ALLEVIATING THE DELETERIOUS EFFECTS OF SALT STRESS ON WHEAT (*TRITICUM AESTIVUM* L.) BY FOLIAR APPLICATION OF GIBBERELLIC ACID AND SALICYLIC ACID

IQBAL, M. S.[1*] – ZAHOOR, M.[1] – AKBAR, M.[1] – AHMAD, K. S.[2] – HUSSAIN, S. A.[1] – MUNIR, S.[1] – ALI, M. A.[1] – ARSHAD, N.[1] – MASOOD, H.[1] – ZAFAR, S.[1] – AHMAD, T.[1] – SHAHEEN, N.[1] – MASHOOQ, R.[1] – SAJJAD, H.[1] – SHAHBAZ, K.[1] – ARSHAD, H.[1] – FATIMA, N.[1] – ANSAR, B.[1] – ISLAM, M.[3]

[1]*Biodiversity Informatics, Genomics and Post-Harvest Biology Lab, Department of Botany, University of Gujrat, Gujrat, Pakistan*

[2]*Department of Botany, University of Poonch, Rawalakot, 12350 Azad Jammu and Kashmir, Pakistan*

[3]*Department of Biotechnology and Genetic Engineering, Hazara University, Mansehra, Pakistan*

[*]*Corresponding author*
e-mail: drsajjad.iqbal@uog.edu.pk; ORCID: https://orcid.org/0000-0002-7728-7922

(Received 31st May 2021; accepted 4th Sep 2021)

Abstract. Plant growth regulators gibberellic acid (GA_3) and salicylic acid (SA) were applied in the form of foliar spray to two varieties of wheat viz., Anaj-2017 and Ujala-2016 to alleviate the deleterious effects of soil salinity. Salt was applied at the concentration of 150mM after 2 weeks of seed germination. Ten treatments including control were used; T0 (control), T1 (150 mM NaCl), T2 (0.5 mM SA), T3 (1.0 mM SA), T4 (100 mg/L GA_3), T5 (150 mg/L GA_3), T6 (150 mM NaCl+0.5 mM SA), T7 (150 mM NaCl+1.0 mM SA), T8 (150 mM NaCl+100 mg/L GA_3), T9 (150 mM NaCl+150 mg/L GA_3). GA_3 and SA were applied after one week of salinity stress and repeated thrice. Morphophysiological, biochemical, and yield parameters were recorded. Findings revealed that both growth regulators promote the growth of plants treated with salt stress. Anthocyanin was promoted by 0.0035% at 100 mg/L GA_3 while glycine betaine was also enhanced by 0.26% in Ujala-2016 at 150 mg/L. It was noted that 1.0 mM salicylic acid and 150 mg/L gibberellic acid enhanced significantly various growth parameters. In conclusion, concentration of 0.1 mM SA and 150 mg/L GA_3 along variety Ujala-2016 recommended for the alleviation of salt stress with better growth and yield for future cultivation.
Keywords: *wheat, salinity, plant growth regulators, physiological, biochemical attributes*

Introduction

Wheat is an annual cereal crop cultivated throughout the world in temperate regions. It belongs to family Poaceae while 40% of the world population consumed it as staple food (Salim and Raza, 2020). It is the major source of calories (21%) and proteins due to it 4.5 billion people depend on it (Nawaz et al., 2013; Saddiq et al., 2021). It is predicted that production increase up to 2% annually till 2050 would be required to meet demands of rapidly growing population (Braun et al., 2010). Further, wheat is the major part of diet consumed regularly that have an impact nutritionally for public health. It has an ability to produce high yield and to tolerate a wide range of conditions. The most prominent factor is the ability of gluten protein of wheat to form viscoelastic dough, which is required particularly to bake leavened bread (Braun et al., 2010).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2001_119134

Salinity stress is the major deleterious factor that limits crop germination, ultimately productivity. Most of the crop plants are vulnerable to salinity because of presence of higher quantity of salts in the soil (Dadshani et al., 2019). One-third of food production of the world is produced by approximately 20% of irrigated land that is under salt stress (Shrivastava and Kumar, 2015). The reactive oxygen species (ROS) under these stress conditions accumulated in leaves, resulted in oxidation of components of cells i.e. proteins, lipids, and chlorophyll. It can cause severe damages to plants, these undesirable ROS reacts with organic molecules triggering the peroxidation of membrane lipid, oxidation of proteins, inhibition of enzymes, RNA and DNA damage (Ahmad, 2010).

Plant growth regulators are organic substances which can hamper alternation in the cellular metabolism of plants. Environmental factors can badly affect the synthesis of plant growth regulators, in return disturbance in plant physiological processes, consequently, inhibit their growth potential. Use of such growth hormones in low concentration can control the growth and development of plants, either by inhibition or by promotion, and allow the physiological processes to take place at their usual rate. Usually these growth regulators or growth substances play their respective role in the control of plant growth and development, growing under stress or normal conditions (Hadia et al., 2020). Gibberellic acid (GA_3) is a growth regulator of plants; regulates various physiological responses that are most beneficial for better germination, photosynthetic activity, and growth of the plant (Sudharmaidevi et al., 2017; Rout et al., 2017). GA_3 stimulates the effective ion uptake in plants resulting in growth enhancement and maintaining the plant metabolism under both normal and stress conditions. The application of auxin also stimulates gibberellin synthesis. Further, GA_3 involved in mineral nutrition and nitrogen metabolism directly (Iqbal and Ashraf, 2013). Similarly, salicylic acid (SA) is the phenolic compound and an important endogenous growth hormone of plants. It plays great role in controlling different biochemical and physiological processes i.e. nitrogen metabolism, growth, flowering, and production of ethylene (Hayat et al., 2010). It has been found that salicylic acid is the main endogenous signal involved in defense responses of plants against environmental stresses including low temperature, pathogen infection, salinity, and ozone (Sawada et al., 2008). Moreover, treatment of SA is supplemented by a temporary increase in water level (Wahid et al., 2007). Considering this, an experiment was performed to find the efficacy of GA_3 and SA for better growth and yield by evaluating various morphophysiological, biochemical attributes, and antioxidant activities to suggest the most suitable variety for future cultivation under salinity hit areas.

Materials and methods

Experimental setup

A pot experiment was conducted at the experimental site of the Department of Botany, University of Gujrat, Gujrat, Pakistan during 2019-2020. During summer the climate of the area is hot and humid and cold in winter. Hottest months include May, June and July. Temperature may range during winter as drop to -2°C and maximum ranged from 40 to 50°C. basically the area is agriculture with plain and fertile land. Mild to warm weather can be observed with occasionally heavy rainfall occurs during winter from mid-November to March. The average annual rainfall is 1000 mm (Iqbal et al., 2021).

Two known wheat cultivars viz., Anaj-2017 and Ujala-2016 were selected but there was no report available against salinity tolerance. Seeds were purchased from Punjab

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2001_119134

Seed Corporation distribution shop from Gujrat city. The earthen pots were of 12-inch width ×11 inch tall. The experiment was arranged in a completely randomized design (CRD) with three replicates. Ten treatments were applied through foliar spray after 14 days of germination (*Table 1*). Data were recorded after 21 days of treatments for morphological and biochemical parameters like shoot length, root length, number of leaves, leaf area, chlorophyll contents, antioxidant activities, carbohydrates, and protein contents.

Table 1. *Detail of the treatments applied on two wheat cultivars with various combinations*

No.	Treatment composition
T0	Control
T1	150mM Sodium chloride (NaCl)
T2	0.5mM Salicylic acid (SA)
T3	1.0mM Salicylic acid (SA)
T4	100mg/L Gibberellic acid (GA3)
T5	150mg/L Gibberellic acid (GA3)
T6	150mM Sodium chloride+ 0.5mM Salicylic acid
T7	150mM Sodium chloride (NaCl) + 1.0mM Salicylic acid (SA)
T8	150mM Sodium chloride (NaCl) + 100mg/L Gibberellic acid (GA3)
T9	150mM sodium chloride (NaCl) +150mg/L Gibberellic acid (GA3)

Pots were filled with sand and then salt treatment was provided. Later, ten seeds were sown in every pot. Plants were covered with plastic sheets to prevent environmental impact and to maintain conditions uniform. Hoagland solution was applied to plants after the one-week interval to fulfill their nutritional needs. The concentration of 500ml Hoagland solution was applied to each pot.

Germination percentage, determination of growth and biomass

After one week of seed germination, the germination percentage was recorded by counting the number of seeds germinated in each pot. Five randomly healthy plants were selected for recording of the data. Roots and shoots were washed with water to clean the sand and weighted in grams (g) by using electric balance (Shimadzu Koyoto, Japan. Model: ELB600). Dry root and shoot biomass were recorded after oven drying at 65°C for three days. Likewise, fresh root and shoot length were recorded in centimeters (cm). The number of leaves and plant flag leaves were measured manually at vegetative stage (*Fig. 1*). Leaf area was measured by following formula; Length × Breadth × 0.75, where 0.75 was the correction factor.

Gas exchange parameters

Gas exchange parameters such as the rate of photosynthesis, CO_2 concentration in the cell, rate of transpiration, degree of stomatal opening, and water use efficiency were measured by using an infrared gas analyzer (IRGA) (Serial No: 32641, Model: LCi Console (ADC) Bioscientific Ltd was used). The estimation was carried out during the time 9:00-11:00 A.M under conditions of saturating light intensity, air relative humidity, leaf temperature, and CO_2 concentration.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2001_119134

Anaj-2017 **Ujala-2016**

Figure 1. *Pot evaluation of both wheat varieties Anaj-2017 and Ujala-2016*

Estimation of photosynthetic pigments

Photosynthetic pigments were measured by following the method of Arnon (1949). Fresh leaves were collected from various treatments. Leaf sample (0.5 g) obtained from one replication was crushed in 4 ml of 80 % acetone. The samples were then filtered and the final volume rose to 10 ml by adding acetone. The supernatant was recovered by vortex and then the absorbance was taken by using Hitachi Spectrophotometer (Hitachi, Model U2001, Tokyo, Japan). Absorbance was measured at 3 wavelengths for chlorophyll a, b, c and carotenoids by the following formula:

Chlorophyll 'a' (mg/g fwt) = [12.7(OD 663) – 2.69 (OD 645)] × V/ 1000 × W

Chlorophyll 'b' (mg/g fwt) = [22.9 (OD645 – 4.68(OD 663) × V/1000 × W

Carotenoids (mg/g fwt) = [7.6(OD 480) – 1.49(OD 510)] × V/1000 × W

W = Fresh leaves weight in grams (g), V = Volume of extract of leaves in milliliter (ml)

Determination of glycine betaine and malondialdehyde (MDA)

Glycine betaine concentration was estimated in flag leaves (Grieve and Grattan, 1983). Leaves (0.5 g) were grinded in 5 ml water toluene solution. After that, the extract was transferred to a test tube and shakes for 24 hours at room temperature through shaker. Sample solution was then filtered. Potassium iodide 0.1 ml and 1 ml of the HCl solution were added into 0.5 ml filtrate and shake for 90 minutes in an ice bath. Followed by addition of super cool H_2O (2 ml). 1, 2-dichloromethane in the quantity of 10 ml was added and kept at room temperature for 2 hrs. Two prominent layers were established. The top layer was removed and discarded and the lower layer was used to measure the absorbance at 360 nm by using a spectrophotometer (PG Instruments LTD, UK. Model: T80 UV/VIS Spectrophotometer UV3000).

Malondialdehyde contents were measured by following Cakmak and Horst (1991). One gram of fresh leaves was crushed in 1% trichloroacetic acid (TCA) at 4 °C. The mixture obtained was then centrifuged at 2000 rpm for 15 minutes. Supernatant was

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2001_119134

recovered and TCA and TBA in the quantity of 3 mL were then added (0.5 by 20%). To prevent the reaction, the samples were kept in a water bath for about 90 minutes at 95 °C. Sample absorbance was measured at 532 nm and 600 nm by using the following formula;

Malondialdehyde (MDA) level = (A532 nm – A 600nm) / 1.56 × 105

Antioxidant enzyme assay

For determining the antioxidant potential, 0.5 g fresh leaves representing each treatment were grinded by the addition of 5ml phosphate buffer (cooled) and placed in an ice bath. Samples were centrifuged at 15000 rpm for 20 minutes at 4 °C. The supernatant was removed and used to study catalase and peroxidase dismutase activity (Chance and Meahly, 1995). For measuring the activity of CAT, solution was prepared with the final volume of 3 ml. In the reaction mixture, 50 mM phosphate buffer solution at neutral pH was added, 5.9 mM H_2O_2, and prepared enzyme extract in the quantity of 0.1 ml. The reaction started with the addition of enzyme extract. For measuring CAT activity, spectrophotometer was set on 240 nm and the change in absorbance was noted after every 20 seconds. For measuring of one-unit CAT activity change in absorbance 0.01 units/mint was considered.

To determine the POD activity, 3 ml solution was prepared that contained final volume. The solution was consisted of 50 mM phosphate buffer, 40 mM water, and 20 mM guaiacol. Enzyme extract of 0.1 mL was added to the solution simultaneously. POD was measured by spectrophotometer at the wavelength of 470 nm wavelength, absorbance change was observed after every 20 sec. Absorbance change 0.01 units/mint was observed to calculate the 1 unit of POD activity.

The superoxide dismutase activity was measured by following Giannopolitis and Ries (1977). Plant leaves (0.5 g) were crushed with 5 ml of phosphate buffer. An extraction sample in the quantity of 3 ml was transferred to the falcon tube. Then 50 μm of Nitro blue tetra-zolium (NBT) was added. Additionally, 13 μm of riboflavin, extract 20-50 ml of enzyme extract, 75 mM EDTA, 13 mM methionine, and phosphate buffer (pH 7.8) were added to the sample. After that, the samples were kept under the fluorescent lamp for about 15 minutes. Absorbance was measured using the spectrophotometer at 560 nm.

Estimation of protein and carbohydrates

To estimate the protein content 0.5 gram fresh leaves were crushed by adding 10 ml of 50 mM potassium phosphate buffer with 7.8 pH. Samples were then centrifuged at 10,000 rpm for 15 min at 4 °C (Bradford, 1996). Supernatant 0.1 ml was mixed with 2 ml of Bradford reagent. Protein content was determined at 590 nm absorbance by using a spectrophotometer.

For the preparation of plant extract (0.5 g), fresh leaves were crushed with 80% ethanol. After that, 10 ml water was added to the sample. For different sample concentrations, the final volume raised to 1 ml. Then H_2SO_4 (90% concentrated) was added in the quantity of 5 ml. Mixture shaking and incubation performed at a temperature of 30 °C for 40 mints. After incubation 1 ml of 5% solution of phenol was added to each test tube. Absorbance was measured at 490 nm by using a spectrophotometer.

Determination of anthocyanin and electrolyte leakage

Leaves 0.2 g was crushed with methanol and HCl at 99:1 ratio (methanol 9 ml: HCl 1 ml). This extract was then centrifuged at 18,000 rpm for 30 minutes at 4 °C. The

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2001_119134

supernatant was then separated and incubated at 4 °C for 24 hours in dark. Readings at two wavelengths viz., 530 nm and OD 657nm were recorded. The following formula was used to calculate the anthocyanin content (Krizek et al., 1993);

Content of Anthocyanin = [OD530-0.25 OD657] ×TV / [dry wt. ×1000]

OD = represents the optical density, TV = total extract volume (ml), Dry wt. = weight of the dry leave tissue.

For the determination of electrolyte leakage, the method of Lutts et al. (1995) was followed. Fresh flag leaves were taken from the plants. Leaves were washed with water and divided into different pieces through a blade or scissor. In Falcon tubes 50 ml double distilled water was added and leaves were dipped in the water. Then, tubes were incubated for 24 hours. After 24 hours EC was measured with the help of an electrolyte leakage meter. The value was named EC_1. Now the sample was autoclaved for 20 minutes at 121 °C and again electrolyte leakage was measured. This value was named EC_2. The final value was calculated with the help of the following formula; Electrolyte Leakage $=EC_1/EC_2 \times 100$.

Yield parameter determination

At physiological maturity following yield parameters were recorded on per plant basis (number of spikes, number of grains, spikelets per plant, and 100 grain weight).

Statistical analysis

Statistical analysis was performed by using Minitab software for determining significant and non-significant values through analysis of variance (ANOVA). For comparing mean values Tuckey's HSD (honestly significant difference) test was used along P values < 0.05 to differentiate one parameter from another by applying different alphabets. Different alphabets showed that this treatment is more significant than other treatment/s as compared to same alphabets on the other treatments.

Results

Germination percentage, growth and biomass of plants

The effect of the plant growth regulators on the germination percentage of wheat was found highly significant ($P \leq 0.001$) (*Fig. 2 A-J*), which showed the exogenous application of 1.0 mM of salicylic acid (SA) and 150 mg/L of gibberellic acid (GA_3) positively influenced the 90% germination percentage in Ujala-2016. Highly significant effects of GA_3 were found on root and shoot length, as well as fresh and dry weight of root and shoot. The fresh and dry weight of root and shoot decreased significantly at 150 mM NaCl stress and increased by the application of GA_3 and SA. The length of the root increased 22 cm by the application of 1.0 mM (SA). The fresh weight of plant root was also increased by 4.53 g in Ujala-2016 by the treatment of 150 mg/L GA_3 while dry root weight was increased 2 g in Ujala-2016 with the application of (150 mM NaCl + 150 mg/L GA_3). The highest number of leaves (30%) and leaf area were observed at a concentration of 150 mg/L GA_3 and 1. 0 mM SA (*Fig. 2 A-J*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2001_119134

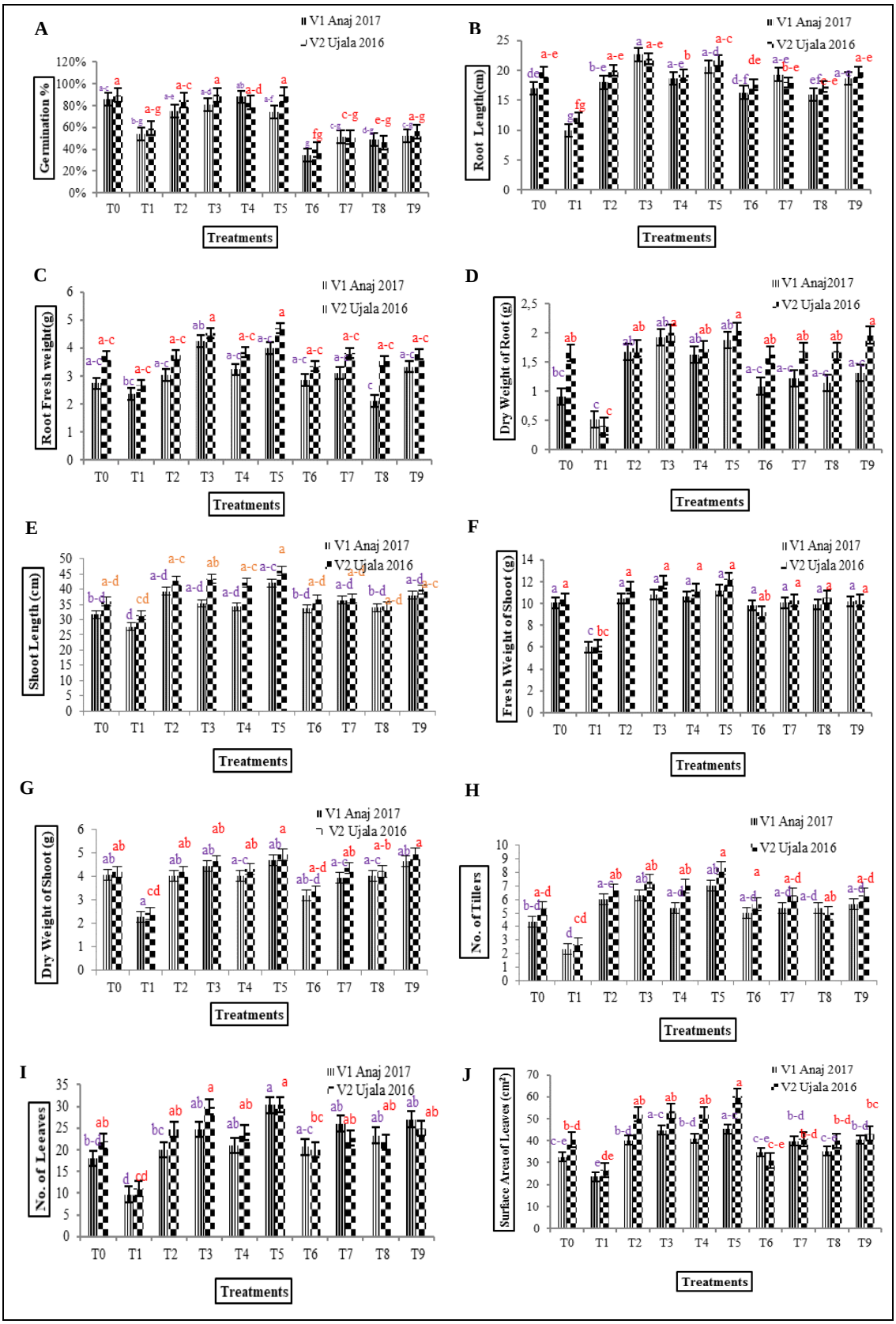

Figure 2. *(A-J): Effect of GA_3 and SA on germination, growth, and biomass production of wheat (Triticum aestivum L.) under NaCl stress (at 150 mM NaCl) and without stress. Values are the means of three replicates*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2001_119134

Gas exchange attributes

Photosynthesis is a significant character of the plant that is reduced considerably under NaCl stress. *Fig. 3 K-O* indicates a substantial decline in the photosynthetic rate of wheat that was 15.9 (µmol CO_2 $m^{-2}s^{-1}$); however, a prominent increase was noticed after the treatment of GA_3 and SA. The photosynthetic rate of wheat was increased by 30.4 (µmol CO_2 $m^{-2}s^{-1}$) by the application of (150 mM NaCl + 150mg/L GA_3) in Ujala-2016. The response of variety was non-significant. The response of varieties on the rate of transpiration of plants was significant, as well as the hormonal response was highly significant ($P \leq 0.001$). Foliar application of gibberellic acid and salicylic acid increases the transpiration rate 1.5 (mmol H_2O m^{-2} s^{-1}) and intercellular CO_2 concentration 206 (µmol CO_2 mol^{-1}) after the treatment of 100 mg/L GA_3 concentration in Ujala-2016. The varieties and hormonal response on the conductance of stomata were non-significant. Results indicate that the stomatal conductance was highest 0.2 (mol H_2O m^{-2} s^{-1}) at 150 mg/L GA_3 in Ujala-2016. The hormonal response was highly significant on the water use efficiency of wheat while the response of variety was non-significant. Water use efficiency was highest at concentration of 150 mM NaCl+ 100mg/L GA_3.

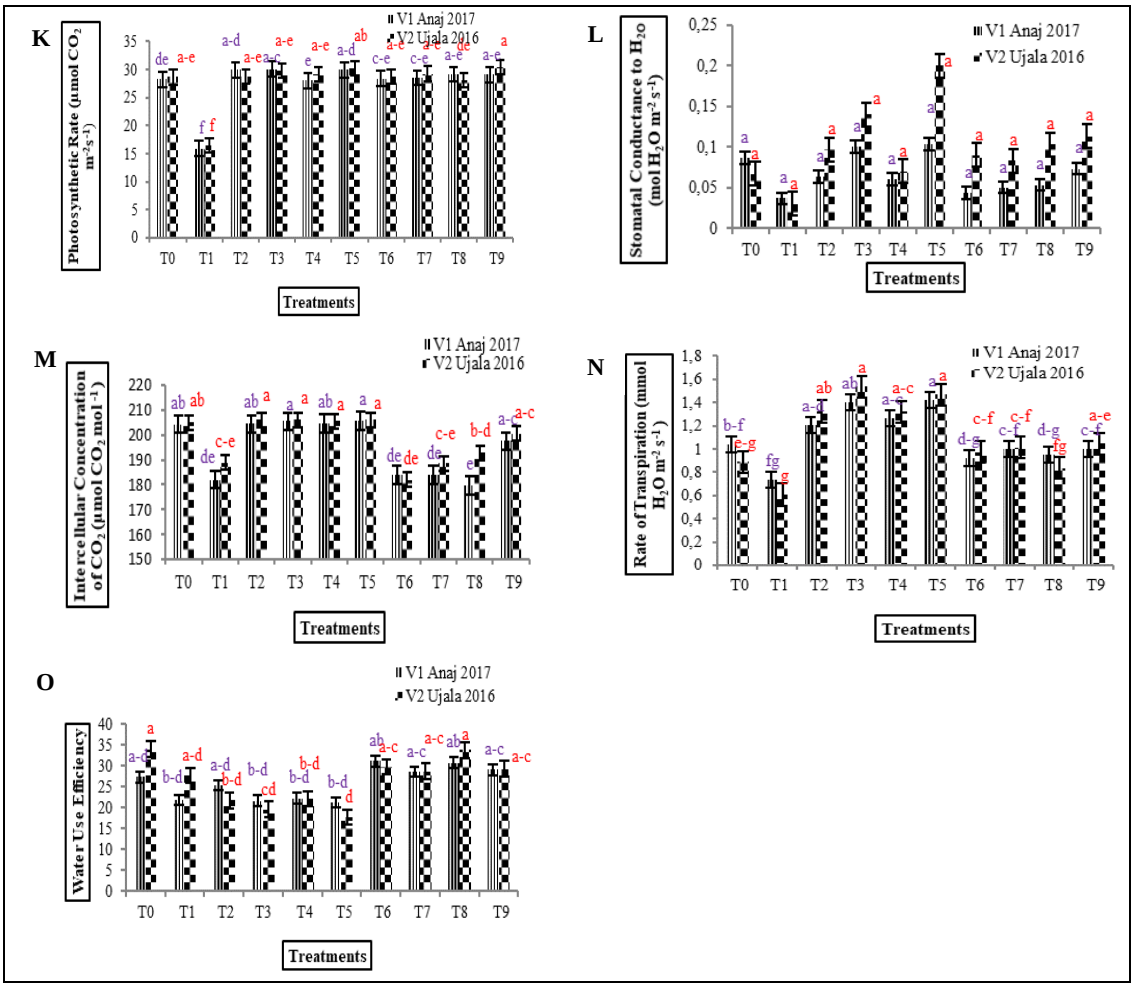

Figure 3. *(K-O): Effect of GA_3 and SA on gas exchange parameters (photosynthetic rate, stomatal conductance, transpiration rate, intercellular CO_2 concentration and water use efficiency) of wheat (Triticum aestivum L.) under NaCl stress (at 150mM NaCl) and without stress. Values are the means of three replicates ± standard error (SE)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2001_119134

Photosynthetic pigments

All photosynthetic pigments including; chlorophyll a, b, total chlorophyll, and carotenoids content were enhanced through the application of SA and GA_3. Highly significant ($P \leq 0.001$) effect of GA_3 and SA was noted on chlorophyll a. Content of chlorophyll b was highest 0.058 (mg/g fwt) at 1.0 mM SA concentration (*Fig. 4 P-R*).

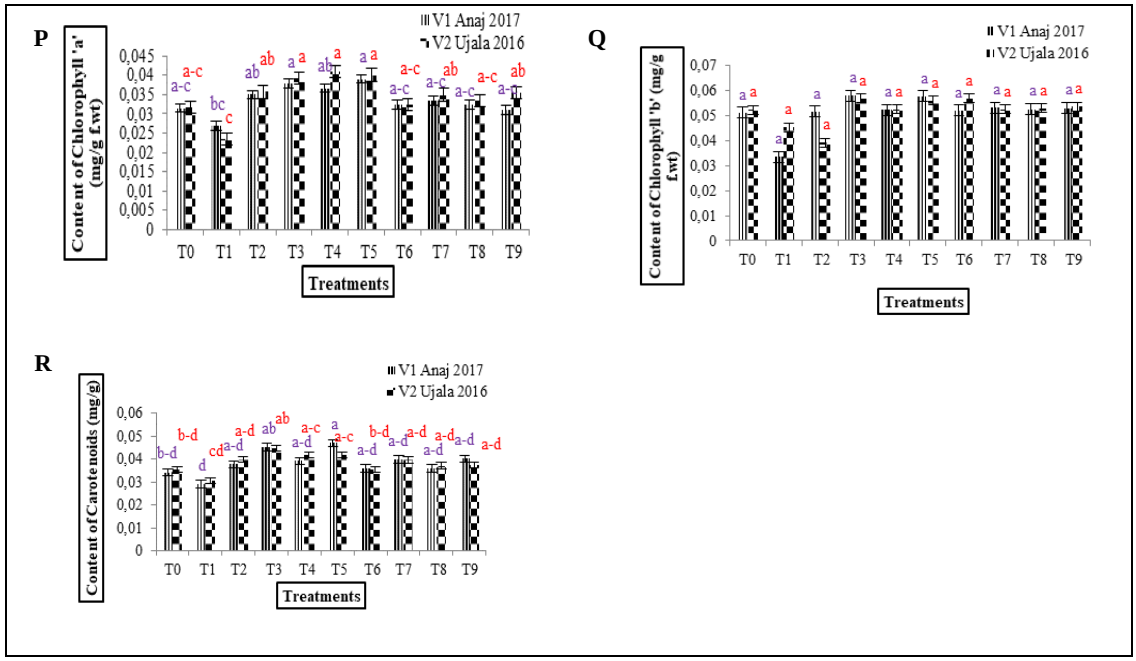

Figure 4. *(P-R): Effect of gibberellic acid GA_3 and SA on photosynthetic pigments (Chlorophyll a, chlorophyll b, and carotenoids) of wheat (Triticum aestivum L.) under NaCl stress (at 150 mM NaCl) and without stress. Values represent the means of three replicates ± standard error (SE)*

Glycine betaine, MDA, carbohydrates, protein, and electrolyte leakage

Highly significant ($P \leq 0.001$) response of SA and GA_3 was observed on all these parameters (*Fig. 5 S-X*). Glycine betaine content was highest 0.3 (µmol/g dry wt.) at 100 mg/L GA_3 and 150 mg/L GA_3 concentration among all treatments. By accumulation of NaCl into plant roots the value of MDA content become higher. On the basis of comparison in both varieties, content of Malondialdehyde was greater in Ujala-2016 than Anaj-2017 due to the accumulation of salt stress. Whereas use of both GA_3 and SA by foliar means demonstrated the positive outcome and reduced the level of Malondialdehyde content. The effect of both GA_3 and SA MDA content was highly significant. Likewise, carbohydrate content was also found highly significant. Plant growth and development is dependent on carbohydrates but under salinity stress it becomes hampered. Although both of the varieties showed reduction in carbohydrate content but Ujala-2016 suffered more as compared to Anaj-2017.

Normal healthy plants have large amount of proteins but under abiotic stress this quantity may suffer and reduction of soluble proteins occur. It was evident from current experiment that NaCl stress was the major cause of depletion of soluble proteins within wheat varieties. The interaction between hormones and varieties were non-significant. On

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2001_119134

the other hand, application of SA and GA_3 proved beneficial in order to enhance the content of total soluble protein in both NaCl affected and non-affected plants.

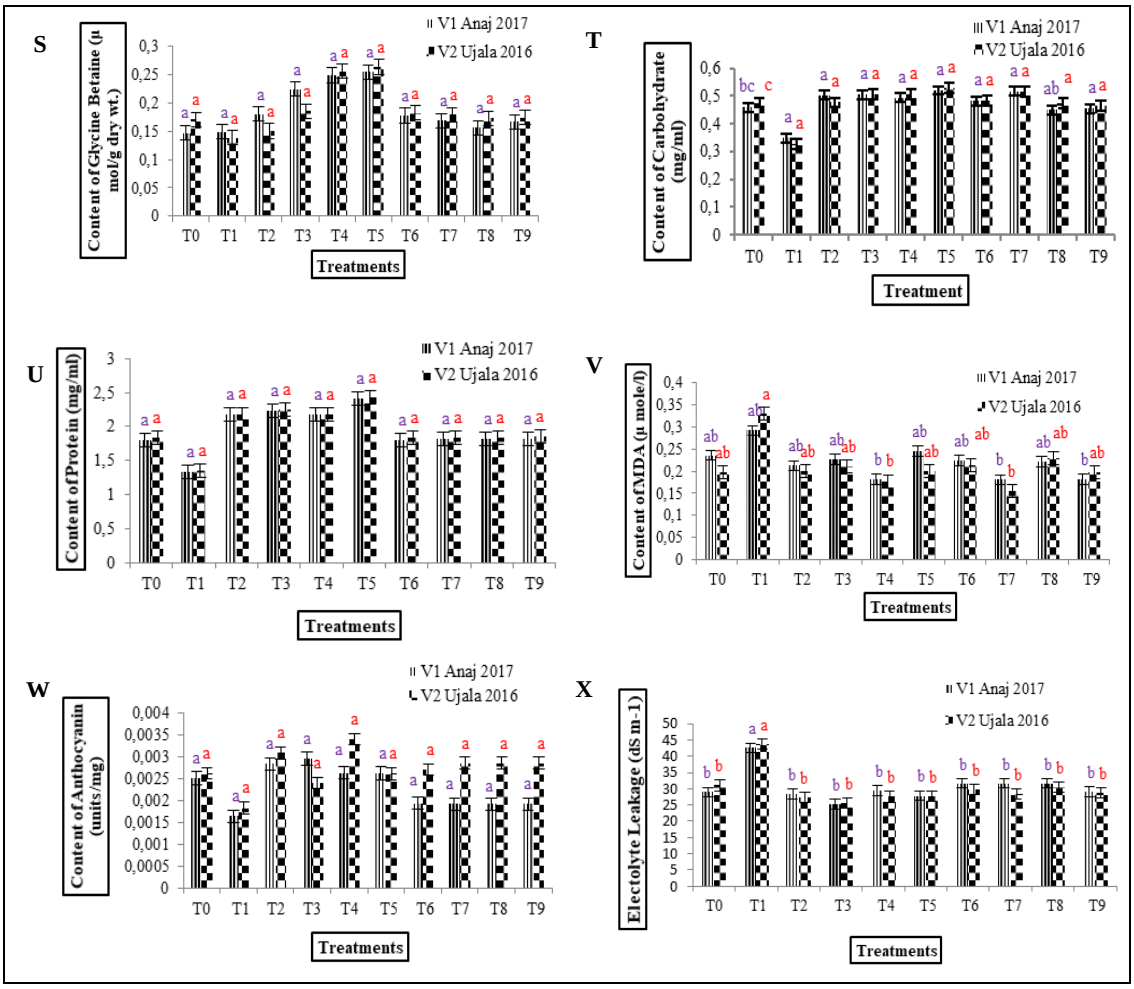

Figure 5. *(S-X): Effect of GA_3 and SA on different biochemical parameters (glycine betaine, carbohydrates, protein, MDA, anthocyanin and electrolyte leakage) of wheat (Triticum aestivum L.) under NaCl stress (at 150 mM NaCl) and without stress. Values represent the means of three replicates ± standard error (SE)*

When plants were subjected to salinity stress then higher level of electrolyte leakage was observed within plant leaves. Ujala-2016 showed higher level of electrolyte leakage than Anaj-2017. The influence of both GA_3 and SA on electrolyte leakage was found highly significant. But by treating NaCl affected plants through GA_3 and SA spray it was noted that the level of electrolyte leakage decreased and reached nearly to control conditions.

Antioxidant activities of enzymes

It was observed that NaCl enhanced antioxidant activities of enzymes including SOD and POD while CAT activity was reduced. The highest SOD activity 0.42 (units/mg protein) was observed at 150 mM NaCl in Ujala-2016 (V_2) when the concentration of NaCl was increased. The activity of POD was also enhanced at the highest concentration

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2001_119134

of NaCl (150 mM NaCl), while minimum POD activity was observed at 0.5 mM concentration of SA. On the other hand, CAT activity was highest 0.3 (units/mg protein) at 150 mg/L GA_3 and is reduced with the rise in the concentration of NaCl as indicated. The hormonal response was significant but the response of varieties was non-significant (*Fig. 6 A1-C1*).

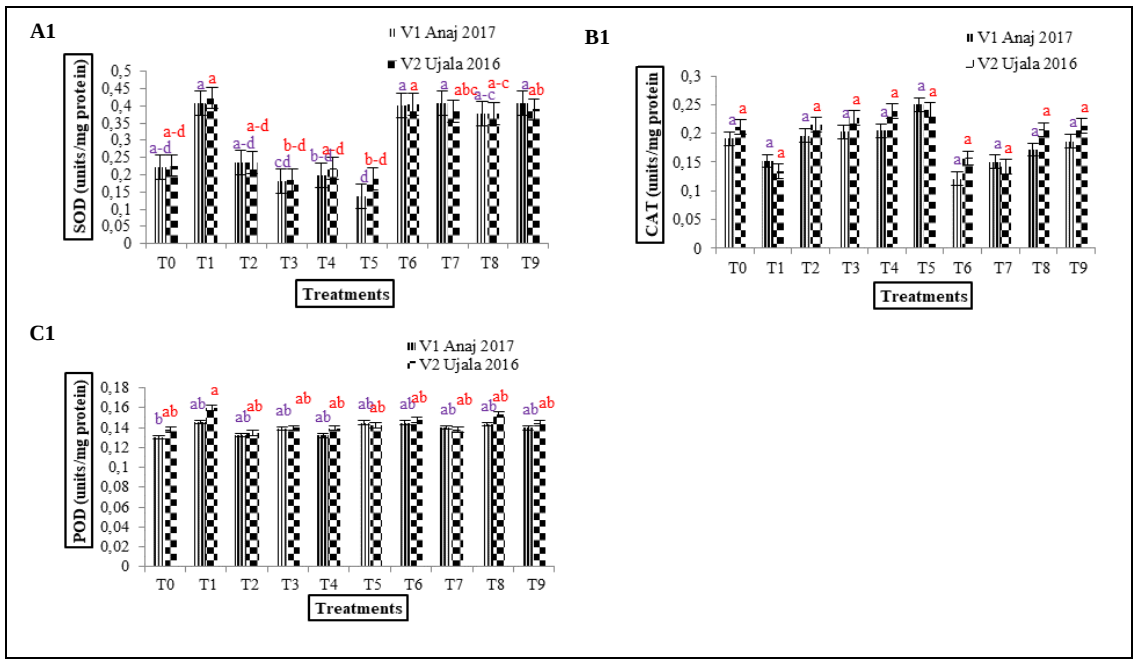

Figure 6. *(A1-C1): Effect of GA_3 and SA on activity of antioxidant enzymes (SOD, POD and CAT) of wheat (Triticum aestivum L.) under NaCl stress (at 150 mM NaCl) and without stress. Values represent the means of three replicates ± standard error (SE)*

Yield parameters

Salt stress substantially reduced the weight of seeds in 100 g value among both varieties of wheat. Plants that were affected with salinity stress showed that seeds were observed with smaller in size. It was noted that the weight of seeds in variety Ujala-2016 was lower than that of Anaj-2017 due to the effect of salt stress. Highest 100 g seed weight 3.2 g was observed in V_2 (Ujala-2016) at a concentration of 1.0 mM SA. The positive impact of both GA_3 and salicylic acid on seed weight were highly significant but between hormones and varieties was non-significant. Use of Gibberellic acid and Salicylic acid by foliar means proved beneficial for increasing the weight of the seeds. Both plant growth stimulators showed highly significant impact on number of spikes/plant but within varieties that was non-significant. It was observed that NaCl is the major cause of reduction in the number of spikes per plant of wheat. The highest number of spikes/plant was recorded at 150 mg GA_3/L. The hormonal response of all yield parameters was highly significant (*Fig. 7 A2-D2*). Both varieties of wheat showed the equal reduction rate of spikes. It was observed that the grain yield was also declined by the addition of salt in plant roots. While it was observed that plants treated by Gibberellic acid and Salicylic acid hormones manifested the positive results and improved the grain yield in plants.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2001_119134

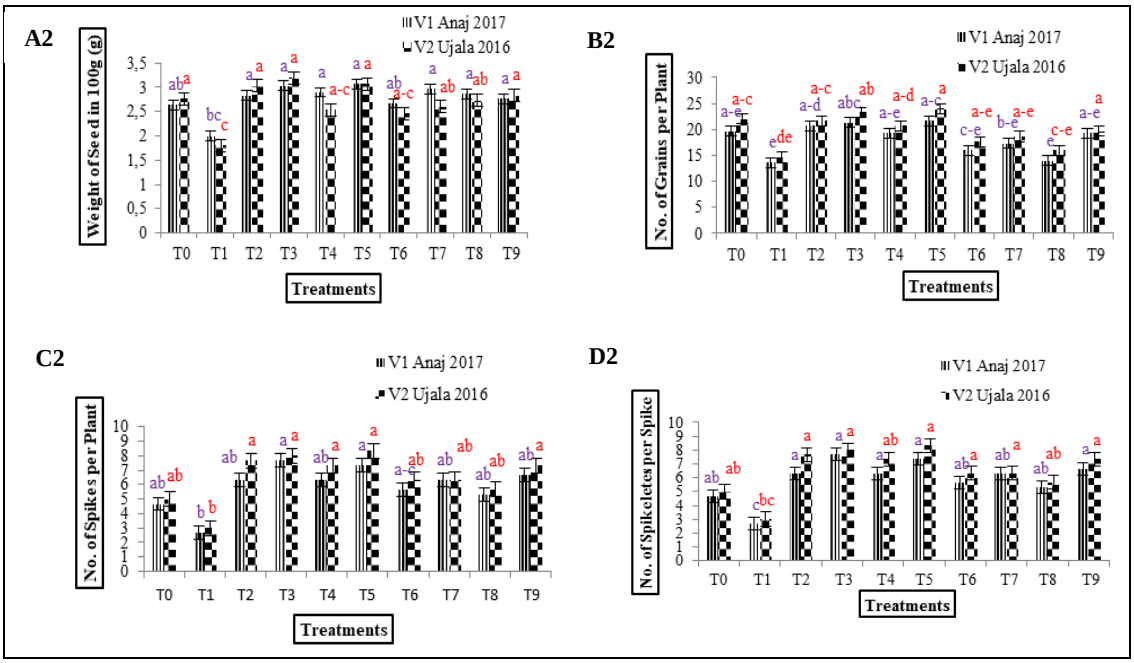

Figure 7. *(A2-D2): Effect of GA_3 and SA on activity of antioxidant enzymes (SOD, POD and CAT) of wheat (Triticum aestivum L.) under NaCl stress (at 150 mM NaCl) and without stress. Values represent the means of three replicates ± standard error (SE)*

Discussion

Published reports showed that the yield of many crops decreased under salt stress but after foliar use of phytohormones such as GA_3 and SA the effect of NaCl can be mitigated and ultimately showed positive effects on plants. Germination of seed is the most important and initial stage for plant growth. The germination of the seeds was affected by the addition of salt in the soil (Mena et al., 2015). In the beginning, plant roots are affected by NaCl because it has direct contact with the soil where NaCl is present. In another study, Ehtaiwesh (2016) reported that mean effect of salinity was significant on plant height, spikelet number, dry weight, grain number, and gain yield etc. further, findings suggested seedling length and dry weight to be considered a selection criterion for salt tolerance. Likewise, Ibrahim et al. (2019) stated that low rate of seed production and quality is threat toward agriculture and industry. GA3 has ability to reduce the rate of transpiration and it also increased the seed weight by maintaining all physiological parameters of the plant. These findings are in agreement with current studies. It is evident that plant photosynthetic mechanism greatly affected by saline environment, resultantly it also reduces the surface area of the leaf and the number of leaves per plant. In the same context, it was observed through the results of the current experiment that the area of leaf in wheat plants decreased when they were subjected to a 150 mM concentration of NaCl. It was evident that salinity stress becomes suppressed in both varieties. The impact of salt stress was also visible on photosynthetic pigments viz., chlorophyll "a", chlorophyll "b", and carotenoids (Zeng et al., 2013). Moreover, Saddiq et al. (2021) advocated that salt stress adversely impacted photosynthesis by damaging chlorophyll pigments and limiting PSII activity while conducting an experiment on winter and spring wheat. In further elaboration it was confirmed that stomatal closing ultimately disturbs the photosynthesis under salt stress.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2001_119134

Further, the conductance of stomata (GS), Intercellular concentration of carbon dioxide (CI), and the total photosynthetic rate (A) were decreased due to NaCl accumulation. Current results were also supported by the findings of Wasaya et al. (2021) who determined that the water stress and salt had harmful effects upon the rate of transpiration, rate of photosynthesis, and conductance of stomata within wheat genotypes. Anthocyanins (flavonoids) are pigments that are soluble in water and are present within the tissues of all plants. Anthocyanins have a vital role in higher plants because they provide the color of fruits and flowers. Moreover, these pigments also played an important role in the antioxidant activities of plants. Current findings also emphasized that the reduction of anthocyanin pigment was occurred by salinity stress. According to Mbarki et al. (2018) higher dry matter production can be maintained in pigmented wheat genotypes under salt stress. Additionally, salt stress showed a deleterious effect on the biochemical parameters of many plants. It was observed that the amount of carbohydrates within the leaves of the wheat plant was depleted by salinization (Hasanuzzaman et al., 2017). Reduction in carbohydrates decreased through the incorporation of sodium chloride in wheat was recorded in the past. NaCl impact on protein contents was also destructive but the exogenous applications of SA and GA_3 have improved the content of protein within salinity-affected plants. Datir et al. (2020) also reported a similar type of results that the treatment of salt stress lowered the quantity of protein in wheat and foliar applied GA_3 raised the amount of proteins among salinity affected plants. Under salt stress, generation of ROS due to Na+ toxicity, which damage biomolecules (e.g., lipids, proteins, and nucleic acids) on the cellular level and alters redox homeostasis is a common phenomenon (Kundu et al., 2018; Sabagh et al., 2021).

Antioxidant activity was also hampered by the application of salt stress but alleviated through the addition of phytohormones in both varieties. It was noted that the catalase enzyme activity was decreased through the addition of salt within the soil but inversely its level was improved after the treatment of Gibberellic acid and Salicylic acid. Current findings were similar to Datir et al. (2020) that stated the activity of catalase enzyme was declined in wheat. Results showed that the level of SOD and POD was enhanced when the concentration of NaCl was greater within the soil. It showed that the highest level of SOD and POD is the indication of stress for plants. While the foliar use of GA_3 and SA proved beneficial for the reduction of SOD and POD levels. The result is in opinion with the findings of Wang et al. (2014) who noticed that the activity of SOD and POD were elevated by the treatment of salt and it can reduce after the application of hormones. Electrolyte leakage of plant leaves between both varieties of wheat showed an elevated level of NaCl stress. Similarly, Mandhania et al. (2006) and Hasanuzzaman et al. (2017) reported that the concentration of electrolyte leakage in the roots and the leaves of wheat were enhanced by raising the amount of salt. The amount of Malondialdehyde was increased due to the accumulation of NaCl within the wheat plant. Our findings are in agreement with the outcomes of Mandhania et al. (2006), who reported that the content of MDA was increased by the application of sodium chloride (NaCl) within the apical portion of leaf in wheat while the application of GA_3 and Salicylic acid decreased the content of Malondialdehyde (MDA).

In the same context, yield parameters also showed adverse effects of salt stress. Results proved that yield parameters such as number of spikes, number of spikelets, seed weight, and number of grains were reduced due to salt concentration. Datir et al. (2020) reported similar results according to their findings all yield parameters like the grain yield, height of spike, weight of spike, the number of seed, the weight of seed, and the number of

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2001_119134

spikelet were declined by the harmful impacts of salinity stress in wheat whereas, the spray of Salicylic acid proved beneficial to ameliorate the harmful effects of salinity stress.

Conclusion

In conclusion both salicylic acid and gibberellic acid promotes growth like root length (22.6 cm), shoot length (42 cm) and shoot fresh weight (11.4 g), physiological characters such as photosynthetic rate (30.1%) and yield of 100 g seed weight (3.7%). The nutritional constituents including protein, carbohydrates were also enhanced by 0.53 mg/mL. Anthocyanin pigment was increased by 0.0035% at 100mg/L GA_3 in Ujala-2016 while glycine betaine by 0.26% at 150 mg/L. Consequently, the concentration of 0.1 mM SA and 150 mg/L GA_3 along variety Ujala-2016 recommended for the alleviation of salt stress with better growth and yield for future cultivation.

Acknowledgements. We are grateful to the staff of botanical garden, University of Gujrat, Gujrat, Pakistan for provision of experimental facilities.

Disclosure statement. The authors declare no conflict of interests.

REFERENCES

[1] Ahmad, M., Ghafoor, A., Asif, M., Farid, H. U. (2010): Effect of irrigation techniques on wheat production and water saving in soils. – Soil and Environment 29: 69-72.
[2] Arnon, D. I. (1949): Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. – Plant Physiology 24(1): 1-15.
[3] Bradford, M. M. (1996): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. – Analytical Biochemistry 72: 248-254.
[4] Braun, H. J., Atlin, G., Payne, T. (2010): Multi-location testing as a tool to identify plant response to global climate change. – Climate Change 1: 115-138.
[5] Cakmak, I., Horst, W. J. (1991): Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). – Plant Physiology 83: 463-468. https://doi.org/10.1111/j.1399-3054.1991.tb00121.x.
[6] Chance, B., Meahly, A. C. (1995): Assay of catalase and peroxidase. – Methods Enzymology 2: 764-775.
[7] Dadshani, S., Sharma, R. C., Baum, M., Ogbonnaya, F. C., Léon, J., Ballvora, A. (2019): Multidimensional evaluation of response to salt stress in wheat. – PLoS One 14(9): e0222659. https://doi.org/10.1371/journal.pone.0222659.
[8] Datir, S., Singh, N., Joshi, I. (2020): Effect of NaCl-induced salinity stress on growth, osmolytes and enzyme activities in wheat genotypes. – Bulletin of Environmental Contamination and Toxicology 104: 351-357.
[9] Ehtaiwesh, A. F. A. (2016): Effects of salinity and high temperature stress on winter wheat genotypes. – PhD Dissertation, Kansas State University, USA, 322p.
[10] Giannopolitis, C. N., Ries, S. K. (1977): Superoxide dismutase occurrence in higher plants. – Plant Physiology 59: 309-314.
[11] Grieve, C. M., Grattan, S. R. (1983): Rapid assay for determination of water soluble quaternary ammonium compounds. – Plant and Soil 70: 303-307.
[12] Hadia, E. H., Slama, A., Romdhane, L., M'hamed, H. C., Abodoma, A. H., Fahej, M. A. S., Radhouane, L. (2020): Morphophysiological and molecular responses of two Libyan

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2001_119134

bread wheat cultivars to plant growth regulators under salt stress. – Italian Journal of Agronomy 15(1633): 246-252. https://doi.org/10.4081/ija.2020.1633.

[13] Hasanuzzaman, M., Nahar, K., Rahman, A., Anee, T. I., Alam, M. U., Bhuiyan, T. F., Oku, H., Fujita, M. (2017): Approaches to enhance salt stress tolerance in wheat. – Wheat improvement, management and utilization. Intech Open Science/Open Minds, pp. 151-187.

[14] Hayat, Q., Hayat, S., Irfan, M., Ahmad, A. (2010): Effect of exogenous salicylic acid under changing environment: a review. – Environmental and Experimental Botany 68: 14-25.

[15] Ibrahim, M. E. H., Zhu, X., Zhou, G., Ali, A. Y. A., Elsiddig, A. M. I., Farah, G. A. (2019): Response of Some wheat varieties to gibberellic acid under saline conditions. – Agrosystems, Geosciences and Environment 2(1): 1-7.

[16] Iqbal, M., Ashraf, M. (2013): Gibberellic acid mediated induction of salt tolerance in wheat plants: growth, ionic partitioning, photosynthesis, yield and hormonal homeostasis. – Environmental and Experimental Botany 86: 76-85.

[17] Iqbal, M. S., Ahmad, K. S., Ali, M. A., Akbar, M., Mehmood, A., Nawaz, F., Hussain, S. A., Arshad, N., Munir, S., Arshad, H., Shahbaz, K., Bussmann, R. W. (2021): An ethnobotanical study of wetland flora of Head Maralla Punjab Pakistan. – Plos One 16(10): e0258167.

[18] Krizek, D. T., Kramer, G. F., Upadhyaya, A., Mirecki, R. M. (1993): UV-B response of cucumber seedlings grown under metal halide and high pressure sodium/deluxe lamps. – Journal of Plant Physiology 88: 350-358.

[19] Kundu, P., Gill, R., Ahlawat, S., Anjum, N. A., Sharma, K. K., Ansari, A. A., Hasanuzzaman, M., Akula, R., Chauhan, N., Tuteja, N., Gill, S. S. (2018): Targeting the redox regulatory mechanisms for abiotic stress tolerance in crops. – In: Wani, S. H. (ed.) Biochemical, Physiological and Molecular Avenues for Combating Abiotic Stress Tolerance in Plants. Elsevier Academic Press, pp. 151-220. doi: 10.1016/B978-0-12-813066-7.00010-3.

[20] Lutts, S., Kinet, J. M., Bouharmont, J. (1995): NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. – Annals of Botany 78: 389-398.

[21] Mandhania, S., Madan, S., Sawhney, V. (2006): Antioxidant defense mechanism under salt stress in wheat seedlings. – Biologia Plantarum 50: 227-231.

[22] Mbarki, S., Sytar, O., Zivcak, M., Abdelly, C., Cerda, A., Brestic, M. (2018): Anthocyanins of coloured wheat genotypes in specific response to salt stress. – Molecules 23: 1518. doi: 10.3390/molecules23071518.

[23] Mena, E., Leiva-Mora, M., Jayawardana, E. K. D., Garcia, L., Veitía, N., Bermudez-Caraballoso, I., Ortiz, R. C. (2015): Effect of salt stress on seed germination and seedlings growth of *Phaseolus vulgaris* L. – Cultivos Tropicales 36: 71-74.

[24] Nawaz, R., Inamullah, Ahmad, H., Din, S. U., Iqbal, M. S. (2013): Agromorphological studies of local wheat varieties for variability and their association with yield related traits. – Pakistan Journal of Botany 45(5): 1701-1706.

[25] Rout, S., Beura, S., Khare, N., Patra, S. S., Nayak, S. (2017): Effect of seed pre-treatment with different concentrations of gibberellic acid (GA_3) on seed germination and seedling growth of *Cassia fistula* L. – Journal of Plant Sciences 5: 135-138.

[26] Sabagh, A. E., Islam, M. S., Skalicky, M., Raza, M. A., Singh, K., Hossain, M. A., Hossain, A., Mahboob, W., Iqbal, M. A., Ratnasekera, D., Singhal, R. K., Ahmed, S., Kumari, A., Wasaya, A., Sytar, O., Brestic, M., Çig, F., Erman, M., Rahman, M. H. U., Ullah, N., Arshad, A. (2021): Salinity stress in wheat (*Triticum aestivum* L.) in the changing climate: adaptation and management strategies. – Frontiers of Agronomy 3: 661932. doi: 10.3389/fagro.2021.661932.

[27] Saddiq, M. S., Iqbal, S., Hafeez, M. B., Ibrahim, A. M. H., Raza, A., Fatima, E. M., Baloch, H., Jahanzaib, Woodrow, P., Ciarmiello, L. F. (2021): Effect of salinity stress on physiological changes in winter and spring wheat. – Agronomy 11: 1193. https://doi.org/10.3390/agronomy11061193.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2001_119134

[28] Salim, N., Raza, A. (2020): Nutrient use efficiency (NUE) for sustainable wheat production: A review. – Journal of Plant Nutrition 43: 297-315.
[29] Sawada, H., Shim, I. S., Usui, K., Kobayashi, K., Fujihara, S. (2008): Effect of salicylic acid on salt sensitivity. – Journal of Plant Sciences 174: 583-589.
[30] Shrivastava, P., Kumar, R. (2015): Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. – Saudi Journal of Biological Sciences 22: 123-131.
[31] Sudharmaidevi, C. R., Thampatti, K. C. M., Saifudeen, N. (2017): Rapid production of organic fertilizer from degradable waste by thermochemical processing. – International Journal of Recycling of Organic Waste in Agriculture 6: 1-11.
[32] Wahid, A., Perveen, M., Gelani, S., Basra, S. M. (2007): Pretreatment of seed with H_2O_2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. – Journal of Plant Physiology 164: 283-294.
[33] Wang, H. M., Xiao, X. R., Yang, M. Y., Gao, Z. L., Zang, J., Fu, X. M., Chen, Y. H. (2014): Effects of salt stress on antioxidant defense system in the root of *Kandelia candel*. – Botanical Studies 55: 57.
[34] Wasaya, A., Manzoor, S., Yasir, T. A., Sarwar, N., Mubeen, K., Ismail, I. A., Raza, A., Rehman, A., Hossain, A., El Sabagh, A. (2021): Evaluation of fourteen bread wheat (*Triticum aestivum* L.) genotypes by observing gas exchange parameters, relative water and chlorophyll content, and yield attributes under drought stress. – Sustainability 13: 4799.
[35] Zeng, J., Chen, A., Li, D., Yi, B., Wu, W. (2013): Effects of salt stress on the growth, physiological responses, and glycoside contents of *Stevia rebaudiana* Bertoni. – Journal of Agricultural and Food Chemistry 61: 5720-5726.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2001_119134