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Role of Ascorbic Acid in Drought Stress Tolerance of Wheat Plant *Triticum aestivum* L.

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Abstract

Drought is a major abiotic stress, severely affecting the physiological processes and growth of plants. The present investigation aimed to evaluate the impacts of drought stress with three periods for irrigation: every 3 days(control), every 6 days, and every 12 days and foliar spray with different concentrations of ascorbic acid (0, 75, 150, 225) mg.L^{-1} on the wheat plant. Pots experiment was conducted as a completely randomized design with three replications in the green house, Department of Biology, College of Education for Pure Science (Ibn Al- Haitham), University of Baghdad, during the growing season, 2018 – 2019. The results indicated that drought stress decreased plant growth and hastened the senescence of flag leaves, which is significantly associated with a decrease in the quality and quantity of grain yield. Foliar spray with ascorbic acid enhanced plant drought tolerance in terms of plant height, shoot dry weight, flag leaf traits (flag leaf area, flag leaf chlorophyll and the concentration of macronutrients such as nitrogen, phosphorous, potassium) and some yield components (spike length, grain weight. pot^{-1} and protein percentage of grains). The results indicated the efficiency of ascorbic acid foliar spray on vegetative growth, flag leaf traits and some yield components of wheat plants affected by drought stress and the superiority to 150 mg.L^{-1} ascorbic acid in which plants can resist the unfavorable drought stress periods(12 days).

Keywords: Drought stress, Ascorbic acid, wheat plant, Flag leaf.

1.Introduction

Wheat plant is the most strategic and major food crop all over the world, and different biotic and abiotic stresses reduce its yield [1,2]. Drought stress, which is abiotic stress, affects different stages of plant growth, is a major restriction to agricultural productivity around the world. It affect photosynthesis which is considered a basic physiological process [3,4]. Photosynthesis is the source of dry matter and grain yield in crop plants, and flag leaf photosynthesis contributes about 30-35% of carbon necessary for grain filling and early senescence limits the yield potential [5]. Drought stress causes oxidative damage through the production of free oxygen radicals in cells These toxic free radicals attack lipids, proteins, and nucleic acid, leading to damage to the integrity of the cell membrane [6]. Drought stress affects plant growth, especially at grain filling, and a clear reduction in the number of leaves and leaf area [7].

Ascorbic acid (AsA), referred to as vitamin C , is a powerful water-soluble antioxidant in plants [8]. ASA act as an adjuster for plant development through hormone signaling and as a coenzyme in reactions such as fats, carbohydrates, and proteins metabolism. Moreover, it involved in the regulation of many important processes such as photoinhibition, cell elongation and differentiation, growth and development, flowering senescence and photosynthesis [9, 10]. [11] referred that ascorbic acid foliar spraying resulted in improvement of the vegetative growth, grains yield and chemical components of barley plants.

This study is an attempt to light more evidence about the effectiveness of ascorbic acid foliar spray in increasing the resistance of wheat plants affected by drought stress.

2. Materials and methods

The experiment was carried out in the green garden, Department of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, during the growing season 2018 – 2019, to assess the effect of drought stress and ascorbic acid foliar spray on vegetative growth and flag leaf traits and some yield components of wheat plants. The experiment was laid out in a completely randomized design CRD with three replications. Healthy uniform grains were sown in the pots with a capacity of 11 kg soil on 11/11/2018. Thinning of seedlings was done after two weeks from germination. Drought stress was conducted in three periods:

The first period was referred to as 3D (irrigation every 3 days) and considered a control treatment. The Second period was referred to as 6D (irrigation every 6 days). Third period was referred to as 12D (irrigation every 12 days).

Plants were foliar sprayed with ascorbic acid (0,75, 150, 225) $mg.L^{-1}$ twice on 26/12/2018 and on 7/1/2019, in the early morning. Four random plants per pot were harvested on 16/1/2019 Plant heights were measured for each treatment and plant samples were oven dried at 65 C° to stable mass, the dry weights of shoots were measured. Some flag leaf traits were calculated: flag leaf area as [12]. flag leaf chlorophyll using SPAD and the concentration of macronutrients, nitrogen as [13], phosphorous as [14], and potassium as [15]. Three plants were harvest on 22/4/2019 and some yield components were calculated: spike length, grains weight. pot^{-1},and protein percentage of grains as [16]. Statistical analysis of the experimental data was done by SAS 2012, and the comparisons of means were done via the least significant difference (LSD) test $p \leq 0.05$.

3. Results and Discussion

Data in **Tables (1, 2)** indicated that increasing drought stress periods from 3 to 12 days caused a significant and gradual reduction in averages of plant height, and shoot dry weight by (22.39, 25.21) % relative to their unstressed control. Spraying ascorbic acid at different concentrations induced a significant increase; the maximum increase was detected at 150$mg.L^{-1}$ by (21.00, 32.26)

%, respectively, more than their controls for both parameters. Worthy to mention the combination of the dual factors, 150 mg.L^{-1} ascorbic acid can minimize the hard effects of drought stress (12 day) on plant height and shoot dry weight their values were increased to 62.84 cm, 3.17gm in comparison with their values 42.00 cm, 1.98 mg at the same drought stress period but without ascorbic acid treatment.

Table 1. Effect of drought stress and ascorbic acid on plant heights (cm).

Drought stress (Day)	**Ascorbic acid concentrations(mg.L^{-1})**				**Drought stress average**
	0	**75**	**150**	**225**	
3D	65.88	68.28	74.20	71.30	69.91
6D	59.00	61.00	64.88	63.50	62.09
12 D	42.00	52.10	62.84	60.08	54.26
LSD 0.05	Drought stress x Ascorbic acid = 1.70				Drought stress = 0.85
Ascorbic acid average	55.63	60.46	67.31	64.96	
LSD 0.05	Ascorbic acid = 0.98				

Table 2. Effect of drought stress and ascorbic acid on shoot dry weights (gm).

Drought stress (Day)	Ascorbic acid concentrations(mg.L^{-1})				Drought stress average
	0	75	150	225	
3D	3.30	3.48	4.07	3.57	3.61
6D	2.82	3.00	3.52	3.34	3.17
12D	1.98	2.57	3.17	3.06	2.70
LSD 0.05	Drought stress x Ascorbic acid = 0.042				Drought stress = 0.021
Ascorbic acid average	2.70	3.02	3.59	3.32	
LSD 0.05	Ascorbic acid = 0.025				

Leaf senescence usually occurs during stress, flag leaf area and the content of chlorophyll were measured in plants to assess the impact of drought stress, both are related to visual symptoms of plant illness. Data in **Tables (3, 4)** showed that averages of flag leaf area and flag leaf chlorophyll were significantly decreased under drought stress: the highest period caused the highest significant decrease for both parameters by (31.62, 16.76) %. Application of ascorbic acid up to 225 mg.L^{-1} induced a significant increase in the average of parameters: the maximum significant increase was achieved at 150mg.L^{-1} by (32.90, 17.43) % more than control plants. The combination of the dual factors was confirmed, 150 mg.L^{-1} ascorbic acid can counteract the unfavorable effects of drought stress (12 days) and raised the value of flag leaf area and chlorophyll, they were 28.83 cm^2, 38.90 SPAD, in comparison with their values 18.19 cm^2, 29.40 SPAD at the same drought stress period but without ascorbic acid treatment.

Table3. Effect of drought stress and ascorbic acid on flag leaf area (cm^2).

Drought stress (Day)	**Ascorbic acid concentrations (mg.L^{-1})**				**Drought stress average**
	0	**75**	**150**	**225**	
3D	32.88	35.28	37.52	36.26	35.48
6D	25.88	29.90	35.92	32.85	31.14
12D	18.19	23.52	28.83	26.50	24.26
LSD 0.05	Drought stress x Ascorbic acid = 1.72				Drought stress = 0.86
Ascorbic acid average	25.65	29.57	34.09	31.87	
LSD 0.05	Ascorbic acid = 0.99				

Table 4. Effect of drought stress and ascorbic acid on flag leaf chlorophyll (SPAD).

Drought stress (Day)	**Ascorbic acid concentrations (mg.L^{-1})**				**Drought stress average**
	0	75	150	225	
3D	39.80	42.77	45.20	41.42	42.30
6D	35.80	37.87	39.20	38.24	37.78
12D	29.40	35.42	38.90	37.12	35.21
LSD 0.05	Drought stress x Ascorbic acid = 1.92				Drought stress = 0.96
Ascorbic acid average	35.00	38.69	41.10	38.93	
LSD 0.05	Ascorbic acid = 1.11				

Data in **Tables (5, 6, 7)** indicated that the average nitrogen, phosphorous and potassium concentrations in flag leaf were reduced and correlated with increasing drought stress periods to 12 days by (24.56, 22.73, 19.40) %, respectively, relative to their unstressed controls. Adding ascorbic acid as foliar spray improved macronutrient concentrations and there was clear superiority to 150 mg.L^{-1}, for giving the highest average by (28.24, 20.00, 29.60) % more than their controls. The combination of the two factors was significant; 150 mg.L^{-1} ascorbic acid reversed the negative effects of 12 days of drought stress on macronutrients concentrations and gave values (2.40, 0.38, 2.75) % compared with their values (1.80, 0.27, 1.61)% at the same drought stress period but without ascorbic acid treatment.

Table 5. Effect of drought stress and ascorbic acid on nitrogen concentrations (%) of flag leaf.

Drought stress (Day)	**Ascorbic acid concentrations(mg.L^{-1})**				**Drought stress average**
	0	**75**	**150**	**225**	
3D	2.67	2.80	3.10	2.84	2.85
6D	2.01	2.40	2.80	2.70	2.48
12D	1.80	2.00	2.40	2.38	2.15
LSD 0.05	Drought stress x Ascorbic acid = 0.055				Drought stress = 0.027
Ascorbic acid average	2.16	2.40	2.77	2.64	
LSD 0.05	Ascorbic acid = 0.032				

Table 6. Effect of drought stress and ascorbic acid on phosphorous concentrations (%) of flag leaf.

Drought stress (Day)	**Ascorbic acid concentrations(mg.L^{-1})**				**Drought stress average**
	0	**75**	**150**	**225**	
3D	0.42	0.43	0.47	0.42	0.44
6D	0.37	0.40	0.42	0.40	0.40
12D	0.27	0.33	0.38	0.37	0.34
LSD 0.05	Drought stress x Ascorbic acid = 0.03				Drought stress = 0.02
Ascorbic acid average	0.35	0.39	0.42	0.40	
LSD 0.05	Ascorbic acid = 0.02				

Table 7. Effect of drought stress and ascorbic acid on potassium concentrations (%) of flag leaf.

Drought stress (Day)	**Ascorbic acid concentrations(mg.L^{-1})**				**Drought stress average**
	0	**75**	**150**	**225**	
3D	2.80	3.01	3.15	2.99	2.99
6D	2.27	2.57	2.77	2.69	2.58
12D	1.61	2.68	2.75	2.60	2.41
LSD 0.05	Drought stress x Ascorbic acid = 0.046				Drought stress = 0.023
Ascorbic acid average	2.23	2.75	2.89	2.76	
LSD 0.05	Ascorbic acid = 0.027				

Tables (8, 9, 10) illustrated that in response to increasing drought stress periods, the average spike length and grains weight. Pot^{-1}, protein percentages of grains were reduced by (23.48, 27.56, 16.70) % compared with their controls. Ascorbic acid foliar spray, up to 225 $mg.L^{-1}$ increased the average of yield components, and the best increase was pronounced at 150 $mg.L^{-1}$ by (19.60, 24.27, 16.94)% more than their controls. Regarding the combination between drought stress and ascorbic acid, 150$mg.L^{-1}$, ascorbic acid can nullify the effect of extreme drought stress period on yield components and gave the values 13.79 cm, 6.48gm 16.89%, in comparison with their values 10.13 cm, 4.21gm, 14.10% at the same drought stress period but without ascorbic acid treatment**.**

Table 8. Effect of drought stress and ascorbic acid on spike lengths (cm).

Drought stress (Day)	Ascorbic acid concentrations($mg.L^{-1}$)				Drought stress Average
	0	75	150	225	
3D	15.02	16.28	17.89	16.40	16.40
6D	13.88	14.29	15.00	14.84	14.50
12D	10.13	12.88	13.79	13.40	12.55
LSD 0.05	Drought stress x Ascorbic acid = 0.74				Drought stress = 0.37
Ascorbic acid average	13.01	14.48	15.56	14.88	
LSD 0.05	Ascorbic acid = 0.43				

Table 9. Effect of drought stress and ascorbic acid on grains weights (gm).pot^{-1}.

Drought stress (Day)	Ascorbic acid concentrations($mg.L^{-1}$)				Drought stress Average
	0	75	150	225	
3D	7.23	7.66	8.20	8.10	7.80
6D	6.10	6.55	7.14	7.21	6.75
12D	4.21	5.52	6.48	6.40	5.65
LSD 0.05	Drought stress x Ascorbic acid = 0.039				Drought stress = 0.020
Ascorbic acid average	5.85	6.58	7.27	7.24	
LSD 0.05	Ascorbic acid =0.023				

Table 10. Effect of drought stress and ascorbic acid on protein percentages (%)of grains.

Drought stress (Day)	Ascorbic acid concentrations($mg.L^{-1}$)				Drought stress average
	0	75	150	225	
3D	17.49	18.52	20.74	19.52	19.07
6D	16.22	17.44	18.29	17.64	17.40
12D	14.10	15.68	16.89	16.79	15.87
LSD 0.05	Drought stress x Ascorbic acid = 0.18				Drought stress = 0.09
Ascorbic acid average	15.94	17.21	18.64	17.98	
LSD 0.05	Ascorbic acid = 0.10				

The results showed a reduction in the growth and yields of wheat plants because drought causes dehydration to leaves mesophyll and reduces its efficiency for using CO_2 and causes photosynthesis inhibition [3]. Drought stress caused oxidative damage, and ROS production was enhanced due to the inhibition of carbon dioxide assimilation and changes in photosystem activities. CO_2 fixation is limited due to stomata closure which in turn leads to a reduced $NADP^+$ cycle [17]. Leaf senescence is correlated with resistance to oxidative stress; the degradative processes due to leaf senescence are chlorophyll damage and leaf area reduction [18]. Plant leaves yellowing is considered the first symptom in chloroplast senescence related to the accumulation of ROS induced by stress; it might be associated with lipids peroxidation of chloroplast membranes, and formation of fatty acids hydroperoxides, and enhances the activity of chlorophyllase, and interferes with protein synthesis binding chlorophyll [19]. Flag leaf can be estimated to be a source of carbon to the plants: high associations were found between flag leaf area and grains yield per plant, and its senescence is likely to affect the remobilization of nutrients from the shoot to the root [20].

Our results indicated that 150 mg.L^{-1} ascorbic acid foliar spray can improve the growth of wheat plants . It can modify flag leaf senescence because ascorbic acid can act as ROS removers or ROS chain breakers, thus quenching oxidative free radicals [21]. The anti-oxidant activity of ascorbic acid is greatly associated with longevity in plants, and the stability of its prime oxidation product, the monodehydroascorbate radical (MDA) and its capacity to terminate radical chains reactions by disproportionation into the non-toxic product AsA dehydroascorbate [22]. Ascorbic acid has great importance during photosynthesis because it is able to donate electrons to PSI and PS II in both normal, and stress conditions [23].Its application on plants improved total chlorophyll rather than through the stimulation of its biosynthesis, or interruption of its degradation because, ascorbic acid act as a substrate for ascorbate peroxidase, and it is able to scavenge ROS produced in the thylakoid membrane ,and the high concentrations of it may be detrimental to plant growth [24] .

4.Conclusions

It could be concluded that ,wheat plants' treatment with ascorbic acid can curiously increase their ability to survive under hard stress through the inhibition of drought stress induced leaf senescence. Ascorbic acid leads to an increase in the concentrations of nutrients in flag leaf and its role in improving osmotolerance through regulating nutrient absorption from soil solution. Results indicated a clear superiority to 150 mg.L^{-1} ascorbic acid in which plants can resist the unfavorable drought stress periods (12 days).

References

1.Curtis, T.; Halford, N.G. Food security: the challenge of increasing wheat yield and the importance of not compromising food safety. *Annals of Applied Biology* **2014**, *164*(3), 354–372.

2. AL-Kazzaz, A.G.M. ; AL-Kareemawi, I.H.K. Foliar Spraying Alphatocopherol Enhances Salinity Stress Tolerance in Wheat Plant *Triticum Aestivum* L. *Ibn Al-Haitham Jour. for Pure & Appl. Sci***. 2021,** *34*(3) ,10-16.

3. Ashraf, M.; Harris, P.J.C. Photosynthesis under stressful environments: an overview. *Photosynthetica* **2013**, *51*(2), 163-190.

4. Hussain N.N.; Jassim Z. R. Effect of Drought Stress on Barley (*Hordeum vulgare* L.) in Germination Stage. *Ibn Al-Haitham Journal for Pure and Applied science* **2015,** *28*(1), 242-252.

5. Sylvester-Bradley, R.; Scott, R.K.; Wright, C.E. *Physiology in the Production and Iimprovement of Cereals*. Home-Grown Cereals Authority Research Review, 18. HGCA, London, **1990.**

6. Movahhedi Dehnavi, M.; Zarei, T.; Khajeeyan, R. ; Merajipoor, M. Drought and Salinity Impacts on Bread Wheat in a Hydroponic Culture: A Physiological Comparison. *J. Plant Physiol. Breeding* **2017,** *7*(1), 61-74.

7. Push Pavalli, R.; Zaman-allah, M.; Turner, N.C.; Baddam, R.; Rao, M.V. ;Vadez, V. Higher flower and seed number Leads to higher yield under water stress conditions imposed during reproduction in chick pea. *Funct. Plant Biol***., 2015**, *42*, 162-174.

8. Macknight, R.C.; Laing, W.A.; Bulley, S.M.; Broad, R.C.; Johnson, A.A.T. ; Hellens, R.P. Increasing ascorbate levels in crops to enhance human nutrition and plant abiotic stress tolerance. *Curr. Opin. Biotech.* **2017**, *44*, 153–160.

9. Zhang, Y. Biological Role of Ascorbate in Plants. (Chapter 2). *Springer*, Briefs in Plant Science. **2013**.

10. Al-Kaisy, W.A. ;Mahadi S. F. Influence of Foliar Application with Abscisic Acid (ABA) and Vitamin C on Some Plant Hormones for Peas Plant. Pisum sativum L. *Ibn Al-Haitham Journal for Pure and Applied science* **2017,** Special Issue, 64-72.

11. Abdo, F.A. ; El-Moselhy, M.A. Response of barley plant to foliar spray with ascorbic acid under different levels of nitrogen application. *Egypt. J. Appl., Zagazig Univ*. **2004,** *19*(2), 111-128.

12. Mckee, G.W. A coefficient for computing leaf area in hybrid corn. *Agron. J.*, **1964,** *5*6(2), 240-241.

13. Chapman, H.D.; Pratt, F.P. Methods of Analysis for Soils, Plants and Waters. *Univ. Calif. Div. Agri. Sci.* **1961**, 161–170.

14. Matt, K.J. Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. *Soil Sci.* **1970,** *109*, 214-220.

15. Page, A.L.; Miller, R. H. ;Kenney, D.R. *Method of Soil Analysis* . 2^{nd} (ed), Agron. 9, Publisher, Madiason, Wisconsin **1982.**

16. Vopyan, V.G. *Agricultural Chemistry*. English translation, Mir puplishers.1^{st} edn. **1984.**

17. Jaleel, C.A.; Manivannan, P.; Wahid, A.; Farooq, M.; Al-Juburi, H.J.; Somasundaram, R. ;Panneerselvam, R. Drought stress in Plant: A review on Morphological characteristics and Pigments composition. *Int. J. Agric. Biol.* **2009**, *11*(1), 100-105.

18. Munnre-Bosch, S. Aging in perennials. *Crit. Rev. Plant Sci* **2007**, *26*, 123-138.

19. Moller, I.M.; Jensen, P.E.; Hansson, A. Oxidative modifications to cellular components in plants. *Annu. Rev. Plant Biol*. **2007,** *58*, 459 - 481.

20. Liu, Y.; Tao, Y.; Wang, Z.; Guo, Q.; Wu, F.; Yang, X.; Deng, M.; Ma, J.; Chen, G.; Wei, Y. and Zheng, Y. Identification of QTL for flag leaf length in common wheat and their pleiotropic effects. *Molecular Breeding*, **2018,** *38*(*11*), 1- 11.

21. Krieger-Liszkay, A. ;Trebst, A. Tocopherol is the scavenger of singlet oxygen produced by the triplet states of chlorophyll in the PSII reaction centre. *J. Exp. Bot.* **2006,** *57*(*8*), 1677–1684.

22. Noshi, M.; Hatanaka, R.; Tanabe, N.; Terai, Y.; Maruta, T. ; Shigeoka, S. Redox regulation of ascorbate and glutathione by a chloroplastic dehydroascorbate reductase is required for high-light stress tolerance in Arabidopsis. *Biosci. Biotechnol. Biochem*. **2016,** *80*, 870–877.

23. Ivanov, B.N. Role of ascorbic acid in photosynthesis. *Biochem. Moscow* **2014,** 79, 282–289.

24. Davey, M.W.; Mantagu, M.V.; Dirk, I.; Maite, S.; Angelos, K.; Smirnoff, N.;. Binenzie, I.J.J.; Strain, J.J.; Favell, D. ; Fletcher, J. Plant ascorbic acid chemistry, function, metabolism, bioavailability and effects of processing. *J. Sci. Food and Agri.* **2000,** *80*, 825 - 850.