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Ascorbic Acid (Asa) improves Salinity Tolerance in Wheat (Triticum Aestivum L.) by Modulating Growth and Physiological Attributes

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Cover Page Footnote

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ASCORBIC ACID (AsA) IMPROVES SALINITY TOLERANCE IN WHEAT (*TRITICUM AESTIVUM* L.) BY MODULATING GROWTH AND PHYSIOLOGICAL ATTRIBUTES

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ABSTRACT

Wheat (*Triticum aestivum* L.) is one of the most important staple foods. High concentration of sodium chloride severely affects plants in general and wheat in specific. In this study the ameliorative effects of ascorbic acid (AsA) against sodium chloride stress were investigated in two commercial wheat cultivars (Galaxy 2013 and Akbar 2019). Experiments were conducted in three replicates. Two levels of salt (0, 150 mM) along with exogenous application of ascorbic acid (0, 30 mM, 60 mM) were applied at three leaves seedling stage. At the establishment of treatments, data regarding physiological, biochemical and yield attributes were recorded and subjected to statistical analysis. The application of AsA significantly ($p \leq 0.05$) improved growth, yield and key physiological attributes in tested wheat varieties under salinity stress. Overall wheat genotype Akbar 2019 showed better growth under salt stress. It is concluded from this study that AsA may be used to mitigate salinity effects in wheat.

Keywords: Osmoprotectants, reactive oxygen species (ROS), salinity, foliar applications.

INTRODUCTION

The increasing population of the world is becoming a threat for food sources in the world (Clair and Lynch, 2010). Wheat (*T. aestivum*) is a main grain cereal consumed worldwide and is a staple food for more than 50% of world population (Mahmood et al., 2020). The production of wheat is decreasing globally due to environmental hazards. Pakistan is at 4th position in Asia and 11th in the world for wheat production (Ahmad et al., 2017). In Pakistan the worst threat for wheat production is drought and salinity (Azooz et al., 2012).

Abiotic stresses like salinity cause reduction in crop yield by causing stomatal closure as a result of which carbon dioxide fixing process slows down and reactive oxygen species are produced. Reactive oxygen species eradicate lipids and proteins of cell and ultimately the cell is killed. Foliarly applied AsA curtails the salinity induce damages in plants (Hasanuzzaman et al., 2013). Plants growing in salt stress also face water deficit, oxidative stress and ionic imbalance.

The AsA is recommended for the improvement of growth because of its effective role in mitigating the negative salinity effects on germination and growth related attributes in plants (Gul et al., 2015). It is an influential antioxidant which eradicates the salinity induced reactive oxygen species (ROS). AsA improves the photosynthetic activity of plants and its application alleviates oxidative stress in plant facing salinity and improves growth.

It has been reported that foliar spray of AsA improves the photosynthetic rate in plants under saline conditions. It was also shown that foliar spray of AsA initiates a rise in stomatal conductance in

combination of induction in intercellular carbon dioxide concentration in plants under saline condition. This is because AsA significantly up-regulates stomatal conductance (Chen and Gallie, 2004). When the stomatal conductivity and the rate of transpiration under salt stress change, then plant water status is also changed. In a previous study it has been reported that glycinebetaine application adjusted plant water status under saline condition to improve stomal conduction and thus higher CO_2 fixation (Raza et al., 2006). The endogenous level of AsA is improved by its foliar spray and plays a key role in regulating stomatal activity and photosynthesis rate.

Foliar applications of AsA caused induction in leaf potassium ions and reduction in K^+/Na^+ ratio in roots and Na^+ level also decreased in shoot under salt stress while the level of Ca^{2+} was increased (Al- Hakimi and Hamada, 2001). The increased level of calcium in cytosol improves enzymatic antioxidants activity (Agarwal et al., 2005) and expression of osmotic response related genes (Pardo et al., 1998). The AsA induced changes in ions may activate the antioxidant system which helps to maintain the ion homeostasis (Mittler, 2002). In sensitive wheat cultivars large amount of proline assembles and is associated with high Na^+/K^+ and Na^+/Ca^{2+} ratios. Proline also works as a ROS killer and minimize oxidative stress promoted by osmotic stress (Hong et al., 2000; Matysik et al., 2002; Cuin and Shabala, 2007; Kaul et al., 2008; Szekely et al., 2008). For stabilizing the reactive oxygen species (ROS) balance in cell, proline metabolism is very essential. The balance between proline synthesis and degradation is a key element for detecting ROS status in the cell (Szabados and Savoure, 2010). There exists anonymity related to proline accumulation and saline stress tolerance relation and it is unclear even with use of various exogenously applied approaches (Ashraf and Foolad, 2007). Salinity increases total soluble protein contents in cell. The increase in protein concentration of different wheat genotypes was recorded under salinity stress (Afzal et al., 2008).

In view of such concerns, it is hypothesized that positive effect of AsA under salt stress in wheat improves growth by the interaction of antioxidants with reactive oxygen species and osmoprotectants like proline and total soluble proteins.

AsA participates in multiple plant functions. It acts as enzyme co-factor and participate in the α-tocopherol synthesis in plants and is considered as one of the major antioxidants in plants. Low AsA level increases sensitivity to salinity stress. In the present study investigations were made to understand whether AsA foliar applications improve salinity toxic effects by improving physiological attributes and adjusting ionic status in commercial wheat cultivars.

MATERIALS AND METHODS

Experiment Details and Seed Treatment

The experiment was conducted in November-February during the year 2019-2020. Seeds of wheat varieties, Galaxy 2013 and Akbar 2019 were collected from Ayub Agricultural Research Institute (AARI), Faisalabad. Glazed pots with diameters 18 cm bottom and 25 cm neck having 45 cm height were filled with 20 kg soil. All pots were placed in botanical garden of Govt. College University, Faisalabad and surface sterilized seeds were sown in these pots.

Seeds were sterilized using $HgCl_2$ solution (01%) and washed with distilled water thrice. Pots were arranged following completely randomized design (CRD) including factorial plane. After germination, uniform number of 05 seedlings were maintained in each pot by thinning. At three leaves stage, the plants were supplemented with 25 ml of 150 mM sodium chloride solution three times in a week. After two weeks of salinity

treatment, AsA (0, 30 and 60 mM) was foliarly applied at alternate days for one week. The treatments were arranged as T1 (Non-Saline), T2 (Non-Saline+ 30 mm AsA), T3 (Non-Saline+ 60 mm AsA), T4 (Saline 150 mm), T5 (Saline+ 30 mm AsA), T6 (Saline+ 60 mm AsA). After two weeks of AsA treatment, gas exchange attributes were studied and samples were taken for chlorophyll, osmoprotectants and ion determination under control and salinity condition.

Osmoprotectants

i. Proline

Bates et al. (1973) protocol was followed and free proline contents of leaf were determined spectrophotometrically.

ii. Total Soluble Proteins

Bradford (1976) method was followed for assessment of total soluble proteins in the wheat cultivars under study.

Leaf Chlorophyll Contents

For the determination of chlorophyll "a" and "b" Arnon (1949) method was followed. Fresh leaf material 01 g was grinded in 80% acetone solution, the volume was raised up to 3 ml. Absorbance was recorded at 645 and 663 nm using spectrophotometer (Hitachi-U-2001, Japan) for chlorophyll determination and final calculations were made with following formula:

$$Chl.a\left(\frac{mg}{g}\right) = \frac{V}{1000} \times W \times [12.7(OD\ 663) - 2.69(OD\ 645)]$$

$$Chl.b\left(\frac{mg}{g}\right) = \frac{V}{1000} \times W \times [22.9(OD\ 645) - 4.68\ (OD\ 663)]$$

W = gram weight of leaf tissue (fresh)

V= Volume (ml) of acetone used in extract

Mineral Nutrients

The 0.1 g dried, root and shoot samples were grinded and placed in a digestion flask followed by addition of 2 ml digestion mixture (H_2SO_4) in each flask. Flasks were kept at room temperature for one night and digestion was made at 150 ºC, then temperature of the hot plate was raised up to 250 ºC until the appearance of fumes. After cooling the flasks, 01 ml of H_2O_2 was added. The described procedure was repeated again and again until the flasks contents turned colorless. The final volume was made up to 50 ml. After filtering the extract, the pure extract was used for mineral ions analysis. Ions contents (K^+, Na^+ and Ca^{2+}) were determined using flame photometer (Jenway, PFP, UK).

Gas Exchange Attributes

Using infrared gas analyzer (ADC, Hoddeson, England), photosynthetic rate, CO_2 stomatal conductance, Gas exchange and water use efficiency (A/E) of newly developed young leaves was measured. The measurements were taken from 10:00 am to 1:00 pm using the following specifications: leaf chamber temperature varied from 25-28 ºC, leaf chamber volume gas flow rate (v) 296 ml/min, ambient CO_2 concentration (C_{ref}) 371 µmol/mol, leaf chamber molar gas flow rate (U) 403 µmol/s and ambient pressure (P) 97.95 kPa, PAR (Q_{leaf}) at leaf surface maximum up to 770 µmol/m2/s.

Yield Attributes

For yield attributes the samples were harvested at maturity and data were recorded for number of spikes per pot, spike length, number of grains and 1000 grain weight.

Statistical Analysis

The collected data were subjected to an analysis of variance (ANOVA) using the statistical software Co-Stat.

RESULTS

Osmoprotectants

Saline stress remarkably increased proline and total soluble proteins content of both wheat genotypes under study as compared to control condition (Table 01). The AsA foliar treatment exhibited significant ($p \leq 0.05$) results both under induced salinity and control conditions.

Leaf Chlorophyll Contents

Salinity caused a considerable ($p \leq 0.05$) decline in chlorophyll "a" and chlorophyll "b" of both wheat genotypes under study in comparison to non-saline stress condition (Table 01). The AsA foliar treatment significantly ($p \leq 0.05$) affected photosynthetic attributes both under saline and non-saline stress condition. Akbar 2019 showed better results than Galaxy 2013 regarding chlorophyll "a" while reverse is true for chlorophyll "b".

Mineral Nutrients

Salinity stress raised root and shoot sodium (Na^+) and caused significant ($p \leq 0.05$) decline in root and shoot Ca^+ and K^+ of both wheat cultivars (Table 2). The AsA foliar treatment significantly ($p \leq 0.05$) affected all except root Na^+ and root K^+ both under saline and non-saline stress condition. Overall, Akbar 2019 was observed better in ion homeostasis.

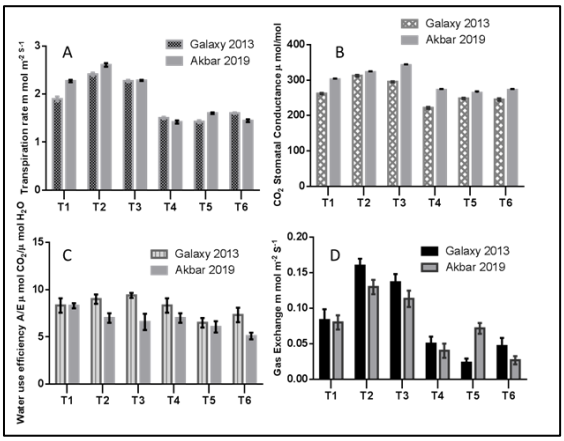

Figure 1: Effect of AsA on (A) transpiration rate, (B) CO2 stomatal conductance (C) water use efficiency A/E (D) gas exchange of two wheat genotypes grown under control and Salt (NaCl) stress conditions.

Gas Exchange Attributes

Saline stress induced a significant ($P \leq 0.05$) reduction in photosynthetic rate (A) and transpiration rate (E) of both wheat varieties (Table 3) under study as compared to non-saline stress condition (Fig.1). Salinity stress caused a substantial ($p \leq 0.05$) decrease in water use efficiency (A/E) of both wheat genotypes under salt stress as compared to control condition. The AsA applications significantly affected the rate of photosynthesis under NaCl stress in contrast to control (Fig.2).

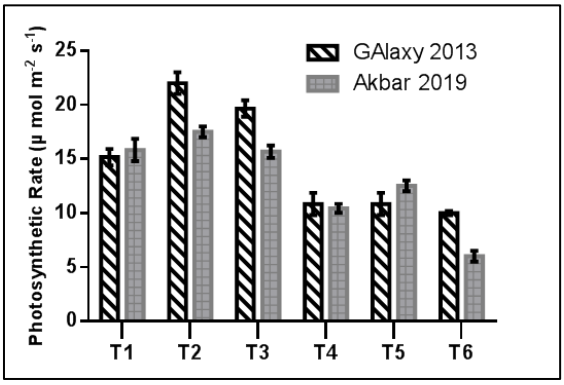

Figure 2: Effect of AsA on photosynthetic rate of two wheat genotypes grown under control and Salt (NaCl) stress conditions.

Table 1: Osmoprotectant and Leaf Chlorophyll Contents in two wheat genotypes grown under saline (150 mM) and nonsaline (Control) condition by exogenously applied AsA (30 mM, 60 mM). The values are expressed as means ± standard deviation of each attribute.

Verities	Treatments	Leaf chlorophyll contents		Osmoprotectants	
		Chl "a" (mg/g FW)	Chl "b" (mg/g FW)	Proline (µM/g FW)	TSP (mg/g FW)
Galaxy 2013	T1	33.93 ± 0.98	37.36 ± 1.39	0.75 ± 0.02	0.29 ± 0.00
	T2	35.66 ± 1.12	51.76 ± 1.94	0.90 ± 0.09	0.25 ± 0.07
	T3	35.17 ± 0.65	43.03 ± 1.84	0.57 ± 0.12	0.24 ± 0.04
	T4	25.35 ± 2.78	25.27 ± 3.40	0.31 ± 0.16	0.34 ± 0.01
	T5	28.55 ± 2.57	43.90 ± 1.55	0.66 ± 0.02	0.34 ± 0.01
	T6	29.10 ± 1.03	29.18 ± 2.59	0.35 ± 0.07	0.31 ± 0.02
Akbar 2019	T1	33.92 ± 1.91	37.60 ± 3.20	0.53 ± 0.09	0.31 ± 0.01
	T2	30.99 ± 1.28	43.48 ± 2.00	0.77 ± 0.02	0.24 ± 0.04
	T3	34.92 ± 1.25	51.24 ± 1.96	0.84 ± 0.05	0.32 ± 0.02
	T4	26.02 ± 1.29	30.53 ± 1.98	0.37 ± 0.07	0.38 ± 0.02
	T5	23.71 ± 1.84	34.39 ± 3.80	0.23 ± 0.05	0.29 ± 0.02
	T6	27.44 ± 1.43	39.13 ± 1.84	0.16 ± 0.015	0.37 ± 0.01

Table 2: Mineral Nutrients in mg/g dry weight (DW) of two wheat genotypes grown under saline (150 mM) and nonsaline (Control) condition under exogenously applied AsA (30 mM, 60 mM). The values are expressed as means ± standard deviation of each attribute.

Verities	Treatments	Mineral Nutrients					
		Shoot Na^+	Root Na^+	Shoot Ca^{2+}	Root Ca^{2+}	Shoot K^+	Root K^+
Galaxy 2013	T1	38.20±4.95	29.01±5.21	46.78±2.19	8.45±1.03	75.63±4.95	37.54±4.95
	T2	34.92±3.94	35.57±3.00	51.78±1.89	5.15±1.10	77.60±3.00	42.80±5.21
	T3	51.99±4.95	39.51±4.10	49.72±4.34	8.45±1.03	61.84±6.01	44.11±4.10
	T4	88.11±3.94	48.05±4.10	36.32±2.44	6.69±0.97	52.65±5.91	32.29±6.01
	T5	73.00±4.95	62.50±13.79	32.18±3.90	3.53±0.51	53.30±6.91	30.32±6.01
	T6	67.75±2.27	59.87±4.10	42.92±2.46	5.45±0	56.59±5.21	23.75±3.00
Akbar 2019	T1	50.02±4.10	40.17±4.95	63.45±3.70	5.18±0.46	75.63±4.10	45.42±4.95
	T2	44.77±1.97	36.23±3.00	51.72±3.20	6.45±0.91	74.97±6.01	44.11±4.10
	T3	44.11±4.95	45.42±9.30	49.85±2.74	8.67±0.62	76.29±5.21	48.05±4.10
	T4	73.00±4.95	57.90±13.40	45.25±3.73	2.38±1.17	60.53±3.94	22.44±3.00
	T5	76.29±3.94	55.27±8.20	41.05±3.34	3.85±1.03	54.62±7.10	36.23±4.95
	T6	68.41±3.94	57.24±3.00	36.12±3.12	6.65±1.03	45.42±4.10	32.95±7.10

Table 3: Analysis of variance (ANOVA) of data showing the changes in different studied attributes of two wheat genotypes, Galaxy 2013 and Akbar 2019 grown under saline (150 mM) and non-saline (Control) condition with exogenously applied AsA (30 mM, 60 mM).

Attribute		SS	DF	MS	F	*P* value
Transpiration Rate	Interaction	0.2938	5	0.05876	69.13	P < 0.0001****
	Treatments	6.206	5	1.241	1460	P < 0.0001****
	Variety	0.0676	1	0.0676	79.53	P < 0.0001****
	Residual	0.0204	24	0.00085		
CO2 Stomatal conductance	Interaction	2082	5	416.5	57.23	P < 0.0001****
	Treatments	29922	5	5984	822.3	P < 0.0001****
	Variety	9184	1	9184	1262	P < 0.0001****
	Residual	174.7	24	7.278		
A/E	Interaction	8.685	5	1.737	5.067	P = 0.0026**
	Treatments	25.66	5	5.133	14.97	P < 0.0001****
	Variety	19.51	1	19.51	56.91	P < 0.0001****
	Residual	8.227	24	0.3428		
Gas Exchange	Interaction	0.006070	5	0.001214	11.58	P < 0.0001****
	Treatments	0.06247	5	0.01249	119.1	P < 0.0001****
	Variety	0.0003674	1	0.0003674	3.503	P = 0.0735 ns
	Residual	0.002517	24	0.0001049		
Photosynthetic Rate	Interaction	55.53	5	11.11	19.88	P < 0.0001****
	Treatments	608.6	5	121.7	217.9	P < 0.0001****
	Variety	27.91	1	27.91	49.97	P < 0.0001****
	Residual	13.41	24	0.5586	19.88	P < 0.0001****
Spikes per pot	Interaction	15.92	5	3.183	7.163	P = 0.0003***
	Treatments	44.14	5	8.828	19.86	P < 0.0001****
	Variety	20.25	1	20.25	45.56	P < 0.0001****
	Residual	10.67	24	0.4444		
Spike Length (cm)	Interaction	0.7447	5	0.1489	1.117	P = 0.3778 ns
	Treatments	20.06	5	4.011	30.08	P < 0.0001****
	Variety	4.767	1	4.767	35.75	P < 0.0001****
	Residual	3.200	24	0.1333		
Number of Grains	Interaction	4651	5	930.1	79.91	P < 0.0001****
	Treatments	40280	5	8056	692.2	P < 0.0001****
	Variety	5575	1	5575	479.0	P < 0.0001****
	Residual	279.3	24	11.64		
1000 Grains Weight (g)	Interaction	5.156	5	1.031	1.631	P = 0.1900 ns
	Treatments	669.2	5	133.8	211.7	P < 0.0001****
	Variety	194.1	1	194.1	307.1	P < 0.0001****
	Residual	15.17	24	0.6322		

Yield Attributes

Saline stress considerably ($p \leq 0.05$) reduced the number of spikes and spike length of both wheat genotypes under NaCl induced salinity as compared to non-saline stress condition (Table 3). Salinity induce a significant ($p \leq 0.05$) decrease in number of grains and 1000 grain weight of both barley genotypes under salt stress as compared to non-saline stress condition. Overall, Akbar 2019 performed better under salt stress in contrast to non-saline condition (Fig.3).

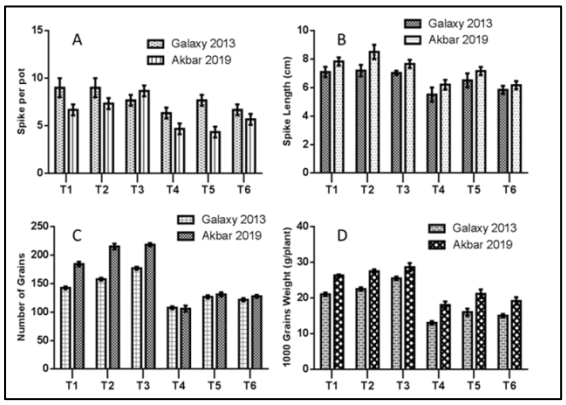

Figure 3: Effect of AsA on (A) spike per pot (B) spike length (C) number of grains (D) 1000 grains weight of two wheat genotypes grown under control and Salt (NaCl) stress conditions.

DISCUSSION

Proline content increased in tested wheat genotypes under saline stress. These results are in agreement with Rehman et al. (2021). Proline is considered as one of the important factor involved in osmoregulation (Yordanov et al., 2003; Hamdia, 2004). An increase in proline level under salt stress may be due to reduction in the rate of Calvin cycle which causes reduction in $NADP^+$ amount and thus resulted in high concentration of ROS, which altogether serve as stimulus against salt stress. So, proline biosynthesis is critical to maintain a low ration of $NADPH/NADP^+$ for ensuring tolerance in wheat plants. In current study salinity raised proline content in both wheat cultivars.

Under NaCl induced salinity, photosynthetic pigments (chlorophyll "a" and "b") were reduced in both wheat genotypes. The results of the present study are in agreement with (Mohsin et al., 2020) where mild and severe salt stress reduced the photosynthetic pigment in wheat. Yadav et al., (2020) conducted a study on pearl millet and wheat and reported the role of salicylic acid and thiourea in reducing the salinity effects. Salinity reduced chlorophyll content by 9.6 and 14.12% at EC_{iw} 8 and 12 dSm^{-1} in pearl millet and similar trend in chlorophyll reduction was observed in wheat. But both supplements increased the chlorophyll content under both stressed and non-stressed conditions, current study is in strong relation with (Yadav et al., 2020). High sodium chloride concentration increased chloroplast membrane damage and caused reduction in the chlorophyll content. Due to high thylakoid membrane sensitivity to NaCl, membrane destruction lead to seepage of chloroplast material. Chlorophyll degrading enzyme activity increased under salinity (Bahrani and Joo, 2012) and destabilizes the chloroplast structure and protein complex pigments facing instability. As a whole, more significant reduction in chlorophyll was seen in Galaxy 2013 as compared to Akbar 2019 genotype.

This destruction shows a significant positive correlation with sodium ions contents and Na+/K+ ratios and a negative correlation with plant survival (Hong et al., 2000; Khedr et al., 2003; Murakeozy et al., 2003; Taji et al., 2004; Hoque et al., 2007, 2008). The soluble protein was increased under salt stress in the present study as reported previously, the reason might be de novo protein synthesis under stress. These accumulated proteins were involved in n osmotic adjustment and may serve as nitrogen source when stress is over.

Salinity stress significantly ($P \leq 0.05$) reduced all yield attributes like the number of spikes per plant, spike

length, 1000 grain weight in both wheat genotypes. The applied salinity stress reduced the photosynthetic activity and photo-assimilates accumulation in grains which reduced the 1000 grain weight. Results of this study are in good agreement with study conducted on wheat under salinity by (Ahmed, 2009; Mensah and Ihenyen, 2009; and Khan et al., 2010). Although, AsA application has improved plant growth. In a study conducted by (Rehman et al., 2021) salinity reduced grain number, spike length, and spike number but glutathione (GSH) enhanced the grain yield and other attributes our study is in strong agreement with them. AsA play an important role in the stomatal regulation (Chen and Gallie, 2004). Salinity is associated with strong antioxidant system (Gossett et al., 1994; Gossett et al., 1996; Mittova et al., 2002; Bor et al., 2003).

CONCLUSION

It is concluded from the results of this study that AsA is key element to induce salinity tolerance in wheat. The AsA application significantly improves photosynthetic pigment, yield attributes and ion balance in the subjected wheat cultivars.

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