth. The results showed that the height of the plant increased with a decrease in the light intensity, and this varies with the results (Yang et al., 2014). They indicated that exposing *Festuca arundinacae* to a low light intensity of 40% of the normal light intensity, stimulated a significant decrease in number of growth parameters, chlorophyll (a & b) contents, and carotenoids levels with the increase of the light stress. The plants under normal light intensity surpassed their relative water content, and this agrees with (Faysal & Ali, 2017) that there was a decrease in the relative water content of the leaves of the fenugreek plant with a decrease in the intensity of light, which leads to a decrease in the osmotic regulation compounds (sugars and proteins). Whereas, the intensity of the light (35%) exceeded on the intensity of light 75% and 100% in the characteristic of the leaf area, and this was consistent with Al-Shalal (2015) the Fenugreek plants grown under light intensity exceeded 45% over plants under full lighting. Ni et al. (1999) explained that shading worked to inhibit the activity of phytochrome B, which leads to the activation of the structure of gibberellin, which has a major role in division and elongation. The results of the current study showed that exposing plants to light stress led to a significant decrease in plant pigments contents, especially from the intensity of light 35%, due to the presence of a close relationship between the decrease in light intensity and the process of photosynthesis. Saifuddin et al. (2010) observed that the most important steps taken by plants for the process of decreasing light intensity are to decrease the pigment chlorophyll and carotenoids contents and decrease stomatal conduction with a decrease in the respiratory rate. On the other hand, spraying plants with AsA achieved a positive effects on most of the studied traits. Ascorbic acid plays multiple roles in plant growth, such as cell division and cell wall expansion, and this is what Adeem & Ahmed (2017) noticed that there was an increase in the height of *Zea mays* plants when sprayed with AsA at a concentration of 200ppm. Also, the relative water content is positively correlated with the phenols and the proline contents and the evidence of the stability of the membranes as observed by Farooq et al. (2013) when wheat seeds were treated with AsA. The results also showed an increase in the leaf area and total chlorophyll. In this case Abdul-Qados (2014) explained the role of AsA as an antioxidant on soybean plants subjected to stress and the effectiveness of AsA is explained by its effect on increasing the growth of the shoot system, which is represented by plant height, leaf area, and in protein and carbohydrate content. Faisal (2020) explained that treatingthe plant with AsA (especially the concentration of 200ppm) can improve growth and reduce the inhibitorydecreasing effect in chlorophyll and carotenoids contents of wheat plants, and he explained the role of AsA in stimulating photosynthesis process, which were reflected positively in carbohydrate manufacturing. In terms of the interactions between the studied factors, we noted that the interaction of AsA with drought and light intensity reduced the inhibitory effect of those factors on the studied traits. AsA can be used to improve some physiological characteristics and to enhance the plant's ability to deal with harsh environmental stresses.

Conclusion

We concluded from this study that spraying the vegetative shoot of wheat plants with ASA had a positive effect on plant growth, and the interaction between ASA with dehydration and light intensity had a ameliorative positive effect on most studied physiological characteristics.

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تأثير العوامل البيئية المختلفة والمعاملة بحامض الاسكوربيك على العديد من المكونات الفسيولوجية في الحنطة *Triticum aestivum* L.

كمال عبد القادر محمد، محمد سعيد فيصل
قسم علوم الحياة - كلية التربية للعلوم الصرفة - جامعة الموصل - الموصل - العراق.

أجريت الدراسة الحالية في جامعة الموصل /كلية التربية للعلوم الصرفة /قسم علوم الحياة حيث نفذت تجربة في البيت الزجاجي وذلك بزراعة صنف الحنطة (بحوث 22) إذ تم تعريض النباتات إلى فترتي الجفاف (6، 9) أيام وثلاثة مستويات من الإضاءة (100، 75، 35%) وتم رش المجموع الخضري بثلاثة تراكيز من حامض الاسكوربيك (0، 100، 200) جزء في المليون لمعرفة تأثيرها على الصفات الفسيولوجية وحللت النتائج إحصائياً باستخدام التصميم العشوائي الكامل (C.R.D) وكررت المعاملات ثلاث مرات. أوضحت النتائج أن للجفاف تأثير سلبي في صفة طول النبات ومساحة الورقة ومحتوى الماء النسبي والكلوروفيل الكلي والكاروتين وخصوصاً فترة الجفاف الثانية وبنسبة بلغت (17.12، 36.02، 9.70، 28.57 و 30.26%) على التوالي قياساً مع معاملة السيطرة، في حين تفوقت النباتات النامية تحت شدة الإضاءة الاعتيادية (100%) في صفة طول النبات والكلوروفيل الكلي بينما حصلت زيادة في صفتي المساحة الورقية ومحتوى الماء النسبي مع إنخفاض شدة الإضاءة مقارنةً مع الإضاءة الاعتيادية. من جانب أخر تداخل حامض الاسكوربيك مع فترات الجفاف وشدة الإضاءة ذو تأثير إيجابي على نمو النبات في أغلب الصفات الفسيولوجية المدروسة.