ISSN 2709–3662 (Print)
ISSN 2709–3670 (Online)
https://doi.org/10.52587/JAF04020
Journal of Agriculture and Food
2023, Volume 4, No.2, pp. 1-11

Ascorbic acid ameliorates drought stress in maize (*Zea mays* l.) grown under field conditions

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Abstract

Drought is a serious abiotic constraint against the growth and production of maize. Different management approaches have been adopted to cope with the negative effects of water deficit. Therefore, a trial was carried out to optimize the concentration of cenolate (ascorbic acid) and to assess its role in ameliorating the negative effects of drought for sustainable crop production. The treatments comprised of (a) three drought levels: D_0= control (no drought), D_1= drought at vegetative stage or 40 days after emergence (skip irrigation), D_2= drought at reproductive or silking stage (skip irrigation), (b) two maize varieties:V_1= FH-1046 (drought tolerant), V_2= FH-1137 (drought-sensitive) and (c) four levels of ascorbic acid (0, 0.5 mM, 1.0 mM and 1.50 mM). The results showed that drought caused a significant reduction in the photosynthetic pigments, shoot biomass and plant growth. However, treatment of ascorbic acid through the foliar method decreased the adverse impacts of drought. Application of ascorbic acid increased the plant vigor and final productivity of maize by improving the antioxidative defense system, stabilizing membrane, maintaining water levels and increasing the contents of photosynthetic pigments. Among cultivars, FH-1046 was comparatively resistant against drought. Foliar

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applied AsA at lower concentration (1.0 mM) showed positive influences in both maize cultivars grown under water-limited conditions.

Keywords: Antioxidant enzymes, Drought levels, Foliar application, Irrigation
Article History: **Received:**17th April 2022; Revised: 15th June, 2023; Accepted: 15rh September, 2023

Introduction

In both natural and agricultural environments, plants are frequently subjected to various biotic and abiotic stresses. Water deficiency, commonly recognized as drought, is characterized as a lack of adequate moisture required for the completion of a plant's life span properly (Zhu, 2002). Moisture deficit is one of the world's utmost severe abiotic stresses and is an extreme restriction on the production and improving the quality of major grown crops for better safety of food stuffs (Shao *et al.,* 2008; Jaleel 2009). Moisture deficit disturbed the normal metabolic processes of plants including biochemical and physiological processes, reduced rate of photosynthesis and damaged to chlorophyll, resulting in reduced plant growth (Zlateve *et al.,* 2006; Malik and Ashraf 2012; Zlatev & Lidon, 2012). Reactive oxygen species (ROS) production is increased under drought conditions that drastically affect the cellular organelles, cellular reactions and plasma membrane in plants (Ahmad *et al.,* 2019). These ROS caused oxidative damage to lipid and protein contents that lead to disturbance in plants' metabolic processes (Rout and Shaw 2001). Maize being an important member of Poaceae family ranks as 3rd main grain crop after rice and wheat. Globally maize is used as the main staple food (Frova *et al.,* 1999). In Pakistan, maize crop is usually grown on an area of 0.95 Million hectares with net production of about 3.5 Mt on annual basis (Li *et al.,* 2010). Nowadays, maize is considered as a crucial crop for food security and enhancing food safety globally (Ahmad *et al.,* 2019). Water deficiency is a major constraint in the productivity of maize in Pakistan (Ali *et al.,* 2018). Under moisture deficit conditions, maize responds differently with cultivars (Ali *et al.,* 2008; Ali *et al.,* 2011; Jabeen*et al.,* 2008; Khodarahmpour 2011). All plants adopted various strategies to cope with the negative effects of moisture deficit (Ashraf 2010; Li *et al.,* 2010). Different genetic factors are stimulated in plants for the anti-oxidant defense that protects the cell from oxidative damage under drought (Ahmad *et al.,* 2019). In such a case, a range of anti-oxidants including both enzymatic and non-enzymatic produced in plants. This process made changes at physiological, cellular and transcriptome levels (Ahmad *et al.,* 2019). Enzymatic anti-oxidants include catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) twisted in plants that scavenge the ROS (Badawi *et al.,* 2004). Similarly, plants also produced non-enzymatic antioxidants including tocopherols and ascorbic acid (vitamin C) to overcome the deleterious effects of ROS (Ahmad *et al*., 2019). Studies revealed that an increase in a number of ascorbic acid (enzyme-substrate) might increase the tolerance against oxidative stress in plants under drought (Dolatabadian *et al.,* 2009).

Foliar application of chemicals to plants is the most significant and appreciable approach. Ascorbic acid is an important anti-oxidant for stressed plants due to its strong antioxidant potential and its role in processes of development including seed germination and bolting (Khan *et al.,* 2011). Studies illustrated that foliar application of ascorbate improved the seed germination, increased the seedling's fresh weight (Tavili *et al.,* 2009). Further, ascorbate maintained the water potential in plants and protected them against abiotic stresses (Athar *et al.,* 2009). In plants, the efficiency of application of ascorbic acid

in saline stress has recently been documented. However, the role of its application and tolerance mechanisms in plants are rarely reported under drought (Athar *et al.,* 2008; Ashraf 2010; Tuna *et al.,* 2013).

Thus, keeping in view of the above discussion, the present research has been investigated to check the efficacy of exogenously applied ascorbic acid on plants and its role in relieving the deleterious effects of moisture deficits on growth, biochemical and physiological attributes of two contrasting maize hybrids.

Materials and Methods

Experimental site

This experiment was conducted at Agronomic Research Farm, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan in 2017-2018. The Soil was clay loam with a medium fertile nutrient status. Physico-chemical properties of soil are described `in Table-1.

Table 1. Physico-chemical properties of soil

Variable	Unit	Value obtained
E.C.	d S m^{-1}	1.28
pH	-	7.90
Available P	Ppm	12.58
Available K	Ppm	282.00
Available N	Ppm	0.07
Saturation	%	32.20
O.M	%	1.02

Experimental details

Field oriented trial was conducted at Student's Research Farm, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan. The experimental area falls in altitude 184 m, latitude 31.40º N, and longitude 73.05º E. Experiment consisted of three factors *viz. (*a) Two contrasting maize cultivars, V_1= FH-1046 (drought tolerant), V_2= FH-1137 (drought-sensitive), three drought levels, D_0 = Control (normally recommended irrigation), D_1= Skipping irrigation at the vegetative stage or 40 days after emergence, D_2= Skipping irrigation at reproductive or silking stage, and (c) Foliar spray with three different concentrations of ascorbic acid (0.5 mM, 1.0 mMand 1.50 mM) and control (no spray). Maize cultivars were selected on the basis of their historical record against drought. FH-1046 is a drought tolerant cultivar (Ali *et al.,* 2018) while FH-1137 is a drought-sensitive cultivar (Ali *et al.,* 2018). The experiment comprised of three replications using RCBD (Randomized Complete Block Design) under a split-split plot sequence. Maize crop was sown in the spring season using recommended seed rate (25 kg ha^{-1}).

Meteorological data during the crop season (Spring 2017-18)

Weather data was collected during crop season from the meteorological observatory in Department of Agronomy, University of Agriculture, Faisalabad. This observatory is situated within 150 m radius to the experimental site. Data allied to mean temperature, mean relative humidity, mean rainfall and mean sunshine hours are given below in Fig 1.

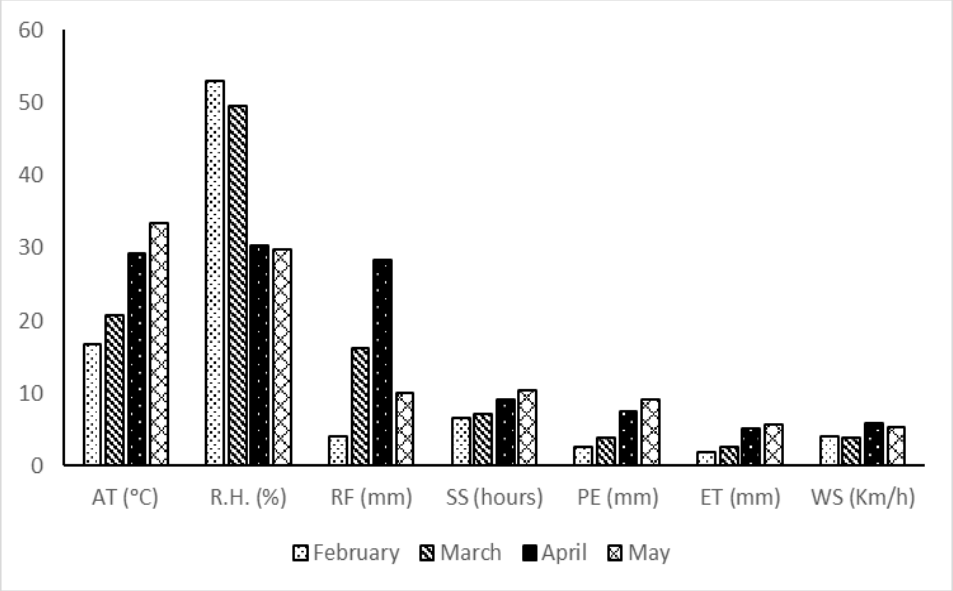

Fig 1. A.T (Average temperature)[ºC], R.H (Relative humidity)[%], R.F (Rainfall)[mm], S.S (Sunshine)[hours], P.E (Pan evaporation)[mm], E.T (Evapotranspiration)[mm], W.S (Wind speed)[Kilometer/hour]

Procedure for recording observation

Morphological attributes

All the morphological parameters like Length of cob (cm), number of cobs per plant, number of grains per cob, cob weight without sheath (g), thousand-grain weight (g) were measured. They were recorded after harvesting the crop through destructive sampling while all biochemical and physiological parameters except the grain protein contents were recorded before one week of harvesting.

Physiological attributes

(i). *Water Potential (-MPa)*: Following the procedure ascribed by Scholander *et al.* (1964) by using "water potential apparatus". Water potential of leaf was determined (Chas W. Cook & Sons. Birmingham B 42, ITT England). (ii). Osmotic Potential (-MPa) of the leaf was determined using calibrated osmometer (Cryoscopicosmometer, Osmomat 030-D, Genatec). (iii). Stomatal Conductance (m mol m^{-2} s^{-1}): A portable and open system, LCA-4 ADC analyzer of infrared gas was used for the measurement of the stomatal conductance. (iv). Canopy Temperature (°C): Infrared temperature sensors (IRIS) was used to record the energy emitted by the plants. (v). Photosynthetic rate (*An*) [µmol m^{-2} s^{-1}]: It tells about how the plants are metabolically active, water use efficiency of plants, and water levels in plants (Singh *et al.*, 2018; Pettigrew, 2004). An infrared gas analyzer (IRGA) was used for photosynthetic rate determination inplants (Singh *et al.*, 2018; Rosolem *et al.*, 2019). This measurement was done through non-destructive sampling (without excising leaf from the parent plant). Three readings were recorded separately for each three plants of one treatment and then averaged. The same procedure was repeated for all other treatments.

(vi). Relative water content (RWC) % were measuredfollowing the protocol by Schonfled*et al.* (1988). Values of DW, TW and FW were used to calculate RWC according to Schonfeld' s equation.

Relative water content (RWC) (%) = [(fresh weight (FW)- dry weight (DW))/ (turgid weight (TW) - dry weight (DW))]*100

(vii). Membrane Stability Index (MSI) %: MSI, Leaf membrane stability index was determined following the protocol designed by Premachandra*et al.* (1990), which was revised by Sairam in 1994. MSI was obtained by using following equation.

$$MSI= 1-(C_1/C_2)\ x100$$

(viii). Leaf chlorophyll contents (mg g^{-1}): Chlorophyll *a* and *b* and their total amount found out using the protocol elaborated by Arnon (1949). A pico-drop spectrophotometer (Hitachi-U-2001, Japan) was used for this purpose. 0.5g leaf sample was dipped in 80% acetone and allowed the final volume up to 5 ml. After filtration, the filtrate sample was run in the spectrophotometer. For chlorophyll *a* and chlorophyll *b*, absorbance was noted at 645 and 663 nm respectively. Using these absorbance readings, chlorophyll *a* and *b* and total contents were determined bythese formulas (Yoshid*et al.* 1976).

Chlorophyll*a* (mg g^{-1}) = [12.7(OD663) – 2.69(OD645)] × Volume/1000 × Weight

Chlorophyll*b* (mg g^{-1}) = [22.9(OD645) – 4.68(OD663)] × Volume/1000 × Weight

Total Chlorophyll (mg g^{-1}) = [20.2(OD645) + 8.02 (OD663)] × Volume/1000 × Weight

Where,

Volume = Volume of the acetone used in extract

Weight = Weight of fresh leaf tissue

Biochemical attributes

(i) Leaf nitrogen contents (m mol g^{-1} dwt^{-1}) were determined using Kjeldahl's apparatus. (ii) Phosphorus content in leaves (m mol g^{-1} dwt^{-1}) were determined using spectrophotometer. 5 ml aliquot was poured into 10 ml of Barton reagents and volume was raised up to 50 ml using double distilled water. After few minutes, colors were developed and phosphorous content was calculated at 420 nm by using a standard curve. (iii): Potassium contents in leaf (m mol g^{-1} dwt^{-1}): 0.1 g ground leaf sample was kept in digestion tube of the flame photometer. 5ml conc. sulphuric acid was poured into these tubes. These were allowed to incubate for 24 h at 25 ^{0}C. Further, 1 ml of 35% concentrated hydrogen peroxide wasadded into tubes. Then, tubes were kept in a digestion block of apparatus at 350 ^{0}C for 30 min. After that tubes were removed and allowed to cool. Again 1 ml of 35% H_2O_2 was poured into tubes and these tubes were placed back inthe block for digestion. This process was repeated till the cooled material was changed to colorless. The collected extract was filtered and used to determine the potassium contents of the leaf by flame photometer.

Antioxidants Extractions: To analyze the activities of antioxidant enzymes, 0.5 g leaf sample was frozen. Frozen leaf was ground and placed into 5ml phosphate buffer, 50 mM, in ice bath. Centrifugation of mixture was done at 15000 revolutions per minute for 15 minutes at 4 °C. Supernatant collected was further used for assessing the activities of antioxidant enzymes.

(iv). Catalase (µmol mg^{-1}protein) was determined using the protocol elaborated by Chance and Maehly 1955). (v). Superoxide dismutase and Peroxidase(µmol mg^{-1}protein): SOD and POD activities were assayed by determining their potential to stop the process of photoreduction of NBT [nitro blue tetrazolium](Giannopolitis and Ries 1977).

(vi). Grain protein content (%): Crude protein contents (%) were determined using the protocol elaborated by Bremner (1964). By using the following formula contents of crude protein were determined.

$$\text{Crude Protein (\%)} = \frac{(V1-V2) \times 100}{100w} \times 100 \times 6.25 \times 14$$

V_1 = Titration of sample (ml)
V_2 = Blank titration (ml)
N = Normality of hydrogen peroxide
W = 100 (weight of sample)

Statistical Analysis

Data regarding physiological, biochemical, quality, yield and other parameters related to yield were taken using standard measures and investigated statistically by ANOVA, Fisher's analysis of variance procedure. All meanstreatments were related by Least Significant Difference test at 0.05 probability level with Statistics 10, software, as elaborated by Steel *et al.* 1997.The graphical presentation of meteorological data was made by MS Excel 2016**.**

Results

Morphological attributes

Data regarding morphological attributes i.e. length of cob (CL), per plant cobs no (NC), per cob grains no (GN), the weight of cob (CW) without sheath, thousand-grainweight(GW), grain (economic) yield (GY), and biological yield (BY) is presented in Table 2. Results in Table 2 predicted that all the morphological attributes wereaffected significantly by applying foliar spray of ascorbic acid under drought stress conditions. Data showed that statistically maximum cob length and number of cobs per plants were obtained under no stress conditions and applying a foliar spray of ascorbic acid @1.5 mM. (19.66 and 1.99 respectively). On the other hand, minimum cob length and number of cobs per plants were obtained under stress conditions at the reproductive stage and without applying ascorbic acid (11.00 and 0.66 respectively). Results presented in Table 2 predicted that maximum grains per cob and cob weight/plant were obtained under no stress conditions and applying a foliar spray of ascorbic acid @1.5 mM. (474.00gand 225.33g, respectively). However minimum no of grains per cob and cob weight per plant was obtained under moisture deficit conditions at the reproductive stage and without applying ascorbic acid (11.00 and 0.66 respectively). Similar trends were also achieved for 1000-grain weight, biological and grain yield (Table 3). Maximum thousand-grain weight, biological and grain yield were recorded under adequate water conditions and applying foliar spray of ascorbic acid @1.5 mM. (274.66 g, 17.14 t/ha and 7.03 t/ha, respectively). However, minimum thousand-grain weight, biological and grain (economic) yield were obtained under stress conditions at the reproductive stage and without applying ascorbic acid (154 g, 10.03 t/ha and 4.17 t/ha, respectively).

Physiological attributes

Data regarding physiological attributes *i.e.* water potential, osmotic potential, canopy temperature, stomatal conductance, relative water content, membrane stability index (MSI), chlorophyll contents of leaf and rate of photosynthesis inTable 3 showed that all the physiological attributes were affected significantly by the applying foliar spray of ascorbic acid under drought stress conditions.Data presented in Table 3showed that minimum water and osmotic potential (WP, OP) were achieved under no drought

conditions and applying ascorbic acid as foliar spray @ 1 mM. (-0.75 MPa and -0.22 MPa, respectively).

Table 2. Influence of foliar application of ascorbic acid on morphological attributes of maize cultivars grown under drought stress conditions

Treatments			**CL**	**NC**	**GN**	**CW (g)**	**1000 GW (g)**	**BY (t ha^{-1})**	**GY(t ha^{-1})**
Varieties	**DS**	AA (mg L^{-1})							
`	**D_0**	**A_0**	15.33d	1.00 d	427.00d-j	200.33de	258.25 b	12.23 f	5.70 c
		A_1	16.33 c	1.66 b	465.67a-c	210.00 c	263.00 b	1416 d	5.80 c
V1		A_2	19.66 a	1.99 a	474.00 a	225.33 a	274.66 a	17.14 a	7.03 a
		A_3	18.33 a	1.66 b	468.00 ab	214.00 b	266.33 a	16,86 b	5.90 b
	D_1	**A_0**	14.05 e	1.00 d	420.00 d-j	179.00 k	219.00 d	12.10 i	5.17 g
		A_1	15.00 de	1.33 c	427.67 d-j	189.00 i	227.15 d	12.60 h	5.30 f
		A_2	16.25 c	2.00 a	440.00 b-f	199.00 e	239.66 c	13.07 f	5.66 d
		A_3	15.16 de	1.66 b	435.33 c-j	195.00 f	234.33 d	12.84 g	5.60 e
	D_2	**A_0**	12.00g	1.00 d	405 f-k	175.00 l	204.00 f	13.30lm	4.27 mn
		A_1	13.25 f	1.00 d	410.33 f-j	179.67 j	209.20 f	11.51 k	4.51 j
		A_2	14.00 e	1.33 c	415.33 e-j	181.67 j	214.00 e	11.67 j	4.75 i
		A_3	14.33 e	1.66 b	418.00 d-j	189.00 i	217.55 e	12.00 i	4.94 h
V2	**D_0**	**A_0**	13.99 ef	1.00 d	437.33 c-g	191.00 h	237.33 c	13.46 e	5.27 f
		A_1	15.00 de	1.05 cd	439.33 b-g	192.67 g	239.33 c	14.11 d	4.38 kl
		A_2	16.12 c	2.00 a	447.33 a-d	202.00 d	247.66 c	14.99 c	4.67 i
		A_3	15.66 d	1.55 b	444.33 b-e	199.00 e	244.33 c	14.14 d	4.48 j
	D_1	**A_0**	12.21 g	1.00 d	402.00 jk	171.00 n	198.33 g	111.11 n	4.28 mn
		A_1	13.00 f	1.33 c	410.00 g-k	173.00 m	201.33 f	11.31 l	4.31 lm
		A_2	13.66 ef	1.66 b	432.00 d-i	178.00 k	208.33 f	12.1 1i	4.38 k
		A_3	13.50 f	1.33 c	415.33 e-j	175.33 l	204.35 f	11.29 lm	4.32 lm
	D_2	A_0	11.00 h	0.66 e	354.00 l	160.33 q	154.00 ij	10.03 o	4.17 o
		A_1	12.00 g	1.00 d	360.67 l	166.00 p	159.00 i	11.04 n	4.21 no
		A_2	13.66 ef	1.05 cd	381.00 kl	172.00 mn	181.66 g	11.60 jk	4.26 mn

		A_3	12.33 g	1.00 d	367.67 l	167.66 o	166.00 h	11.11 mn	4.22 no

The means not sharing the common letter differ significantly

Table 3. Effects of foliar-applied ascorbic acid on physiological attributes cultivars of maize grown under drought stress conditions

Treatments			**WP MPa)**	**OP(-MPa)**	**CT (°C)**	**SC (mmol m^{-2} s^{-1})**	**RWC (%)**	**MSI (%)**	**LCC (mg g^{-1})**	**PR (µmol m^{-2} s^{-1})**
Varieties	**DS**	**AA (mg L^{-1})**								
V1	D_0	A_0	0.88 l	0.30 ij	31.00 f	73.66 e	82.00 d	80.00 c	3.05 e	15.04 d
		A_1	0.83 mn	0.26 l	29.27 fg	76.66 b	85.00 b	83.00 b	3.55 c	16.33 c
		A_2	0.75 p	0.22 n	27.66 h	78.66 a	90.00 a	88.00 a	4.04 a	19.14 a
		A_3	0.77 o	0.24 m	28.33 g	77.33 b	88.00 b	86.00 a	3.75 b	17.00 b
	D_1	A_0	0.98 i	0.33 g	33.34 d	63.66 k	75.00 j	73.00 f	2.55 h	13.66 f
		A_1	0.96 j	0.31 hi	32.05 e	65.66 i	78.33 g	76.66 de	2.84 g	14.00 e
		A_2	0.85 mn	0.26 l	29.66 fg	71.66 f	80.00 e	78.00 cd	3.05 e	16.33
		A_3	0.92 k	0.28 jk	30.00 fg	66.66 h	78.00 h	76.00 de	3.05 e	15.00
	D_2	A_0	1.25 e	0.41 c	36.33 b	61.66 m	70.00 mn	71.00 fg	2.05 n	12.33 g
		A_1	1. 14 f	0.38 d	35.00 c	63.66 k	72.00 l	72.00 gh	2.25 k	14.00 e
		A_2	1.04 h	0.34 fg	33.66 d	66.66 h	77.33 i	75.00 e	2.54 h	16.33 c
		A_3	1. 09 g	0.36 a	34.00 cd	64.66 j	75.00 j	73.00 f	2.29 j	15.66 d
V2	D_0	A_0	0.92 k	0.32 gh	33.33 d	70.66 a	78.00 gh	76.00 de	2.84 g	11.14 h
		A_1	0.88 l	0.29 ij	32.66 e	71.66 f	79.00 f	76.66 de	2.95 f	12.09 g
		A_2	0.82 n	0.26 l	30.33 fg	74.66 d	80.00 e	78.00 cd	3.55 c	15.05 d
		A_3	0.85 m	0.28 kl	31.00 f	73.66 e	80.00 e	78.00 cd	3.15 d	13.33 f
	D_1	A_0	1.05 h	0.36 ef	36.00 b	62.66 m	70.33 m	68.33 hi	2.15 m	10.00 i
		A_1	1. 05 h	0.34 g	34.00 cd	62.66 l	72.33 l	70.33 gh	2.25 k	11.33 h
		A_2	0.96 ij	0.31 hi	32.66 e	65.66 i	73.33 k	71.33 fg	2.35 i	13.33 f
		A_3	1.03 h	0.32 gh	33.00 d	63.67 k	72.33 l	70.33 gh	2.29 j	12.19 g
	D_2	A_0	1.53 a	0.46 a	38.33 a	59.66 n	67.33 o	65.33 j	2.06 n	9.33 j
		A_1	1.46 b	0.43 b	36.00 b	61.66 m	79.33 n	67.33 hi	2.15 m	10.03 i

		A_2	1.35 d	0.38 d	34.00 cd	64.66 j	70.33 m	68.33 hi	2.25 k	12.00 g
		A_3	1.39 c	0.41 c	35.66 c	62.33 l	70.33 m	68.33 hi	2.22 l	11.00 h

The means not sharing the common letter differ significantly

Table 4. Effects of foliar applied ascorbic acid on biochemical attributes of maize cultivars grown under drought stress conditions

Treatments			LN (mmol g^{-1} dwt^{-1})	LP (m mol g^{-1} dwt^{-1})	LK (m mol g^{-1} dwt^{-1})	SOD (µmol mg^{-1}protein)	POD(µmol mg^{-1}protein)	CAT (µmol mg^{-1}protein)	PC (%)
Varieties	DS	AA(mg L^{-1})							
V1	D_0	A_0	14.00 d	3.18 f	3.11 e	180.33 e	10.99 b	120.00 d	10.95 f
		A_1	16.00 b	3.58 c	3.53 c	185.66 de	11.33 ab	125.66 d	11.35 c
		A_2	18.33 a	4.01 a	3.92 a	190.33 cd	11.83 a	139.66 c	11.85 a
		A_3	16.55 ab	3.70 b	3.62 b	188.66 d	11.55 a	130.45 c	11.55 b
	D_1	A_0	12.66 ef	2.82 i	2.76 h	233.00 ab	10.70 b	150.00 b	10.88 g
		A_1	13.00 e	2.94 h	2.88 g	255.33 ab	10.95 b	157.00 b	10.95 f
		A_2	14.33 d	3.05 g	2.99 f	270.33 a	11.12 ab	165.00 a	11.15 d
		A_3	13.17 e	3.01 g	2.94 fg	250.66 ab	10.98 b	160.33 a	11.00 ef
	D_2	A_0	9.14 h	2.23 n	2.15 mn	201.66 c	10.33 c	90.00 f	10.05 m
		A_1	11.22 g	2.35 m	2.27 l	205.00 bc	10.11 c	102.00 e	10.15 l
		A_2	13.33 e	2.94 k	2.48 g	220.33 b	10.25 c	106.00 e	10.30 jk
		A_3	12.25 ef	2.40 lm	2.34 k	210.25 bc	10.23 c	105.66 e	10.25 k
V2	D_0	A_0	11.25 g	3.02 g	2.96 f	150.66h	10.50 b	118.00 de	10.55 i
		A_1	12.33 ef	3.24 e	3.16 e	160.00 g	10.55 b	120.25 d	10.60 i
		A_2	13.25 e	3.32 d	3.26 d	164.33 fg	10.77 b	125.45 d	10.75 h
		A_3	15.67 c	3.33 d	3.28 d	170.33 f	11.00 ab	125.33 d	11.05 e
	D_1	A_0	10.63 gh	2.24 n	2.18 m	201.25 c	10.00 c	135.33 c	10.05 m
		A_1	11.55 g	2.45 l	2.38 k	202.00 c	10.20 c	140.23 c	10.60 i
		A_2	13.25 e	2.65 j	2.60 i	208.33 bc	10.33 c	148.33 bc	10.35 j
		A_3	13.25 e	2.55 k	2.48 j	205.33 bc	10.33 c	142.25 bc	10.35 j
	D_2	A_0	7.00 i	2.02 p	1.95 p	190.99 cd	9.00 f	82.23 g	9.05 p

		A_1	9.00 h	2.13 o	2.05 o	195.25 cd	9.50 df	90.00 f	9.55 o
		A_2	12.00 f	2.23 n	2.16 mn	207.25 bc	10.00 cd	105.00 e	10.05 m
		A_3	9.33 h	2.18 o	2.10 no	202.33 c	9.72 d	96.33 f	9.75 n

The means not sharing the common letter differ significantly

Regarding canopy temperature (CT), minimum canopy temperature (27.66) were recorded under no stress conditions and applying foliar spray of ascorbic acid @1.5 mM (38.33), (Table 3).

Table 3depicted that maximum stomatal conductance (SC) and relative water content (RWC) were recorded under no drought conditions and applying ascorbic acid as foliar spray @ 1 mM. (78.66 mmol $m^{-2}s^{-1}$and 90.0 %, respectively). Contrastingly, minimum stomatal conductance and relative water content were noted under stress conditions at the reproductive stage and without applying ascorbic acid (59.66 and mmol $m^{-2}s^{-1}$and 67.33%, respectively). Data regarding membrane stability index(MSI), chlorophyll contents of leaf (LCC) and photosynthetic rate (PR) showed that maximum membrane stability index, leaf chlorophyll content and photosynthetic rate were achieved no drought conditions and applying ascorbic acid as foliar spray @ 1 mM. (88.00%, 4.04 mg g^{-1} and 19.14µmol m^{-2} s^{-1}, respectively). However, minimum membrane stability index, leaf chlorophyll content and photosynthetic rate were obtained under stress conditions at the reproductive stage and without applying ascorbic acid (65.33%, 2.06 mg g^{-1} and 9.33 respectively).

Biochemical attributes

Data regarding biochemical attributes i.e. leaf nitrogen (LN), leaf phosphorous (LP), leaf potassium contents (LK), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and protein contents (PC). Table 4 showed that all the biochemical attributes were affected significantly by applying foliar spray of ascorbic acid under drought stress conditions. Significantly maximum leaf nitrogen, leaf phosphorous and leaf potassium contents were recorded under no drought conditions and applying ascorbic acid as foliar spray @ 1 mM. (18.33 mmol $g^{-1}dwt^{-1}$, 4.01 mmol $g^{-1}dwt^{-1}$ and 3.92 mmol $g^{-1}dwt^{-1}$, respectively). However, minimum leaf nitrogen, leaf phosphorous and leaf potassium contents were obtained under stress conditions at the reproductive stage and without applying ascorbic acid (7.00 mmol $g^{-1}dwt^{-1}$, 2.02 mmol $g^{-1}dwt^{-1}$ and 1.95 mmol $g^{-1}dwt^{-1}$, respectively). Data regarding superoxide dismutase, peroxidase, catalase and protein contents are presented in Table 4, which depicted that maximum superoxide dismutase, peroxidase, catalase and protein contents were recorded under no drought conditions and applying ascorbic acid as foliar spray @ 1 mM. (190.33 µmol mg^{-1}protein, 11.83 µmol mg^{-1}protein, 139.66 µmol mg^{-1}protein and 6.85%, respectively). However minimum superoxide dismutase, peroxidase, catalase and protein contents were obtained under stress conditions at the reproductive stage and without applying ascorbic acid (µmol mg^{-1}protein, 9.00 µmol mg^{-1}protein, 82.23 µmol mg^{-1}protein and 4.05%, respectively).

Discussion

Drought caused a marked reduction in maize biomass and final yield due to reduction in stomatal conductance, chlorophyll concentrations, gas exchange attributes; net photosynthesis, and transpiration rates of plants (Jabeen *et al.,* 2008; Elhafid *et al.,* 1998; Shah and Paulsen 2003; Flexas *et al.,* 2004; Ali and Ashraf 2011). The reduction in chlorophyll contents might be due its degradation and photo-oxidation under moisture

deficit (Anjum *et al.,* 2011; Farooq *et al.,* 2013; Hassan *et al.,* 2013). Chlorophyll degradation occurred because of chlorophyllase activities increased under drought (Mihailovic *et al.,* 1997).

Studies showed that ascorbic acid application improved maize growth under abiotic stresses. Because AsA enhanced cell division, maintained water level and modulated the plants' cell cycle (Tuna *et al.,* 2013; Athar *et al.,* 2009; De Pinto *et al.,* 1999). Exogenously applied AsA at lower concentration increased the contents of photosynthetic pigments, elevated the photosynthetic activity and ameliorated the negative effects of reactive oxygen species produced under moisture deficit situation (Noman *et al*., 2015; Malik and Ashraf 2012). ROS caused degradation of photosynthetic pigments, lipid peroxidation, obstruction of cell division/expansion and protein denaturation (Sairam and Saxena 2000; Anjum*et al.,* 2011; Arora *et al.,* 2002).

Ascorbic acid is a strong indispensable antioxidant that scavenges the adverse effects of ROS and free radicals, thus protected the photosynthetic pigments from degradation under drought (Ashraf 2009). The scavenging effect of AsA against ROS was prominent might be due to its anti-oxidant vitamin nature and its endogenous synthesis, translocation and accumulation in leaves. Foliar applied ascorbic acid increased the photosynthetic and transpiration rates, contributed to more biomass production and luxuriant growth in wheat and okra under water stress (Amin *et al.,* 2009).

The literature revealed that moderate drought reduced the SOD and POD activities in maize. Moreover, their activities were increased under severe stress. In addition, more production of these antioxidant enzymes, POD and SOD, occurred in maize with exogenous use of AsA under drought (Tuna *et al.,* 2013; Darvishan *et al.,* 2013). These effects were exactly the same to Reddy *et al.* (2004) described that AsA application contributed to more production of SOD/POD and ameliorated the adverse effects of free radicals ultimately resulted in affluent plant growth. Oxidative stress caused a significant reduction in protein contents of maize under water stress. However, protein contents were increased in maize hybrids by foliar feeding of ascorbate. This increment in protein contents was due to improved activities of SOD, catalases and POD caused by elevated levels of AsA in leaves (Rout & Shaw, 2001).

It was also reported that AsA application through foliar spray also stabilized the cell membrane, improved the MSI (membrane stability index), and increased the relative water contents in maize. Improvement in relative water contents in maize was due to fact that elevated levels of AsA in plants protected its membrane from degradation under stress (Ahmad *et al*., 2014).

Yield related characters including the length of cobs, per cob grains no., 100-grain weight of maize were improved by foliar application of AsA. Enhanced grain yield due to the exogenous application of AsA might be due to increased antioxidant activity and membrane stabilization that have assisted the maize crop to maintain normal metabolic processes under drought (Ahmad *et al.,* 2014).

Basra *et al.* (2006) documented that AsA performed better at its lower concentration compared to its higher concentration. In an experiment, priming of rice seeds was done in a solution of ascorbic acid at two different concentrations (10 and 20 ppm). Seedlings treated with a lower concentration of ascorbate (10 ppm) were superior in all the morphological traits. However, higher concentration of AsA reduced the germination percentage, seedling weights and shoot length which proved that AsA had stimulatory

influences at lower concentration and inhibitory influences at higher concentration (Basra *et al.*, 2006).

Conclusion

In conclusion, foliar application of Ascorbic Acid alleviated the adversities of moisture deficits at growth stages of maize by stabilizing membranes, increasing the actions of POD and SOD, catalase enzymes and, by improving the growth. These changes ultimately resulted in enhanced maize productivity and yield under drought. Foliar application of AsA at a lower concentration (1 mM) is the most acceptable, effective, economical and eco-friendly treatment as it has shown stimulatory effects in growth enhancement of maize under drought.

Acknowledgments

Authors are grateful to the authority of University of Agriculture, Faisalabad, Pakistan for laboratory facilities.

Disclosure statement

There is no conflict of interest.

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Citation

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