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FOLIAR APPLICATION OF SORBITOL IS A SHOTGUN APPROACH TO ALLEVIATE THE ADVERSE EFFECTS OF SALINITY STRESS ON TWO VARIETIES OF WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

The current experiment was designed to alleviate the detrimental effects of salt stress on two varieties of Wheat i.e. Johar 2016 (V_1) and Anaj 2017 (V_2). Sorbitol was used by foliar application method to bring under control the negative impacts of NaCl stress on plants. Salt was applied at concentration of 150mM and Sorbitol was used in three concentrations (50, 75 and 100mM). Sorbitol application was done three times on plants with the interval of one week. All the morphological, physiological and yield parameters were recorded. The experimental design was completely randomized and for the statistical analysis, CoStat software was used. The findings of the experiment concluded that the application of sorbitol on plants proved beneficial in salt stress as well as under normal conditions. Foliar application of sorbitol helped to ameliorate the negative impacts of NaCl stress on plants. It was noticed that sorbitol caused a significant increase in the growth of plants in salt stress and normal conditions. Almost all the growth parameters were increased after sorbitol's application. The results concluded that the 50mM and 75mM concentration of sorbitol gave the best results to overcome the effects of NaCl stress. All the treatments of sorbitol improved the growth parameters of plants under normal conditions but the best results were observed at 50mM and 75mM.

Key words: Salt stress, Sorbitol, Wheat, Antioxidants, Glycine betaine.

Introduction

The cultivated Wheat (*Triticum aestivum* L.) belongs to family Poaceae which is the family of sweet grasses. Wheat is commonly known as king of cereals because it is the main cereal crop in various parts of the Globe. It is the most important and extensively grown food cereals throughout the globe, due to its broad adaptability along with the nutritive values quality than other cereals crops. Similarly, it also stands first in terms of production and acreage. An important role is played by wheat with reference to food, economic production and nutrition across the world and it is considered as a strategic crop (Barutcular *et al*., 2017; Darwish *et al*., 2018; Jahan *et al*., 2019). It is extensively cultivated crop around the world. Wheat is also considered as the 1st domesticated food crop and in the major civilizations in West Asia, Europe, and North Africa it is the main staple food from past 8,000 years.

In Pakistan cultivation of wheat is done on an area of more than 9.0 mega hectares (Khan *et al*., 2017). The cultivation of wheat is performed in Pakistan and different countries around the world in order to fulfill the demand for food. Even though, the production potential of wheat is lower than its productivity per hectare because of various environmental factors that cause a reduction of crop yield and among these factors the major one is salt stress (Iqbal *et al*., 2018). Salt stress causes apparently around 60% reduction in production and yield of wheat (Xie *et al*., 2016). The production and yield of wheat are the important elements of wheat's supply. For the security of food adequate availability of food at the country, provincial and household is the main concern. In world markets any change in wheat demand and supply can affect the well-being of taxpayers, farmers, consumers and of those people who depend directly or indirectly on agriculture (Khan *et al*., 2008; Hong-juan *et al*., 2017).

Salinity is extremely harmful that limits the growth of plants and affects the productivity of Wheat (Khaliq *et al*., 2015; Barnawal *et al*., 2017). As reported by FAO/LPNMS (Land and Plant Nutrition Management Service) a large cultivated land's portion is affected by salt stress around the world that's why the land cannot be cultivated (Yassin *et al*., 2019). Salt affected areas are exceeding 800 Mha that is up to 6% of the entire worlds land area (Saddiq *et al*., 2019). In arid and semi-arid areas salt stress is a severe threat to productivity of agriculture (Babu *et al*., 2012). The yield of some main crops reduces up to 50% in the arid and semi-arid areas of the globe and its reason is salinity stress (Dugasa *et al*., 2016). Due to NaCl stress osmotic potential of soil increased that cause the movement of water in soil from low salt concentrated areas (plant tissues) because in soil salt concentration is higher (Khan *et al*., 2017). Physiology of crop at cellular and plant level disturbed due to osmotic effect (Shahid *et al*., 2011).

Sorbitol is a main product of photosynthesis in the Rosaceae family and the metabolism of sorbitol is strongly related to the yield and quality of fruit (Teo *et al*., 2006). The growth and development of plant sorbitol act as energy sources and carbon backbone. It also works as a signaling molecule that involves in the regulation of plants growth and helps against different environmental stress as well. According to a recent study, in apple plant sorbitol provide resistance against pathogenic fungus named as *Alternaria alternata* by regulating the expression of NLR (Nucleotide-binding Leucine-rich repeat) gene (Meng *et al*., 2018). Sorbitol's exogenous

application is effective to alleviate detrimental effects of NaCl stress on plant growth and also on other various parameters like, lipid peroxidation, ROS generation and membrane damage especially in salt sensitive rice crop. Interestingly in some cases these osmoprotectants increase growth and other physiological parameters in salt tolerant rice. Sorbitol accumulation is helpful against salinity, drought and chilling stress tolerance in many species of plants (Gupta & Huang, 2014). So the observations and findings indicate that application of sorbitol is an interesting and alternative method that can be used to enhance the salt stress tolerance level of salt sensitive crops (Theerakulpisut & Gannula, 2012). The Current study was designed in order to ameliorate the detrimental effects of salt stress through foliar application of Sorbitol on two varieties of Wheat.

Materials and Methods

Location of research work was the Botanical Garden of University of Gujrat, Department of Botany. Overall there were eight treatments including control and for each treatment there were four replicates. Salt treatment was applied at the time of sowing. After two weeks of germination foliar method was used for application of sorbitol. Application was done 3 times after one-week interval. The experiment was arranged in Completely Randomized Design (CRD).

Seeds of two varieties Johar 2016 (V_1) Anaj 2017 (V_2) were obtained from Punjab Seed Corporation Gujranwala Center. For seed sowing plastic pots containing river sand were used and placed in a nesting house. Irrigation of the plants was performed with Full length Hoagland's nutrient solution and its application was performed one time in one week. Salt stress application was done at the time of seed sowing and sorbitol was applied after 2 weeks of germination. Following treatments were used in this experiment. T0 = Control, T1 = NaCl (150 mM), T2 = Sorbitol (50 mM), T3 = Sorbitol (75 mM), T4 = Sorbitol (100 mM), T5 = Sorbitol (50 mM) + (NaCl 150mM), T6 = Sorbitol (75 mM) + (NaCl 150 mM), T7 = Sorbitol (100 mM) + (NaCl 150 mM). Data of morphological, physiological, biochemical and yield parameters were recorded after 3 weeks of Sorbitol application.

Germination percentage and other morphological parameters were recorded. Infra-Red Gas Analyzed (IRGA) was used to find the photosynthetic rate (A), Inter cellular carbon dioxide concentration (Ci), transpiration rate (E), and stomatal conductance to water (gs). For the calculation of water use efficiency, photosynthetic rate was divided by transpiration rate.

Glycine betaine content was calculated by method of Grieve & Grattan (1983). Water toluene solution of 5ml was used to grind the 0.5 g of leaves. Extract was then transfer to test tube and shake for 24 hours at temperature of 25°C with the help of shaker. After shaking sample solution was filtered with filter paper. Filtrate of 0.5 ml was taken and 0.1 ml solution of KI and 1ml solution of hydrochloric acid (HCl) was added in it. The prepared sample mixture was again shaked in ice bath for 90 minutes and then super cool water of 2ml was added. Afterward 10 ml of 1, 2 dichloromethane was added. Sample mixture was then left for about two minutes at room temperature so that air can pass through the sample. At last two films were formed in the sample. The top layer was removed and discarded and lower layer was used to measure the absorbance at 360 nm by using spectrophotometer.

Photosynthetic Pigments were recorded by Arnon method (1949). 0.5 g of leaf sample was taken and crushed with 4ml of 80 % acetone. Afterward sample solution was filtered by mean of filter paper. Acetone was added again to prepare 10ml final volume. Then vortex blend was used in order to take supernatant. Hitachi spectrophotometer (Hitachi, Model U2001, Tokyo, Japan) was used to measure the absorbance. Measurement of absorbance was done at three wavelengths and for different chlorophyll contents following formula was used.

Chlorophyll 'a' (mg/g f.wt.) = [12.7(OD 663) – 2.69 (OD 645)] X V/ 1000 X W

Chlorophyll 'b' (mg/g f.wt.) = [22.9 (OD645 – 4.68(OD 663) X V/1000 X W

Carotenoids (mg/g f.wt) = [7.6(OD 480) – 1.49(OD 510)] X V/1000 X W

W = Weight of fresh leaves tissues in grams

V = Volume of leaf extract in ml

For anthocyanin content the technique of Krizek *et al*., (1993) was used. For this technique methanol and HCl solution was prepared in 99:1 concentration. (99 ml methanol+ 1 ml HCl. 3ml of this solution was then used to crush 0.2g of leaves samples. The extract which prepared then centrifuged for 30 minutes at 18,000 RPM and the temperature will be 4°C. Supernatant was then separated and incubated at 4°C for 24 hours in dark area. Spectrophotometer was then use to take reading at 2 wavelength 530nm and OD 657nm. Following formula was used to calculate the anthocyanin content.

Anthocyanin content = [OD530-0.25 OD657] X TV / [dry wt. X 1000]

OD = Optical density

TV = Total volume of the extract (ml)

Dry wt. = weight of the dry lead tissue

For the determination of MDA content Cakmak & Horst (1991) method was adopted. For this procedure 1-gram fresh leave of the plant was taken and crushed with 1% (w/v) TCA at temperature 4°C. Afterward the mixture was centrifuged for fifteen minutes at 2000 rpm. When the

centrifuge process was completed supernatant was taken and 3ml of TCA & TBA was added (0.5 by 20%). Sample was placed in water bath for 90 minutes at temperature 95°C to stop the reaction. Absorbance of the sample was measured at 532 nm. And for nonspecific values absorbance was calculated at 600nm. The MAD content was the measure with the aid of following formula:

MAD level = (A532 nm – A 600nm) / 1.56 X 105

For preparing plant extract fresh leaves of 0.5g were crushed with 80% of ethanol. After crushing the leaves 10ml of water was added in the sample. After preparing different aliquots final volume was made 1ml and then 5ml concentrated sulfuric acid (H_2SO_4) was added in each sample. The samples mixture was then shaked and incubated at 30°C temperature for 40 minutes. After incubation 1ml solution of 5% phenol was added in each test tube. Wavelength of 490 nm was set on spectrophotometer and absorbance was observed (Rasool *et al*., 2010).

For the estimation of protein content 0.5g of fresh leave was crushed. For crushing 10ml of 50mM KPO4 potassium phosphate buffer was used. The pH of buffer was maintained 7.8 and crushing of leaves was performed at cool environment. Afterward samples were centrifuged for 15 minutes at 10,000rpm at 4°C temperature (Bradford, 1976). The supernatant of 0.1 ml was then mixed with Bradford reagent of 2ml. protein content was then determined with the help of spectrophotometer at absorbance of 590 nm.

For antioxidant activity 0.5g of fresh leaves samples was collected from each treatment and grinded with 5 ml of phosphate buffer. Phosphate buffer was cooled before using. After grinding the sample mixture was placed in ice bath. Then samples were centrifuged for 20 min at 15000 rpm and temperature was maintained at 4°C. Supernatant was separated. This supernatant was use to obtain both CAT and POD activity with the help of Chance & Meahly (1995) technique.

For CAT activity measurement reaction solution was prepared with final volume of 3ml. In the reaction solution 50mM phosphate buffer solution with pH 7 was used, 5.9mM H_2O_2 and prepared enzyme extract 0.1ml was added. The reaction initiated through adding the enzyme extract. For the measurement of CAT activity spectrophotometer was set on 240nm and the change in absorbance was measure after every 20 second. For the measurement of one unit of CAT activity variation in absorbance of 0.01 units per minutes was measured.

POD activity was measures. The reaction solution was prepared with final volume of 3ml containing phosphate buffer 50mM, guaiacol 20mM., and H_2O_2 40mM then 0.1 ml of enzyme extract was added in the reaction solution. Spectrophotometer was set to the wavelength of 470nm and after every 20 seconds the change in absorbance was measured. For the measurement of one-unit POD activity change in absorbance was measured at the rate of 0.01 units per minute. Each enzyme activity was stated on the concentration of the proteins in the extract for this method of Bradford (1976) was used.

Sod activity was evaluated with the help of Giannopolitis & Ries (1977) method. 0.5 g of leaf samples was crushed with 5ml of phosphate buffer. Falcon tubes were taken and from the prepared mixture 3ml of sample was added in it. Afterward, Nitro blue tetre-zolium (NBT) 50µm, methionine 13mM, EDTA 75mM, enzyme extract 20-50 ml, phosphate buffer of pH 7.8 50 mM, and riboflavin 13µm, was mixed. Then the sample mixture was place under fluorescent lamp for 15 minutes. Spectrophotometer was used and absorbance was measured at 560nm.

For the calculation of electrolyte leakage, the technique of Lutts *et al*., (1996) was used. Fresh flag leaves were taken from the plants. Leaves were washed with water and cut into different pieces. In falcon tubes 50 ml double distilled water was taken and leaves was placed in the water. Tubes were then incubated for 24 hours. After 24 hours EC was measure with the help of electrolyte leakage meter. The value was named as EC1. Now the sample was autoclaved for 20 minutes at 121°C and again electrolyte leakage was measure. This value was named as EC2. Final value was calculated with the aid of following formula:

EC1/EC2 x 100

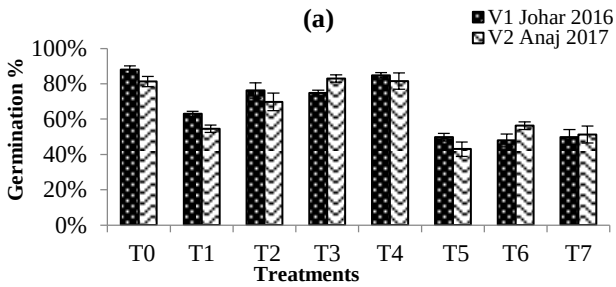
Yield Parameters like, Number of Spikes/Plant, Number of Grains/Spike, Number of Spikelets/ Spike, 100g Grain Weight (g)

Statistical analysis

Experimental design was completely randomized and there were total four replicates, for each treatment. Experimental data was statistical analyzed for comparison of means with the help of software CoStat, by using Analysis of Variance (ANOVA) Technique.

Results and Discussion

Morphological parameters: Germination percentage was the first parameter that was recorded after salt application and results proved that the rate of germination was reduced in salt treated plants (Fig. 1a). Roots of plants are directly in contact with saline soil and as a result, roots are the first part of the plant that get affected with stress. Results of our experiment proved that salinity stress declined the length of root and shoot as well as fresh and dry weight when treated with salt stress (Fig. 1b, c, d) and Sorbitol's treatment improved growth of salt effected plants. ANOVA results also showed that the sorbitol treatment was highly significant (Table 1). The same reduction in root and shoot length of *Brassica rapa* after salt application was noticed by Jan *et al*., (2016). Findings of Tanveer *et al*., (2020) also proved that salt stress declined the dry and fresh weight of root and shoot of *Solanum lycopersicum* L. *u*nder salt stress conditions and treatment of calcium declined the detrimental effects of NaCl stress. The number of tillers of both varieties reduced when the plants subjected to NaCl stress and foliar application of sorbitol significantly increased the number of tillers of salt affected as well as normal plants (Fig. 1h), Sorbitol treatment was highly significant in statistical analysis (Table 1). These findings also fit with the findings of Shahzad *et al*., (2016) where no. of tillers of wheat decreased with increasing NaCl concentration.



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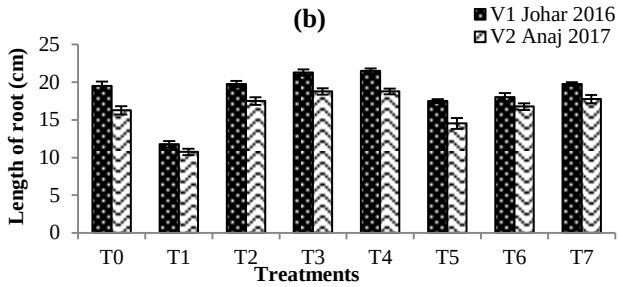

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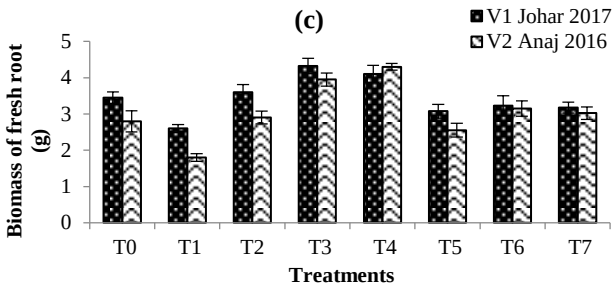

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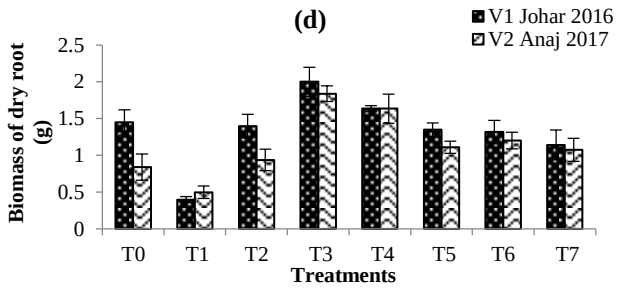

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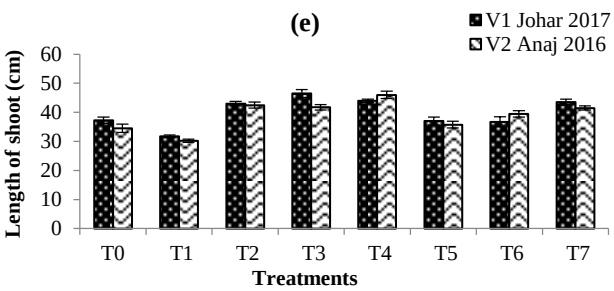

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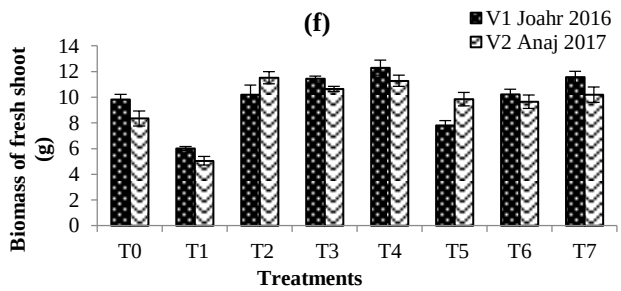

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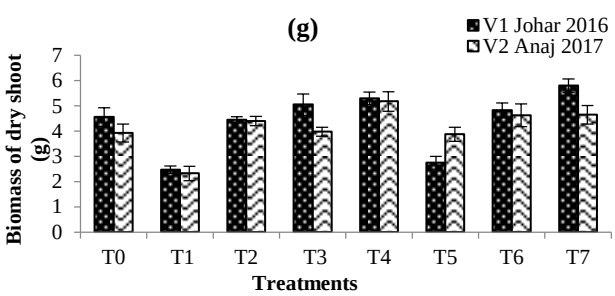

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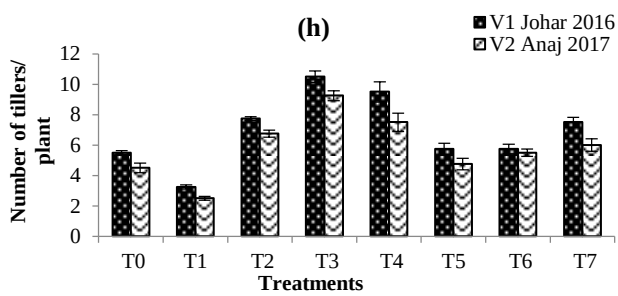

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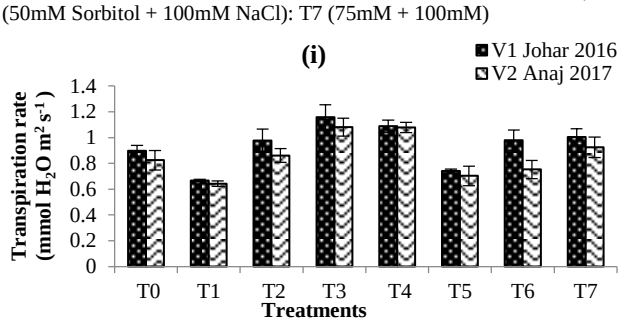

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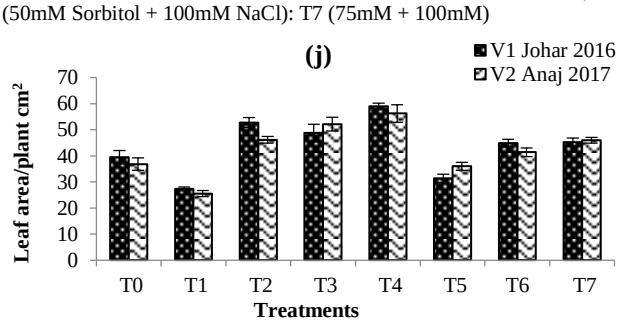

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Fig. 1. Effect of Foliar application of Sorbitol on various morphological parameters of Wheat.

Saline condition greatly affects the photosynthetic activities of plant as a result leaf surface area as well as the number of leaves reduced in plant. It was analyzed from our results that leaf area of wheat reduced when plants exposed to 150mM NaCl. Number of leaves of both varieties also reduced with salt application and Sorbitol application showed positive result in both parameters (Fig. 1i, j). ANOVA results for both parameters were also highly significant for Sorbitol application (Table 1).

Physiological parameters: Reduction in stomatal conductance (gs), net photosynthesis rate (A), intercellular carbon dioxide concentration (Ci) was observed from data and water use efficiency was observed by salt treatment and foliar application of sorbitol improved all these parameters (Fig. 2a, b, c, d). For all these parameters results of statistical analysis of the treatment of Sorbitol is highly significant (Table 2). These findings were same as the findings of Zou *et al*., (2016). They found that all these gaseous exchange parameters were reduced in Wheat after salt treatment.

Biochemical parameters: Salinity stress has an adverse effect on the biochemical parameters of the various plants. The data herein proved that NaCl declined the concentration of carbohydrate in the leaf of wheat varieties. Foliar treatment proved favorable for the plants grown under salinity stress. Carbohydrate content reached up to normal level after all treatments of sorbitol under stress conditions (Fig. 3a). ANOVA results are also highly significant (Table 4). These results were correlated with the consequences of Sadak *et al*., (2013). He found that salinity stress cause reduction in content of carbohydrate when Wheat exposed to NaCl stress. Reduction of the protein content after salt treatment was also observed from our experimental data and foliar treatment of sorbitol elevate the level of protein in salt affected plants (Fig. 3b). Foliar spray of Sorbitol was significant for plants (Table 4). Same consequences were noticed by Deivanai *et al*., (2011) where Rice plants treated with salt stress reduced the level of protein and exogenous treatment of Proline enhanced the concentration of proteins in salt affected plants. Gul *et al*., (2017) also observed the same results. He found that salt application cause reduction in carbohydrate and protein content of *Spinacia oleracea* but application of sorbitol helps the plant to reduce harmful effect of salt stress and increase carbohydrate and protein concentration.

Results of our experiment proved that reduction in photosynthetic rate occurred by salt application because salt stress causes a reduction of photosynthetic pigment (chlorophyll ‘a’ ‘b’ and carotenoids). Application of Sorbitol improved all these parameters of plant in salt stress and normal condition (Fig. 3c, d, e) and ANOVA results for sorbitol application are also significant (Table 4). Our findings were correlated with Sadak *et al*., (2013) and Najar *et al*., (2019). Sadak *et al*., (2013) concluded that in *Triticum aestivum* salt stress declined the photosynthetic activity by effecting the concentration of photosynthetic pigments. A significant reduction of chlorophyll a, b and carotenoids was observed by increasing concentration of salt stress. Najar *et al*., (2019) concluded that the gaseous exchange parameters and photosynthetic pigments reduced with salt treatment in *Medicago truncatula*.

Anthocyanins are the pigments that are present in plants these are also involved in various antioxidant activities. Results of this experiment confirmed that salt stress caused reduction in anthocyanin pigment of wheat leaves (Fig. 3l). Significant result obtained from AVOA for the treatment of Sorbitol on plants (Table 4). Trivellini *et al*., (2014) found the same results. He observed the reduction of anthocyanin content in Hibiscus due to salt stress.

From the current study, it was proved that antioxidant activity of wheat plants changes with salt treatment. The activity of CAT was reduced with the application of NaCl stress in the soil and after foliar spray of Sorbitol, its concentration increased (Fig. 3f). Sorbitol application was significant in ANOVA (Table 4). Dogan *et al*., (2011) found the similar results where salt treatment declined the concentration of CAT in Soybean plants. The results of our experiment showed that POD and SOD increased with the increasing level of salt in the soil (Fig. 3g, h). These results indicate that the high concentration of POD and SOD represents stressful conditions in the plants. In this aspect, the foliar application of Sorbitol reduced the concentration of POD and SOD. These findings were in agreement with Daoud *et al*. (2018) results where increasing salt concentration increased the SOD activity in Wheat and then foliar application of silicon proved useful to overcome the harmful effect of stress by reducing SOD activity.

The consequences of this experiment proved that salinity stress increased electrolyte leakage of leaves of both wheat varieties but foliar spray of sorbitol helped the plant to increase El level (Fig. 3i) and ANOVA showed that sorbitol application is highly significant (Table 4). These findings were in consistence with the findings of Wu *et al*., 2017, According to them when *Lolium perenne* subjected to salt stress it was noticed that EL of plant increased. Exogenous application of 24-Epibrassinolide reduced the electrolyte leakage of plant. Hniličková *et al*., (2019) also concluded that salt stress increased the electrolyte leakage in *Lactuca sativa*, *Tetragonia tetragonoides* and *Portulaca oleracea*. MDA content is also an indicator of salinity stress in plants. After application of salt stress on Wheat, it was proved that MDA content increased as compared to control treatments and application of sorbitol reduced the MDA content in plants (Fig. 3k). Similar results were also documented from recent research performed by Zhang *et al*., (2020). According to their research, salt stress increased the MDA content in *Cucumis sativus* and exogenous application of Melatonin reduced the MDA content. Theerakulpisut & Gannula (2012) also concluded that the treatment of NaCl stress on Rice (*Oryza Sativa* L.) increased the content of MDA and treatment of Sorbitol and Trehalose helped to reduce the MDA content.

Table 1. Means squares (MS) from the analysis of variance (ANOVA) of morphological parameters of two varieties of wheat (*Triticum aestivum* L.) under salt stress.

Source of variance	df	Germination percentage of	Root length (cm)	Biomass of fresh root (g)	Biomass of dry root (g)	Shoot length (cm)	Biomass of fresh shoot (g)	Biomass of dry shoot (g)	Number of tillers	Number of leaves	Leaf area cm^2
Salt	1	3150.01***	64 ***	1.9251 ns	0.2704 ns	60.06 ns	40.64 **	12.119 **	2.640 ns	50.7656 ns	973.284***
Sorb	3	2157.93***	66.916 ***	2.9243 **	2.247 **	289.72 ***	31.45 ***	5.233 *	58.72***	295.723***	911.660***
Var	1	0.01562 ns	81 ***	1.9951 ns	0.5700ns	16 ns	2.03 ns	1.395 ns	19.140**	172.265**	10.9065 ns
Salt × Sorb	3	1862.47***	64.916 ***	3.8389 ***	2.1611 **	138.89 ***	27.00 ***	8.757**	30.098***	252.057***	641.560 ***
Salt ×Var	1	0.7656 ns	0.25 ns	0.0351 ns	0.0042 ns	30.25 ns	0.10 ns	0.0047 ns	0.0156ns	0.14062 ns	104.601 ns
Sorb × Var	3	190.05 ns	1.08 ns	0.2522 ns	0.2116 ns	10.25 ns	3.03 ns	1.2132 ns	0.3489ns	10.0572ns	7.7451 ns
Salt × Sorb × Var	3	161.557 ns	1.833 ns	0.2039 ns	0.2876 ns	7.5 ns	4.91 ns	1.8626 ns	0.8906 ns	74.1822*	18.405 ns
Error	48	208.078	3.510	0.60015	0.38208	21.0104	3.42	1.4719	1.9427083	17.58854	66.6262
Total	**63**										

Table 2. Means squares (MS) from the analysis of variance (ANOVA) of physiological parameters of two varieties of wheat (*Triticum aestivum* L.) under salt stress.

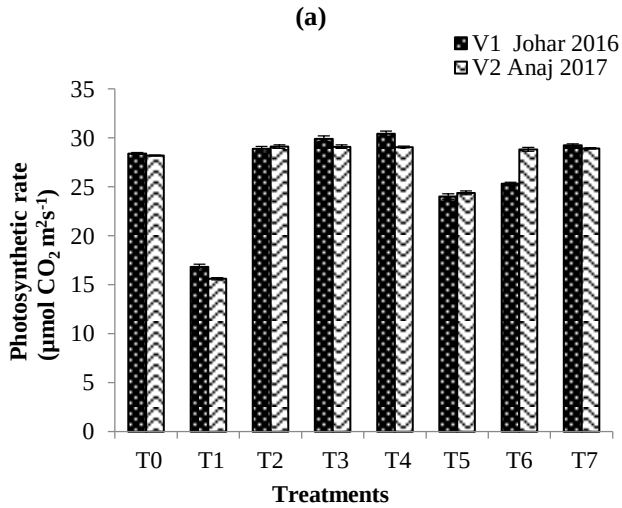
Source of variance	df	Photosynthetic rate (µmol CO2 $m^{-2}s^{-1}$)	Intercellular CO_2 Conc. (µmol CO_2 mol -1)	Intercellular CO_2 concentration	Stomatal conductance to H_2O (mol H_2O m^{-2} s^{-1})	Water use efficiency
Salt	1	228.01 ***	1640.25 ***	0.06375 ns	6.8906 ns	25.869 ns
Sorb	3	150.112***	791.658***	0.18381*	0.0046**	65.631 ns
Var	1	0.01ns	0.20249ns	0.094556ns	0.0011ns	692.54*
Salt × Sorb	3	164.60***	779.118***	0.260502*	0.0101***	356.13ns
Salt ×Var	1	4.3056 *	145.805 *	0.007656ns	0.0031ns	0.3122ns
Sorb× Var	3	4.4608 **	17.1408 ns	0.0159ns	8.3489ns	99.219ns
Salt × Sorb × Var	3	5.1339 ***	25.9222ns	0.00718ns	1.0989ns	205.15 ns
Error	48	0.75333	24.48135	0.0640	8.046875	156.86
Total	**63**					

Table 3. Means squares (MS) from the analysis of variance (ANOVA) for yield parameters of two varieties of wheat (*Triticum aestivum* L.) under salt stress.

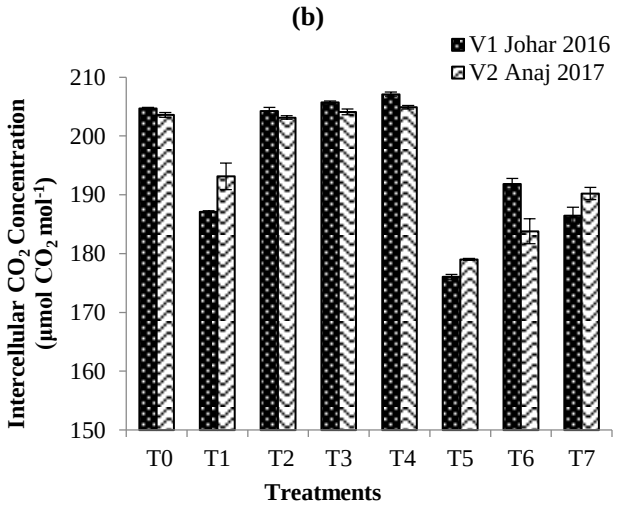
Source of variance	df	No of spikes/Plant	No of grains/Spike	No of spikelet/Plant	100 Grains weight (gram)
Salt	1	5.64062 ns	92.6406 ***	112.890***	0.58140*
Sorb	3	42.0156***	28.473958***	38.557***	1.22265***
Var	1	13.1406**	37.51562***	62.015***	0.09765ns
Salt × Sorb	3	19.3489***	41.01562***	38.057***	0.93432***
Salt ×Var	1	0.39062 ns	0.14062ns	0.0156ns	0.08265ns
Sorb × Var	3	4.4322916 ns	4.30729ns	1.0989ns	0.05807ns
Salt × Sorb × Var	3	5.68229 *	2.432291ns	2.3489ns	0.33640*
Error	48	1.73437	2.880208	3.1614	0.0823437
Total	**63**				

Table 4. Means squares (MS) from the analysis of variance (ANOVA) for biochemical parameters of two varieties of wheat (*Triticum aestivum* L.) under salt stress.

Source of variance	df	Carbohydrates contents (mg/ml)	Protein contents (mg/ml)	Chlorophyll 'a' content (mg/g f.wt)	Chlorophyll 'b' content (mg/g f.wt)	Carotenoids content (mg/g f.wt.)	Catalase (CAT) (units/mg protein)	POD (units/mg protein)	SOD (units/mg protein)	Electrolyte leakage (dS m^{-1})	Glycine betaine content (µ mol/g dry wt.)	Malondialdehyde (MDA) content (µmole/l)	Anthocyanin content (mg/L)
Salt	1	0.0083 **	0.0310 ns	2.8942 **	3.0756 bs	4.0481 ns	4.1006 ns	0.0010**	0.0087ns	190.961 ***	0.0138*	0.02556**	2.078*
Sorb	3	0.0414***	0.7890**	3.038***	4.6070*	2.7688***	0.018***	0.0011***	0.0202**	102.507 ***	0.0100**	0.0125**	1.8128*
Var	1	0.0211***	0.1683 ns	5.6437 ns	5.6109 *	1.8564 ns	0.0010ns	4.6225 ns	0.0206*	55.5565*	0.0042ns	0.01846*	1.4683ns
Salt × Sorb	3	0.0012***	0.7855**	2.428***	5.8520 **	1.5607**	0.0054ns	0.0010***	0.0187*	178.156***	0.0073*	0.01169**	5.4801ns
Salt ×Var	1	0.0012ns	0.0148 ns	2.1275 ns	1.1138 ns	6.6015 ns	3.0625ns	7.6562 ns	0.0028ns	14.831 ns	6.8062ns	3.16406 ns	4.378ns
Sorb× Var	3	9.6839ns	0.0132 ns	8.1384 ns	1.056ns	4.6895ns	2.9560ns	4.5262*	0.0024ns	13.7473 ns	1.3968ns	0.00131 ns	7.115ns
Salt×Sorb×Var	3	8.4334ns	0.0432 ns	7.2609 ns	1.692ns	1.6223ns	5.1977ns	1.3660ns	0.0014ns	19.727 ns	1.9518ns	1.5640ns	2.8470ns
Error	48	0.0010	0.16826	3.1280	1.1220	3.4752	0.00236	1.2994	0.0047	12.8930	0.0020	0.0025	4.6839
Total	**63**												



T0 (Control): T1 (100 mM NaCl): T2 (25 mM Sorbitol): T3 (50 mM Sorbitol): T4 (75 mM Sorbitol): T5 (25mM Sorbitol+100 mM NaCl): T6 (50mM Sorbitol + 100mM NaCl): T7 (75mM + 100mM)



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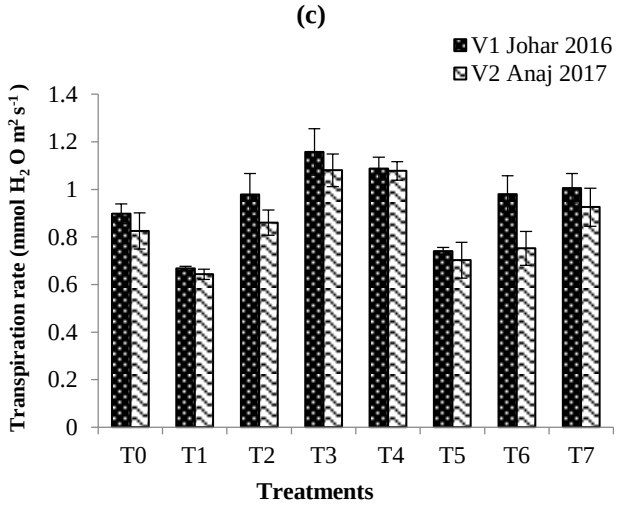

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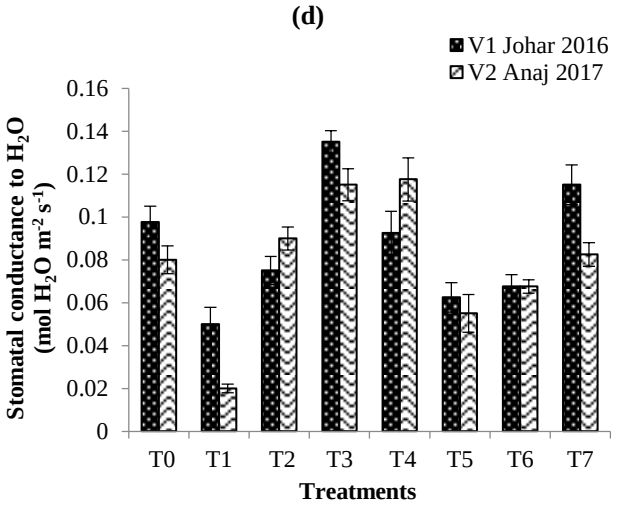

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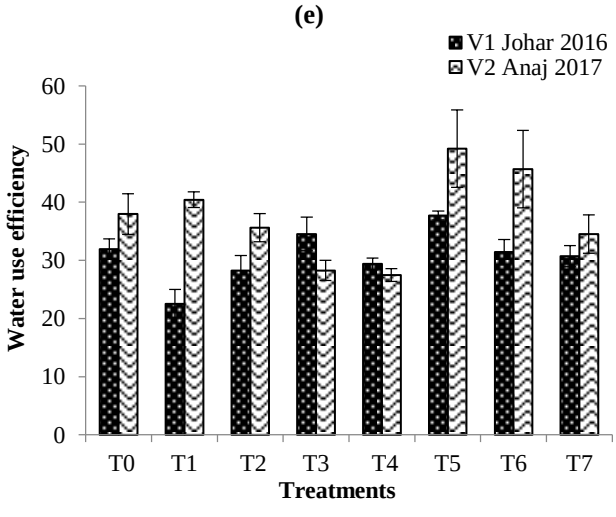

T0 (Control): T1 (100 mM NaCl): T2 (25 mM Sorbitol): T3 (50 mM Sorbitol): T4 (75 mM Sorbitol): T5 (25mM Sorbitol+100 mM NaCl): T6 (50mM Sorbitol + 100mM NaCl): T7 (75mM + 100mM)

Fig. 2. Effect of Foliar application of Sorbitol on various Physiological parameters of Wheat.

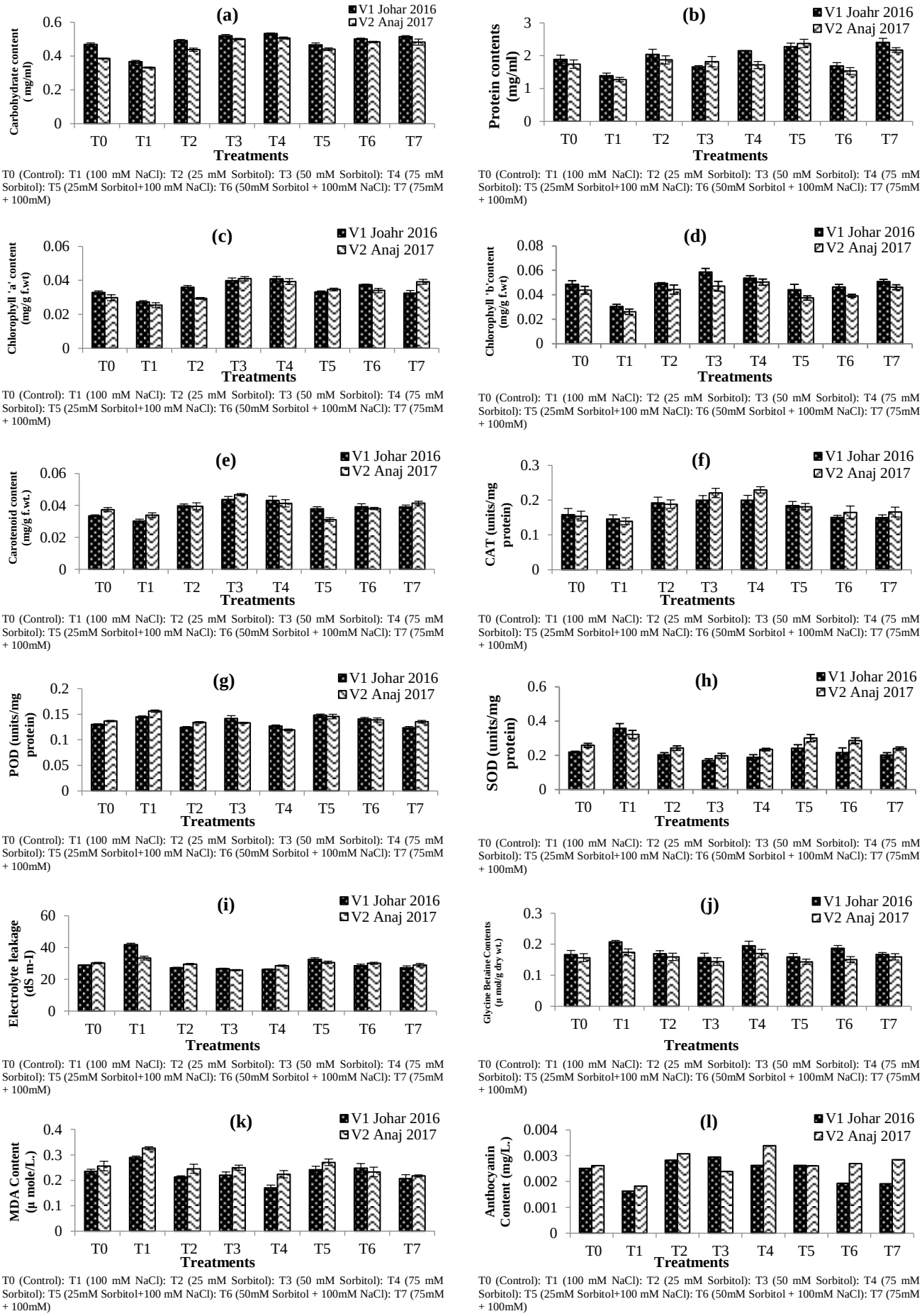

Fig. 3. Effect of Foliar application of Sorbitol on various Biochemical parameters of Wheat.

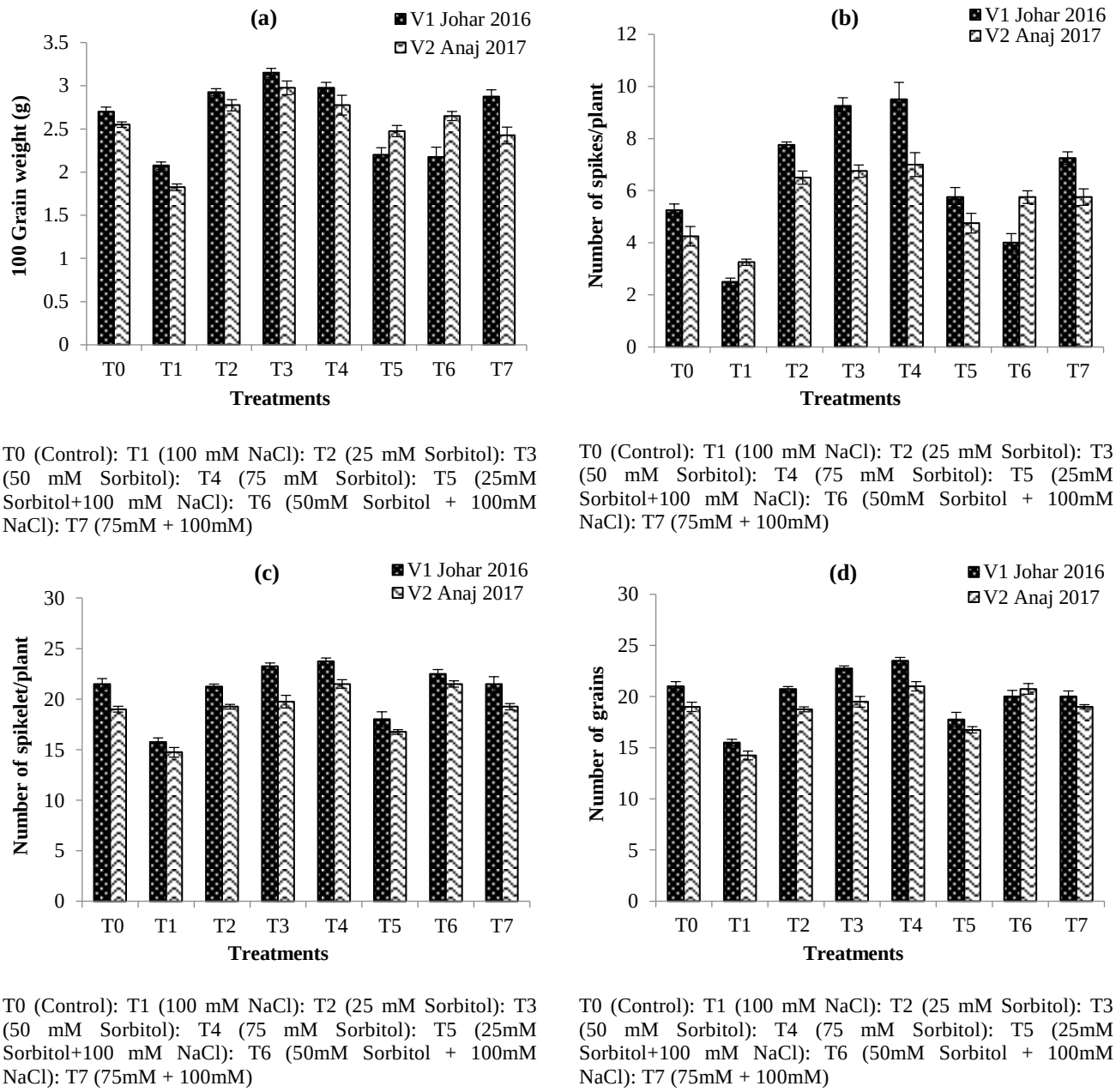

Fig. 4. Effect of foliar application of Sorbitol on various Yield parameters of Wheat.

Yield parameters: As salinity stress affected adversely on all parameters of wheat plant and all these parameters directly influenced the yield of crop. It was noticed that salt stress declined all yield parameters such as weight of seed, no. of spikes, no. of spikelet and no. of grain of wheat crop (Fig. 4a, b, c, d). Sorbitol application significantly increased yield of salt affected as well as normal plants and ANOVA results were also highly Signiant (Table 3). These findings of our experiment are in consistence with the results of Gul *et al*., (2019). They determined that NaCl stress declined the weight of gain and no. of grains in Wheat. Shahzad *et al*., (2016) also noticed declined no. of spikes, no. of spikelet and grain weight and grain number in Wheat after salt treatment. The results of our experiment were also correlated with the results of Yadav *et al*., (2020) where NaCl stress cause reduction in the grain yield of Wheat but Foliar application of salicylic acid increased the yield of crop and help to mitigated effects of salt stress.

Conclusion

Wheat is an important crop around the globe and in order to meet food security challenges in future appropriate wheat production is necessary. Overall sorbitol's application by foliar method proved useful for wheat plants. The results of sorbitol on both varieties were positive. So the use of Sorbitol for the cure of salt stress can be useful in this aspect. Despite sorbitol's application on non-salt treated plants also proved useful therefore its application can also be performed in order to increase crop yield.

References

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant Physiol.*, 24(1): 1.

Babu, M.A., D. Singh and K.M. Gothandam. 2012. The effect of salinity on growth, hormones and mineral elements in leaf and fruit of tomato cultivar PKM1. *J. Anim. Plant Sci.*, 22(1): 159-164.

Barnawal, D., N. Bharti, S.S. Pandey, A. Pandey, C.S. Chanotiya and A. Kalra. 2017. Plant growth-promoting rhizobacteria enhance wheat salt and drought stress tolerance by altering endogenous phytohormone levels and TaCTR1/TaDREB2 expression. *Physiol. Plant*, 161(4): 502-514.

Barutcular, C., A. El Sabagh, M. Koc and D. Ratnasekera. 2017. Relationships between grain yield and physiological traits of durum wheat varieties under drought and high temperature stress in mediterranean environments. *Fresen. Environ. Bull*., 26(6): 4282-4291.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72(1-2): 248-254.

Cakmak, I. and W.J. Horst. 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiol. Plant*, 83(3): 463-468.

Chance, B. and A.C. Meahly. 1995. Assay of catalase and peroxidase. In Methods in enzymology. (pp. 764-775). New York: Academic press. Inc.

Daoud, A.M., M.M. Hemada, N. Saber, A.A. El-Araby and L. Moussaf. 2018. Effect of silicon on the tolerance of wheat (*Triticum aestivum* L.) to salt stress at different growth stages: case study for the management of irrigation water. *Plants*, 7(2): 29.

Darwish, M.A.H., W.Z.E. Farhat and A. El Sabagh. 2018. Inheritance of some agronomic characters and rusts resistance in fifteen f2 wheat populations. *Cerc. Agron. Mold.*, 51(1): 5-28.

Deivanai, S., R. Xavier, V. Vinod, K. Timalata and O.F. Lim. 2011. Role of exogenous proline in ameliorating salt stress at early stage in two rice cultivars. *J. Stress Physiol. Biochem*., 7(4): 157-174.

Dogan, M. 2011. Antioxidative and proline potentials as a protective mechanism in soybean plants under salinity stress. *Afr. J. Biotech*., 10(32): 5972-5978.

Dugasa, T., B. Bebie, R.P.S. Tomer and J. Barnabas. 2016. Effect of seed priming on salt tolerance of bread wheat (*Triticum aestivum* L.) varieties. *J. Sci*., 6(3): 139-153.

Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol*., 59(2): 309-314.

Grieve, C.M. and S.R. Grattan. 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant Soil*, 70(2): 303-307.

Gul, H., M. Farman, L. Anwar Hussain, M. Irshad and M. Azeem. 2017. Exogenously applied sorbitol alleviates the salt stress by improving some biochemical parameters in spinach (*Spinacia oleracea* L.). *Int. J. Biol. Biotech*., 14 (4): 677-686.

Gul, M., A. Wakeel, D. Steffens and S. Lindberg. 2019. Potassium-induced decrease in cytosolic Na^+ alleviates deleterious effects of salt stress on wheat (*Triticum aestivum* L.). *Plant Biol*., 21(5): 825-831.

Gupta, B. and B. Huang. 2014. Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. *Int. J. Geno*., 1-18.

Hniličková, H., F. Hnilička, M. Orsák and V. Hejnák. 2019. Effect of salt stress on growth, electrolyte leakage, Na+ and K+ content in selected plant species. *Plant Soil Environ*., 65(2): 90-96.

Hong-juan, J., Y. Yan-jie and Z. Guo-qiang. 2017. Hydrogen peroxide involved in tip growth of wheat (*Triticum aestivum*) root hairs by high concentration chlorogenic acid. *Can. J. Agri. Crops*, 2(1): 22-33.

Iqbal, M., S. Irshad. M. Nadeem, T. Fatima and A. B. Itrat. 2018. Salinity effects on wheat (*Triticum aestivum* L.) characteristics - a review article. *Int. J. Biosci*., 12(3): 131-146.

Jahan, M.A.H.S., A. Hossain, A. Jaime, T. Da Silva, A. EL Sabagh and M.H. Rashid. 2019. Effect of naphthaleneacetic acid on root and plant growth and yield of ten irrigated wheat genotypes. *Pak. J. Bot*., 51(2): 451-459.

Jan, S.A., Z.K. Shinwari and M.A. Rabbani. 2016. Agro-morphological and physiological responses of *Brassica rapa* ecotypes to salt stress. *Pak. J. Bot.*, 48(4): 1379-1384.

Khaliq, A., M. Zia-ul-Haq, F. Ali, F. Aslam, A. Matloob and A. Navab. 2015. Salinity tolerance in wheat cultivars is related to enhanced activities of enzymatic antioxidants and reduced lipid peroxidation. *Clean–Soil, Air, Water*, 43(8): 1248-1258.

Khan, A., M. Shafi, J. Bakht and S. Anwar. 2017. Effect of salinity and seed priming on growth characters of wheat varieties. *Sarh. J. Agri*., 33(3): 435-446.

Khan, M.J., S. Ahmad and K.N. Saddozai. 2008. Economic analysis of wheat profitability in Peshawar Valley, NWFP. *Pak. J. Social Life Sci*., 6(2): 112-117.

Krizek, D.T., G.F. Kramer, A. Upadhyaya and R.M. Mirecki. 1993. UV-B response of cucumber seedlings grown under metal halide and high pressure sodium/deluxe lamps. *Physiol. Plant.*, 88(2): 350-358.

Lutts, S., J.M. Kinet and J. Bouharmont. 1996. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Ann. Bot*., 78(3): 389-398.

Meng, D., C. Li, H.J. Park, J. González, J. Wang, A.M. Dandekar and L. Cheng. 2018. Sorbitol modulates resistance to Alternaria alternata by regulating the expression of an NLR resistance gene in apple. *Plant Cell*, 30(7): 1562-1581.

Najar, R., S. Aydi, S. Sassi-Aydi, A. Zarai and C. Abdelly. 2019. Effect of salt stress on photosynthesis and chlorophyll fluorescence in Medicago truncatula. *Plant Biosys.*, 153(1): 88-97.

Rasool, R., B.A. Ganai, S. Akbar, A.N. Kamili and A. Masood. 2010. Phytochemical screening of *Prunella vulgaris* L.-an important medicinal plant of Kashmir. *Pak. J. Pharm. Sci*., 23(4): 399-402.

Sadak, M.S., E.M.A. Elhamid and H.M. Mostafa. 2013. Alleviation of adverse effects of salt stress in wheat cultivars by foliar treatment with antioxidants I. changes in growth, some biochemical aspects and yield quantity and quality. *J. Agri. Environ. Sci*., 5(13): 1476-1487.

Saddiq, M.S., S. Iqbal, I. Afzal, A.M. Ibrahim, M.A. Bakhtavar and M.B. Hafeez. 2019. Mitigation of salinity stress in wheat (*Triticum aestivum* L.) seedlings through physiological seed enhancements. *J. Plant Nutr*., 42(10): 1192-1204.

Shahid, M.A., M.A. Pervez, R.M. Balal, R. Ahmad, C.M. Ayyub and T. Abbas. 2011. Salt stress effects on some morphological and physiological characteristics of okra (*Abelmoschus esculentus* L.). *Soil Environ*., 30(1): 66-73.

Shahzad, M., Z.A. Saqib, F. Hafeez, M. Bilal, S.A. Khan and S. A. Asad. 2016. Growth-related changes in wheat (*Triticum aestivum* L.) genotypes grown under salinity stress. *J. Plant Nutr*., 39(9): 1257-1265.

Tanveer, K., S. Gilani, Z. Hussain, R. Ishaq, M. Adeel and N. Ilyas. 2020. Effect of salt stress on tomato plant and the role of calcium. *J. Plant Nutr*., 43(1): 28-35.

Teo, G., Y. Suzuki, S.L. Uratsu, B. Lampinen, N. Ormonde and W.K. Hu. 2006. Silencing leaf sorbitol synthesis alters long-distance partitioning and apple fruit quality. *Proceed. Nat. Acad. Sci*., 103(49): 18842-18847.

Theerakulpisut, P. and W. Gunnula. 2012. Exogenous sorbitol and trehalose mitigated salt stress damage in salt-sensitive but not salt-tolerant rice seedlings. *Asian J. Crop Sci*., 4: 165-170.

Trivellini, A., B. Gordillo, F.J. Rodríguez-Pulido, E. Borghesi, A. Ferrante and P. Vernieri. 2014. Effect of salt stress in the regulation of anthocyanins and color of Hibiscus flowers by digital image analysis. *J. Agri. Food Chem*., 62(29): 6966-6974.

Wu, W., Q. Zhang, E. Ervin, Z. Yang and X. Zhang. 2017. Physiological mechanism of enhancing salt stress tolerance of perennial ryegrass by 24-epibrassinolide. *Front. Plant Sci.*, 8: 1017.

Xie, J., Y. Dai, H. Mu, Y. De, H. Chen and Z. Wu. 2016. Physiological and biochemical responses to NaCl salinity stress in three Roegneria (Poaceae) species. *Pak. J. Bot.*, 48(6): 2215-2222.

Yadav, T., A. Kumar, R.K. Yadav, G. Yadav, R. Kumar and M. Kushwaha. 2020. Salicylic acid and Thiourea mitigate the salinity and drought stress on physiological traits governing yield in pearl millet-wheat. *Saudi J. Biol. Sci.*, 27(8): 2010-2017.

Yassin, M., A. El Sabagh, A.M.M. Mekawy, M.S. Islam, A. Hossain and C. Barutcular. 2019. Comparative performance of two bread wheat (*Triticum aestivum* L.) Genotypes Under Salinity Stress. *Appl. Ecol. Environ. Res*., 17(2): 5029-5041.

Zhang, T., Z. Shi, X. Zhang, S. Zheng, J. Wang and J. Mo. 2020. Alleviating effects of exogenous melatonin on salt stress in cucumber. *Sci. Hort.*, 262: 109070.

Zou, P., K. Li, S. Liu, X. He, X. Zhang, R. Xing and Pengcheng, Li. 2016. Effect of sulfated chitooligosaccharides on wheat seedlings (*Triticum aestivum* L.) under salt stress. *J. Agri. Food Chem.*, 64(14): 2815-2821.

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