EFFECT OF NEPHTHYL ACETIC ACID FOLIAR SPRAY ON AMELIORATION OF SALT STRESS TOLERANCE IN MAIZE (*ZEA MAYS* L.)

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Abstract. The present study was aimed to evaluate the effect of naphthyl acetic acid (NAA) on chlorophyll, protein, proline, sugar and carotenoid contents along with certain enzymatic activities, namely peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APOX) in two selected varieties i.e. Iqbal and Pahari of maize crop induced under salt stress of different concentrations (100mM, 80mM, 60mM, 40mm and 20mM). The experiment was conducted at the Department of Botany, Bacha Khan University Charsadda, Pakistan during maize growing season, 10-09- 2015. The result of the study revealed that different concentrations of NaCl affect the physiological and biochemical parameters. The anti-oxidative enzymes, such as peroxidase (POD), superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APOX), and sugar, proline, and proteins were found maximum in treatment T2, T4, T10 and T12 in the Pahari variety which on physiological basis was found more tolerant to the saline condition and regarding the response to exogenous application of naphthyl acetic acid (NAA) the variety Iqbal was more sensitive to salt stresses.
Keywords: *nephthyl acetic acid, selective growth, antioxidant enzymes, action, salt stress tolerance*

Introduction

Maize (*Zea mays* L.) is one of the most important crops which are used as breakfast and the oil of maize for diabetic patient all over the world. Maize is also used as food

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for animals (Hussain et al., 2010). It is important measure salt stress tolerance of plants because up- or down-regulation of salt changes physiological mechanisms in the plants (Tas and Basar, 2009). The salt was effect resolute at seed earlier stage of wheat from a decrease in germination percentage, fresh and dry weight of shoots and roots due to the translocation of several nutrient ions (Afzal et al., 2005). The presence of salt in the soil of marshlands or when salt is already a part of the soil is called primary or main salinization, but some plants are growing and adapted to saline soil. Minor or secondary salinization occurs in the soil where salt concentration is low and sometimes soil becomes saltier because of poor and low irrigation (Zhu, 2007). Soil salinization is one of the major factors of soil squalor or soil salinity. Soil salinization reached about 19.5% of the irrigation land and 2.1% of the dryland agriculture current in the world (FAO, 2000). In Pakistan, after wheat, cotton, and rice, maize is the fourth largest grown crop. In Pakistan one million hectares of maize production produce about 3.5 million metric tons. Punjab contributes 39% of the total area under maize and 30% of total production, Khyber Pakhtunkhwa contributes 56% of the total area production, Sindh contributes 63% of the production and Baluchistan 5% of the total area (PARC, 2010*).* Salt stress creates both ionic as well as osmotic stress on plants (Parvaiz and Satyawati, 2008). Salt stress is a major abiotic stress that can affect plant morphology and physiology, in this way the fresh and dry parts of shoots and roots are decreased perhaps due to the high concentration of salt ions or water-related qualities (Hajer et al., 2006). The leaf chlorophyll imbalance due to salt stress (abiotic stress) reduces photosynthesis (Turan et al., 2007). Salt stress is caused by high concentration of Na^+ and Cl^- in the environment that reduce the fertility of the soil so plant roots can't grow in the saline soil (Rasool et al., 2013). When maize, wheat, and rice etc. seeds are sown in saline soils, salt (Na^+ and Cl) cause many adverse symptoms on seed germination, as result less number of seeds germinate, plant growth is stunted, height of the plant becomes short, leaves are smaller and thicker than in normal size of plants and the color is dark green and bluish. When plants grow in saline soils, the various plant parts e.g. leaves, fruits, roots, stems etc. are very small and so they affect quality and quantity of fruits, vegetables, and agricultural products e.g. high concentration of Na^+ also decrease sugar production in sugar crops (Storey et al., 1977). Salinity causes two major effects osmotic stress and ionic toxicity in plants, these two major effects osmotic stress, and ionic toxicity affect the various processes in the plant (Yadav et al., 2011).

To use growth-stimulating chemicals (Naphthalene acetic acid) for the increase of plant production and also stimulate plant physiologists the world (Ahmad et al., 2010). To study the upward transport of nutrients due to growth regulator chemical Naphthalene acetic acid (NAA), these growth regulator chemical Naphthalene acetic acid (NAA), ameliorate growth of the physiological and proteins, carbohydrate, sugar etc. the of plants (Iqbal et al., 2009; Tůmová et al., 2018). Growth regulators or hormones stimulate and promote growth in plants. Naphthalene acetic acid (NAA) is a growth controller and also increases the production and yield of plants. Naphthaleneacetic acids (NAA) are synthetic growth regulator hormones which ameliorate the potential of plants with suitable concentration. Naphthaleneacetic acid (NAA) also affects the growth, yield, and production of tomatoes plants. (Jahan and Fattah, 1991). Keeping in view all the above information, the present study will be conducted to screen maize (Pahari and Iqbal) accessions for salt stress tolerance. In addition, physiological and biochemical variations are also assessed for some physiological traits that could be used as selection criteria for future breeding programs.

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Aims of the study

The present study was aimed at assessing the physiological mechanism of adaptation to salt tolerance in maize at the vegetative stage with variable levels of salt stress (20, 40, 60, 80, and 100mM of salt, accompanied by the application of naphthyl acetic acid (NAA) foliar spray). The reactions of maize to salt stress were assessed on the basis of selectivity of growth responses, osmoregulation, and antioxidant enzyme actions.

Materials and methods

Experimental design

The experiment was conducted during the 10- 09- 2015 corn growing season in the greenhouse of the Botanical Department of Bacha Khan University Charsadda (latitude 34.1509', east longitude 71.735'E, 908 feet above sea level). Seeds of two seed selections, Iqbal and Pahari, are from the Crop Research Institute Pirsabaq Nowshehra (CCRI). Seeds were sterilized with 5% oxychloride and 95% ethanol before planting and then rinsed three times with distilled water. After soaking, the seeds were sown in plastic pots (14 cm below the inner diameter, inner diameter 18.5 cm, height 15.6 cm, thickness 0.5 cm), filled with 3 kg of air-dried soil and sand (3:1), and placed in triplicate. The plants were exposed to 20, 40, 60, 80 and 100 mM salts for about 15 (26- 09- 2015) days after germination in an incubator with an average day and night temperature of 25°C (10 hours) and 16°C (14 hours), respectively. In the group experiments, all treatments were sprayed with NAA for 1 week; control plants were usually irrigated with distilled water. After 15 days, leaf samples were collected for analysis.

Chemical analysis of rhizospheric soil

Soil pH and electrical conductivity (EC)

The pH of the rhizospheric soil was measured by preparing a 1:1 (soil: water) suspension (McKeague, 1978; McLean, 1982). Air-dried soil samples (10 g) were mixed in 10 ml of distilled water and stirred for 1 hour in a magnetic stirrer for uniform mixing, and then the suspension was filtered through Whatman No. 42 filter paper. The pH of the filtrate was measured with an Electrical pH meter while the EC was recorded using an electrical conductivity (EC) instrument. The readings were measured in micro-siemens per centimeter (μS/cm). Soil Moisture (10 g) was taken from a uniform depth, 6 inches from the surface of the pot. The dry weight was determined by drying the soil in an oven at 70°C for 72 hours to constant weight.

Soil moisture content

The soil (10 g) was removed from the same depth, i.e. 6 inches from the surface of the pot. The dry weight was determined by drying the soil in an oven at 70°C for 72 hours to constant weight. The soil moisture content is calculated by the following formula:

$$\text{Parentage moisture content} = \frac{(\text{Fresh weight of soil} - \text{the Dry weight of soil}) \times 100}{\text{Fresh weight of soil}} \qquad \text{(Eq.1)}$$

Field capacity of the rhizospheric soil

The field capacity of rhizospheric soil was determined by the following method:

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$$\text{Parentage Field Capacity} = \frac{(\text{Weight of wet soil (g)} - \text{Weight of dry soil (g)}) \times 100}{\text{Weight of dry soil}} \quad \text{(Eq.2)}$$

The agronomic character of maize under drought stress

Detailed agronomic characterization of maize under drought stress was performed, including:

- Root length
- Root fresh weight
- Root dry weight
- Root moisture content
- Shoot length
- Shoot fresh weight
- Shoot dry weight
- Shoot moisture content
- Number of leaves
- Leaf fresh weight
- Leaf dry weight
- Leaf moisture content
- Germination
- Leaf area
- Root/shoot ratio
- Vigorous index

Physiological and biochemical analysis

- The protein content of leaves was determined following the method of Lowry et al. (1951) using BSA as standard.
- Sugar estimation of fresh leaves was done following the method of Dubois et al. (1956).
- Chlorophyll content of leaves was determined by the method of Arnon (1949).
- The proline content of leaves was measured by the method of Bates et al. (1973).
- Peroxidase (POD) activity was determined by the method of Vetter et al. (1958) as modified by Gorin and Heidema (1976).
- Superoxide dismutase (SOD) activity was determined by measuring the inhibition of the photochemical reduction of nitro-blue tetrazolium (NBT) using the method of Beauchamp and Fridovich (1971).
- Ascorbate peroxidase (APX) activity was determined according to Asada and Takahashi (1987).
- Catalase (CAT) was measured according to Asada and Takahashi (1987), with modification.

Statistical analysis

The data were analyzed statistically by the Analysis of Variance technique (Steel and Torrie, 1980) and comparison among treatment means was made by Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

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Results

Physicochemical characteristics of rhizospheric soil

The effect in *Table 1*, *Equations 1 and 2* shows that the extreme pH of the treatment for T6 (60 mM) was recorded in Iqbal and Pahari compared to the control (untreated). A decrease in the pH and EC of the soil collected from Iqbal and Pahari was noted in T10 (100 mM + NAA), reflecting the positive role of NAA in maintaining EC and pH under induced salt stress. In Iqbal and Pahari, the maximum moisture content percentage and field capacity percentage for T8 (80 mM + NAA) treatment were reported; T6 reported the minimum for both varieties.

Table 1. *Effect of salinity on field water holding capacity, moisture content, soil pH and soil conductivity of maize (Zea mays L.)*

Treatments	Field Capacity (%)		Moisture content (%)		Soil pH		Electric Conductivity (S/m)	
	VI	V2	VI	V2	VI	V2	VI	V2
Tl	18.20824±0.01	13.91949±0.02	15.05717±0.012	11.13425±0.013	7.3±0.012	7.3±0.013	700±0.071	302.5±0.072
T2	12.27679±0.04	11.19778±0.05	10.93439±0.06	10.070 15±0.07	7.5±0.06	7.4±0.07	995±0.02	700±0.03
T3	13.971 17±0.04	30.04483±0.05	12.23155±0.06	23.09645±0.07	7.05±0.06	7.1±0.07	1000±0.08	995±0.09
T4	18.69354±0.04	11.5757±0.05	15.74942±0.01	10.37475±0.02	7.6±0.01	7.1±0.02	1263±0.015	1000±0.016
T5	20.62099±0.027	18.5961±0.028	16.41353±0.08	15. 44271 ±0.09	7.25±0.08	7.25±0.09	1246±0.02	1263±0.03
T6	14.26551±0.08	0.813335±0.09	12.48453±0.015	0.806773±0.016	7.4±0.015	7.2±0.016	761±0.05	1246±0.05
T7	18.25423±0.08	18.52983±0.07	15.28907±0.02	15.36283±0.03	7.2±0.02	7.3±0.03	1510±0.033	761±0.034
T8	23.75444±1.01	1.993725±1.02	19.19482±0.06	1.954753±0.07	7.4±0.06	7.2±0.07	750±0.045	1510±0.046
T9	14.82779±0.06	22.93297±0.07	11.84361±0.06	18.6276±0.07	7.15±0.06	7.15±0.07	720±0.075	750±0.08
T10	11.34148±0.06	1.588586±0.07	10.18621±0.011	1.56374±0.012	7.4±0.011	7.1±0.012	1080±0.027	720±.028
T1l	19.4188±1.03	15.44447±1.02	15.98667±0.034	12.66257±0.035	6.9±0.034	7.05±0.035	670±0.035	1080±0.036
T12	15.73781±0.01	11.52747±0.02	13.59781±0.015	10.33599±0.016	7.1±0.015	7.10±0.016	1846±0.071	670±0.072

V1= (Iqbal), V2= (Pahari), Tl concentration) T6= (60mM + NAA). (Control + NAA). (100mM NaCl concentration), T2= (l00mM + NAA), T3= (80mM NaCl concentration), T4= (80mM + NAA), T5= (60mM NaCl T7= (40mM NaCl concentration) T8= (40mM + NAA) T9= (20mMNaCl concentration) T10 (20mM + NAA), T11= (Control), T12= (control +NAA)

Agronomic characteristics

The results in *Table 2* show that the maximum germination rate, leaf area, root activity and leaf area index (LAI) had been measured or calculated of (LAI = leaf area / ground area, m^2/ m^2) were recorded in the two varieties treated T12 (control + NAA), while the minimum germination rate was reported of T4 (80 mM + NAA). The results in *Table 3* indicate that the T12 treatment of the two varieties reported maximum shoot length, shoot fresh and dry weight, and moisture content, while T5 (60 mM) reported the minimum shoot length, indicating that NAA increased the shoot length under salt stress. The results in *Table 4* indicate that the maximum root length and root fresh and dry weight were recorded of T3 (40 mM) treatment in Iqbal, while the minimum values were reported in T5 and T6 (60 mM + NAA). The data in *Table 5* shows that treatment of T12 in both varieties reported maximum leaf number and leaf fresh and dry weight, and reported the smallest in T3 (60 mM) and T4. A detailed physiological and biochemical evaluation of these varieties revealed significant differences in these varieties under salt stress.

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Table 2. *Effect of salinity on root length, root freshness and dryness, and moisture content (Zea mays L.)*

Treatments	Root length (cm)		Root Fresh weight (g)		Root Dry weight (g)		Moisture content (%)	
	VI	V2	VI	V2	VI	V2	VI	V2
Tl	4.4±0.007	2.5±0.008	0.2015±0.05	0.143±0.06	0.1115±0.06	0.078±0.07	0.09±0.02	0.065±0.03
T2	5.9±0.017	3.5±0.018	0.235±0.035	0.24±0.036	0.115±0.015	0.129±0.016	0.12±0.06	0.111±0.07
T3	2±0.013	3.05±0.014	0.148±0.027	0.1275±0.028	0.0915±0.06	0.0425±0.07	0.0565±0.06	0.085±0.07
T4	5.5±0.01	2.5±0.02	0.141±0.02	0.09±0.03	0.099±0.011	0.082±0.012	0.042±0.06	0.008±0.07
T5	3±0.02	3.5±0.03	0.226±0.02	0.122±0.03	0.0855±1.01	0.0545±1.02	0.1405±0.02	0.0675±0.03
T6	6±0.034	2±0.035	0.213±0.045	0.414±0.046	0.12±0.013	0.321±0.014	0.093±0.05	0.093±0.06
T7	3.25±0.034	3±0.036	0.2085±0.02	0.234±0.03	0.138±0.011	0.162±0.012	0.0705±0.06	0.072±0.07
T8	3±0.001	2.5±0.002	0.244±0.027	0.13±0.028	0.138±0.01	0.05±0.02	0.106±0.04	0.08±0.05
T9	1.8±0.04	3±0.05	0.274±0.05	0.125±0.06	0.116±0.011	0.0905±0.012	0.158±0.17	0.0345±0.18
T10	5±0.015	2.3±0.016	0.165±0.01	0.112±0.02	0.076±0.015	0.09±0.016	0.089±0.04	0.022±0.05
T11	3.4±0.05	3.25±0.06	0.384±0.035	0.143±0.036	0.2815±0.01	0.078±0.02	0.1025±0.02	0.065±0.03
T12	4.8±0.024	3.3s±0.025	0.432±0.027	0.148±0.028	0.394±0.034	0.05±0.035	0.038±0.06	0.098±0.07

V1= (Iqbal), V2= (Pahari), Tl concentration) T6= (60mM + NAA), T7= (Control + NAA).: (l00mM Nacl concentration), T2= (l00mM + NAA), T3= (80mM Nacl concentration), T4= (80mM + NAA), T5= (60mM Nacl (40mM Nacl concentration) T8= (40mM + NAA) (T9= (20mM Nacl concentration) T10 (20mM + NAA), T11 = (Control), T12= (control+ NAA)

Table 3. *Effect of salinity on shoot length, shoot fresh weight, dry weight and moisture content of maize (Zea mays L)*

Treatments	Shoot length (cm)		Shoot Fresh Weight (g)		Shoot Dry Weight (g)		Moisture content (%)	
	VI	V2	VI	V2	VI	V2	VI	V2
Tl	10.1±0.026	13.9±0.026	0.038±0.06	0.0725±0.07	0.024±0.05	0.05±0.06	0.014±0.001	0.02±0.002
T2	12.3±0.04	15±0.04	0.056±0.012	0.069±0.013	0.02±0.037	0.06±0.038	0.036±0.05	0.009±0.06
T3	11.5±0.01	17±0.01	0.0615±0.08	0.108±0.09	0.045±0.01	0.0655±0.02	0.0165±0.042	0.228±0.03
T4	0.036±0.05	21.6±O.17	0.074±0.08	0.123±0.09	0.07i0.011	0.09±0.012	0.004±0.05	0.039±0.02
T5	9.15±0.04	18±0.04	0.0465±1.01	0.12±02	0.0265±0.08	0.0675±0.08	0.02i0.034	0.0525±0.035
T6	17.5±0.02	15±0.02	0.083±0.06	0.255±0.07	0.024±0.027	0.094±0.028	0.059±0.04	0.161±0.05
T7	17±0.02	20.15±0.02	0.1105±0.05	0.193±0.06	0.0305±0.035	0.0555±0.036	0.08±0.03	0.1375±0.04
T8	10.5±0.07	22.7±0.07	0.047±0.015	0.143±0.016	0.011i0.017	0.012±0.018	0.036±0.027	0.131±0.028
T9	9.75±0.02	18.75±0.02	0.1±0.034	0.1165±0.035	0.04±0.016	0.0775±0.017	0.06±0.01	0.039±0.02
T10	12.7±0.01	21±0.01	0.075±0.015	0.133±0.016	0.071±0.05	0.074±0.06	0.004±0.02	0.059±0.03
T11	18.2±0.06	34.25±0.06	0.3435±0.06	0.481±0.07	0.0545±0.033	0.061±0.034	0.289±0.034	0.42±0.035
T12	30±0.02	26.5±0.02	0.395±0.034	0.348±0.035	0.099±0.023	0.12±0.024	0.296±0.02	0.228±0.03

V1= (Iqbal), V2= (Pahari), Tl= concentration) T6= (60mM + NAA), (Control + NAA). = (l00mM Nacl concentration), T2= (l00mM + NAA), T3= (80mM Nacl concentration), T4= (80mM + NAA), T5= (60mM Nacl T7= (40mM Nacl concentration) T8= (40mM + NAA) T9= (20mM Nacl concentration) T10 (20mM + NAA), Tl1= (Control), T12= (control+ NAA)

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Table 4. *Effect of salinity on leaf number, leaf fresh and dry weight, and moisture content (Zea mays L.)*

Treatments	No of leaves (cm)		Leaf Fresh Weight (g)		Leaf Dry Weight (g)		Moisture content (%)	
	VI	V2	VI	V2	VI	V2	VI	V2
Tl	2±0.06	3±0.07	0.011±0.012	0.0325±0.013	0.0065±0.01	0.029±0.02	0.0045±0.07	0.0035±0.08
T2	2±0.01	3±0.02	0.01±0.024	0.013±0.025	0.007±0.027	0.009±0.028	0.003±0.01	0.004±0.02
T3	2.5±1.03	3.5±1.04	0.01±0.001	0.03±0.002	0.0065±0.02	0.0205±0.03	0.0035±0.06	0.0095±0.07
T4	3±0.04	4±0.05	0.008±0.033	0.032±0.034	0.002±0.03	0.029±0.04	0.006±0.02	0.003±0.03
T5	2.5±0.05	4±0.06	0.0155±0.014	0.0205±0.015	0.0095±0.033	0.0175±0.034	0.006±0.071	0.003±0.072
T6	2±0.015	3±0.016	0.013±0.024	0.065±0.025	0.007±0.17	0.052±0.18	0.006±0.02	0.013±0.03
T7	3±0.015	3±0.016	0.045±0.04	0.056±0.05	0.0315±0.04	0.0495±0.05	0.0135±0.017	0.0065±0.018
T8	3±0.06	4±0.07	0.019±0.047	0.037±0.048	0.011±0.02	0.03±0.03	0.008±0.06	0.007±0.07
T9	2±0.06	3.5±0.07	0.0335±0.023	0.0295±0.024	0.00845±0.034	0.023±0.035	0.025±0.021	0.0065±0.022
T10	3±1.03	3±1.04	0.028±0.08	0.042±0.09	0.02±0.07	0.033±0.08	0.008±0.02	0.009±0.03
T11	3.5±0.06	4±0.07	0.0825±0.027	0.079±0.028	0.04775±0.02	0.0765±0.03	0.03475±0.06	0.0025±0.07
T12	4±0.015	4±0.016	0.07±0.023	0.098±0.024	0.067±0.06	0.09±0.07	0.003±0.043	0.008±0.044

V1= (Iqbal), V2= (Pahari), Tl= (l00mM Nacl concentration), T2= (1mM + NAA), T3= (80mM Nacl concentration), T4= (80mM + NAA), T5= (60mM Nacl concentration) T6= (60mM + NAA), T7= (40mM Nacl concentration) T8= (40mM + NAA) T9= (20mM Nacl concentration) T10 (20mM + NAA), Tl1= (Control), T12= (Control + NAA)

Table 5. *Effect of salinity on leaf area, germination rate, vigor index and the root-shoot ratio of maize (Zea mays L.)*

Treatments	Leaf area (cm)		Germination (%)		Vigor's index		Root Shoot ratio (%)	
	VI	V2	VI	V2	VI	V2	VI	V2
Tl	2.225±0.05	7.375±0.06	3.5±0.012	2±0.013	184.14±0.07	219.25±0.08	4.905±0.012	1.947±0.013
T2	2.25±0.01	7±0.02	4±0.06	4±0.07	290.28±0.01	101.25±0.02	4.197±0.06	3.479±0.07
T3	2.975±0.01	7.8±0.02	3.5±0.06	3.5±0.07	169.425±0.06	378.63±0.07	2.290±0.06	2.280±0.07
T4	2.88±0.07	7.49±0.08	3±0.01	3±0.02	278.85±0.02	265±0.03	1.906±0.01	0.732±0.02
T5	3.25±0.06	12.25±0.07	3±0.08	3.5±0.09	96.2±0.071	104.25±0.073	3.401±0.08	1.048±0.09
T6	2.75±0.04	10.2±0.05	3±0.015	3±0.016	315±0.02	180±0.03	2.567±0.015	1.624±0.016
T7	12.75±0.03	12.325±0.04	3.5±0.02	3±0.03	217.5±0.017	229.95±0.018	3.434±0.02	1.269±0.03
T8	5.04±0.027	7.02±0.028	3±0.06	3±0.07	94.5±0.06	132±0.07	5.192±0.06	0.901±0.07
T9	3.46±0.023	5.75±0.024	2.5±0.06	3.5±0.07	96.25±0.021	113.5±0.022	1.576±0.06	1.507±0.07
T10	6.4±0.037	7.5±0.038	3±0.011	3±0.012	254±0.02	232±0.03	2.2±0.011	0.843±0.012
T11	9.45±0.05	18.5±0.06	2.5±0.034	2.5±0.035	16±0.06	144±0.07	1.191±0.034	0.922±0.035
T12	27±0.023	20.5±0.024	2 ±0.015	2±0.016	576±0.043	96.6±0.044	1.094±0.015	0.426±0.016

V1= (Iqbal), V2= (Pahari), Tl= (l00mM Nacl concentration), T2= (l00mM + NAA), T3= (80mM Nacl concentration), T4= (80mM + NAA), T5= (60mM Nacl concentration) T6= (60mM + NAA), T7= (40mM Nacl concentration) T8= (40mM + NAA) T9= (20mM Nacl concentration) T10 (20mM + NAA), Tl 1= (Control), T12= (Control + NAA)

Chlorophyll a/b ratio

The chlorophyll a/b ratio (mg/g) of the selected maize varieties Iqbal and Pahari during the vegetative phase was evaluated (*Figure 1*). The results showed that the maximum chlorophyll a/b ratio was found for T6 (60 mM + NAA) in Iqbal and T12 (control + NAA) in Pahari. In Iqbal, the chlorophyll a/b ratio content of the vegetative phase of T12 was statistically similar to T9 (100 mM), T10 (100 mM + NAA) and T11

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DOI: http://dx.doi.org/10.15666/aeer/1702_18171834

(control), at $P < 0.05$. The chlorophyll a/b ratios of the two varieties were the lowest, T1 (20 mM), T3 (40 mM), T4 (40 mM + NAA) and T8 (80 mM + NAA), at $P < 0.05$, indicating that NAA did not have a role in induction. The positive effect of improving chlorophyll a content under salt stress.

Total chlorophyll content

The results in *Figure 2* show that the total chlorophyll content (mg/g) is the largest of the T11 treated (control) out of the two varieties, while in Pahari, the value of T12 (control + NAA) is significantly higher than that of T9 (100 mM) and T10 (T10). Similarly, (100 mM + NAA) at $P < 0.05$ at the nutritional stage. The minimum total chlorophyll content in two varieties of T1 (20 mM), T2 (20 mM + NAA), T5 (40 mM) and T6 (40 mM + NAA) was reported, at $P < 0.05$.

Carotenoid

A comparative study of carotenoid content (µg/g) was carried out at the nutrient stage (*Figure 3*). The results showed that the maximum carotenoid content of the two varieties was reported in the T12 (control + NAA) treatment. In Iqbal, the carotenoid content of T12 (control + NAA) was significantly similar to the T11 (control) of the vegetative phase, at $P < 0.05$. The lowest carotenoid content in the two varieties was T1 (20 mM), T2 (20 mM + NAA), T3 (40 mM) and T5 (60 mM), at $P < 0.05$, indicating that NAA did not have a role in carotenoid content. The positive role enhanced and influence the content is induced under salt stress.

Sugar

The sugar content (µg/g) was evaluated at the nutrition stage (*Figure 4*). The results showed that the maximum sugar content was reported for both T12 (control + NAA) treatments, while in Iqbal, the carotenoid content of T12 was significantly similar to T5 (20 mM), T7 (80 mM) and T11, at $P < 0.05$ nutritional stage. The lowest sugar content of the two varieties was reported as T1 (20 mM), T2 (20 mM + NAA) and T3 (40 mM), at $P < 0.05$.

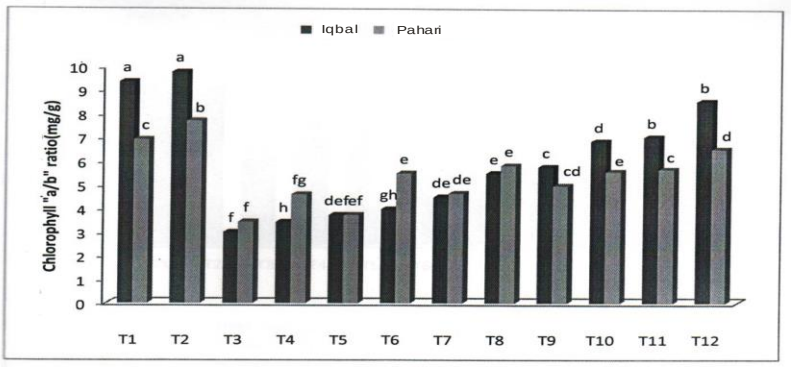

Figure 1. *Chlorophyll "a/b" ratio (mg/g) of the selected maize varities i.e. Iqbal and Pahari at vegetative stage acid induced soil salinity of l00mM, 80mM, 60mM, 40mM, 20mM with nephthyl acetic acid foliar spray. Tl= (l00mM NaCl concentration) T2= (l00mM + NAA) T3= (80mM NaCl concentration) T4= (80mM + NAA) T5= (60mM NaCl concentration) T6= (60mM + NAA) T7= (40mM NaCl concentration) T8= (40mM + NAA) T9= (20mM NaCl concentration) T10= (20mM + NAA) Tl1= (control) T12= (Control + NAA)*

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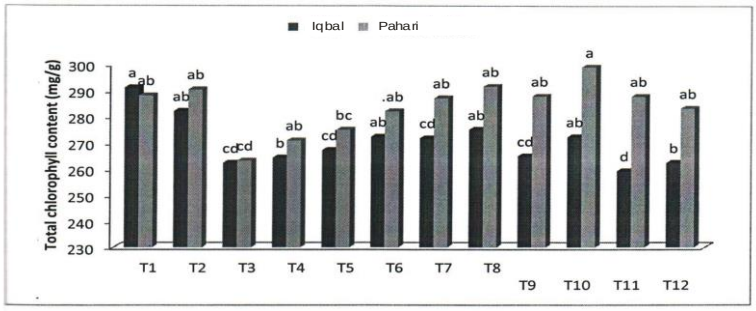

Figure 2. *Total Chlorophyll Content (mg/g) of the selected maize varities i.e Iqbal and Pahari at veget2: under induced soil salinity of l00mM, 80mM, 60mM, 40mM, 20mM with nephthyl acetic acid foliar spray. Tl= (l00mM NaCl concentration) T2= (l00mM + NAA) T3= (80mM NaCl concentration) T4= (80mM+NAA) T5= (60mM NaCl concentration) T6= (60mM + NAA) T7= (40mM NaCl concentration) T8= (40nM NAA) T9= (20mM NaCl concentration) T10= (20mM + NAA) Tl1= (control) T12= (Control - NAA)*

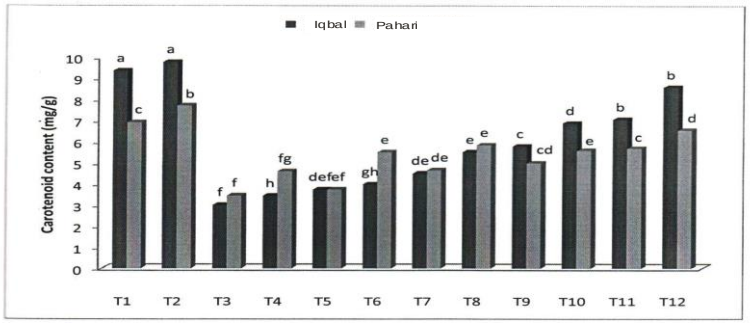

Figure 3. *Carotenoid content (mg/g) of the selected maize varities i.e. Iqbal and Pahari at vegetative stage under induced soil salinity of l00mM, 80mM, 60mM, 40mM, 20mM with nephthyl acetic acid foliar spray. Tl= l00mM NaCl concentration) T2= (l00mM + NAA) T3= (80mM NaCl concentration) T4= (80mM + NAA) T5= 60mM NaCl concentration) T6= (60mM + NAA) T7= (40mM NaCl concentration) T8= (40mM + NAA) T9= 20mM NaCl concentration) T10= (20mM + NAA) Tl1= (control) T12= (Control + NAA)*

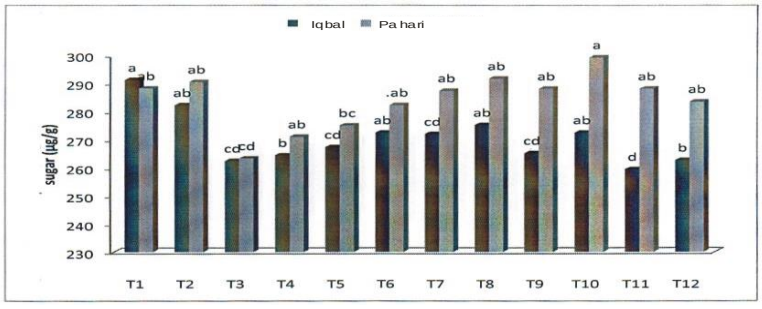

Figure 4. *Sugar content (ug/g) of the selected maize varities i.e Iqbal and Pahari at vegetative stage under - induced soil salinity of l00mM, 80mM, 60mM, 40mM, 20mM with nephthyl acetic acid foliar spray. Tl= 100mM NaCl concentration) T2= (l00mM + NAA) T3= (80mM NaCl concentration) T4= (80mM + NAA) T5= 60mM NaCl concentration) T6= (60mM + NAA) T7= (40mM NaCl concentration) T8= (40mM + NAA) T9= (20mM + NaCl concentration) T10= (20mM + NAA) T11 = (control) T12= (Control + NAA)*

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Proline

A comparison of proline content (μg/g) was carried out at the nutrient stage (*Figure 5*). The results showed that the maximum proline content was detected of T10 treatment in both varieties, while the carotenoid content of T10 (100 mM + NAA) was reported in Pahari. In the vegetative phase, it was significantly similar to T4 (40 mM + NAA), T5 (60 mM) and T9 (100 mM), at $P < 0.05$. The lowest proline levels of T3 (40 mM), T4 (40 mM + NAA), T6 (60 mM + NAA) and T8 (80 mM + NAA) were reported, at $P < 0.05$, indicating that NAA is active in the enhancing effect of induction of sugar content changes under salt stress

Protein

Protein content (μg/g) was assessed during the nutrition phase (*Figure 6*). The results showed that the maximum protein content was reported in T10 (100 mM + NAA) treated Pahari and Iqbal. Pahari protein content T10 (100 mM + NAA) Iqbal is significantly similar to T2 (20 mM + NAA), T4 (60 mM + NAA), T7 (80 mM) and T12 (control + NAA) cultivars with the same vegetative phase at $P < 0.05$. The lowest protein content of T7 (80 mM), T3 (40 mM), T5 (60 mM) and T6 (60 mM + NAA) was reported, at $P < 0.05$, both of which indicated that NAA did not induce protein content under salt stress influences.

Anti-oxidant enzymes

Various biological processes in organisms result in reactive oxygen species (ROS) which cause oxidative stress. In response to such oxidative stress, organisms can deploy superoxide dismutase (SOD) and catalase (CAT) to scavenge ROS so as to protect the cellular homeostasis (Balaban et al., 2005). Plants have enzymatic and non-enzymatic antioxidant mechanisms that counteract the adverse effects of salinity. The former includes enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX) and glutathione reductase (GR). The latter includes compounds such as ascorbate, glutathione, flavonoids, and vitamins C and E (Noctor and Foyer, 1998). Thus, an imbalance between the production of free radical species and the cellular antioxidant defence system will produce an appearance of oxidative stress. Cell damage caused by excess free oxygen free radicals has been explained as a result of changes in the cell membrane produced by acid oxidation of the lipid bilayer, a process known as lipid peroxidation. This produces changes in chemical composition and deterioration of cell membrane ultrastructure, reduces their fluidity, alters their permeability, and inactivates enzyme and membrane channel receptors (Mansour and Salama, 2004).

Peroxidase

Comparison of POD (OD/mint/g) in the selected maize varieties including Iqbal and Pahari at the vegetative stage was made from the plant samples collected from the designed experiment (*Figures 7,8*). The results showed that the T12 (control + NAA) treatment of both varieties reported the maximum POD, while the POD activity of Pahari, T12 (control + NAA) was significantly similar to T2 (20 mM + NAA) and T4 (40 mM + NAA). T6 (60 mM + NAA) and T8 (80 mM + NAA) at $P < 0.05$ at the vegetative stage. The lowest POD activity among the two varieties was T1 (20 mM), T7

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(80 mM) and T9 (100 mM + NAA), at $P < 0.05$, indicating that NAA has a positive role in the improvement of POD activity under salt stress induction.

Superoxide dismutase

The SOD activity (OD/min/g fw) in the selected maize varieties including Iqbal and Pahari at the vegetative stage was made for the plant samples collected from the designed experiment (*Figure 9*). The results showed that the maximum SOD was reported for both T12 (control + NAA) treatments, while in Iqbal, the SOD activity of T12 (control + NAA) was significantly similar to T2 (20 mM + NAA) and T4 (40 mM + NAA) and T6 (60 mM + NAA) in the vegetative phase at $P < 0.05$. It was reported that T9 (100 mM + NAA), T7 (80 mM), T10 (100 mM + NAA) and T11 (100 mM + NAA) had the lowest POD activity in two varieties, at $P < 0.05$, indicating that NAA acts to induce SOD activity under salt stress.

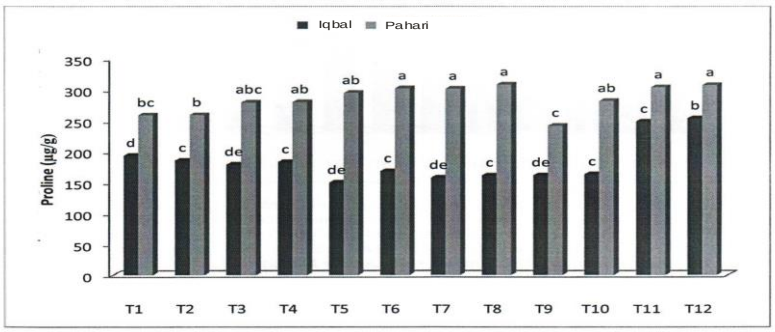

Figure 5. *Proline content (u.g/g) of the selected maize varities i.e. Iqbal and Pahari at vegetative stage under induced soil salinity of l00mM, 80mM, 60mM, 40mM, 20mM with nephthyl acetic acid foliar spray. Tl = 100mM NaCl concentration) T2= (l00mM + NAA) T3= (80mM NaCl concentration) T4= (80mM + NAA) T5= 60mM NaCl concentration) T6= (60mM + NAA) T7= (40mM NaCl concentration) T8= (40mM + NAA) T9= 20mM NaCl concentration) T10= (20mM + NAA) Tl1= (control) T12= (Control + NAA)*

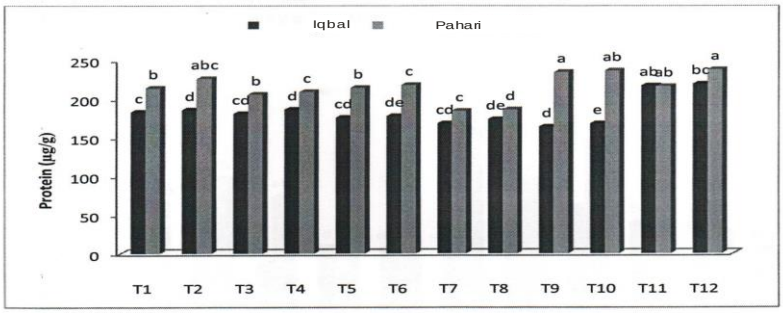

Figure 6. *Protein content (ig/g) of the selected maize varities i.e. Iqbal and Pahari at vegetative stage under induced soil salinity of l00mM, 80mM, 60mM, 40mM, 20mM with nephthyl acetic acid foliar spray. Tl= I l00mM NaCl concentration) T2= (l00mM + NAA) T3= (80mM NaCl concentration) T4= (80mM + NAA) T5= (60mM NaCl concentration) T6= (60mM + NAA) T7= (40mM NaCl concentration) T8= (40mM + NAA) T9= (20mMNaCl concentration) T10= (20mM + NAA) Tl1= (control) T12= (Control + NAA)*

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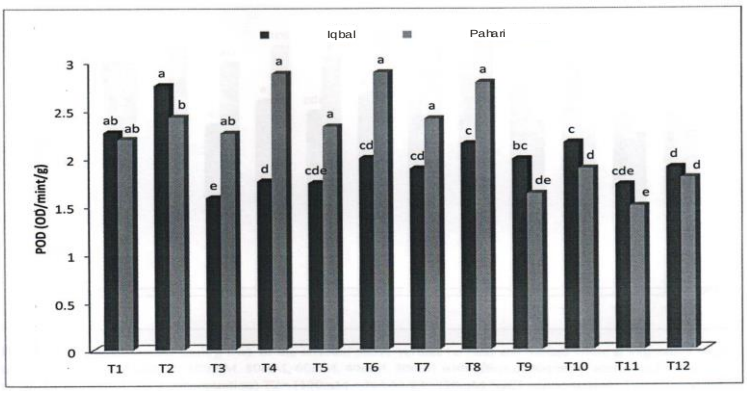

Figure 7. *Peroxidase (OD/mint/g) of the selected maize varities i.e Iqbal and Pahari at vegetative stage under induced soil salinity of l00mM, 80mM, 60mM, 40mM, 20mM with nephthyl acetic acid foliar spray. Tl= (l00mM NaCl concentration) T2= (l00mM + NAA) T3= (80mM NaCl concentration) T4= (80mM + NAA) T5= (60mM NaCl concentration) T6= (60mM + NAA) T7= (40mM NaCl concentration) T8= (40mM + NAA) T9= (20mM NaCl concentration) T10= (20mM + NAA) Tl 1= (control) T12= (Control + NAA)*

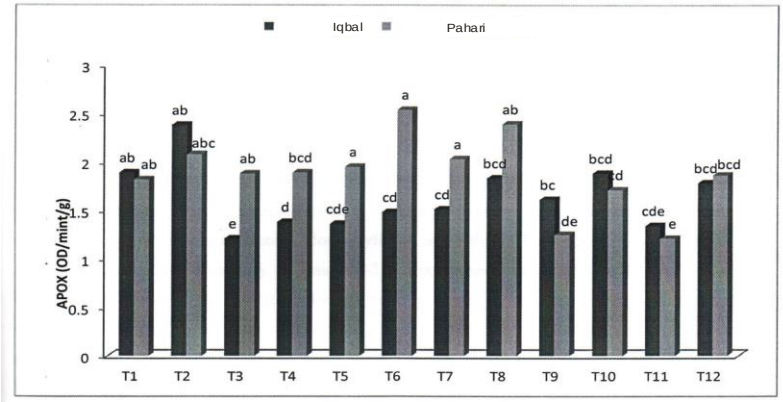

Figure 8. *Ascorbate peroxidase(OD/mint/g) of the selected maize varities i.e. Iqbal and Pahari at vegetative stage under induced soil salinity of 100Mm,80Mm,60rnM,40Mm,20Mm with nephthyl acetic acid foliar spray. V1= Variety 1 (Iqbal) V2= Variety 2 (Pahari) Tl= (l00Mm Salt solution) T2= (l00mM solution sprayed) T3=(80mM Salt solution) T4=(80mMSalt solution sprayed) T5= (60Mm Salt solution) T6= (60Mm Salt solution sprayed) T7=(40Mm Salt solution) T8=(40Mm Salt solution sprayed) T9=(20Mm Salt solution) T10=(20Mm Salt solution sprayed) T11 = (control) T12=(Control spray)*

Catalase

A comparison of CAT activities (OD/min/g fw) in the selected maize varieties including Iqbal and Pahari at vegetative stage were made for the plant samples collected from the designed experiment (*Figure 10*). The results showed that the maximum CAT was reported in the T12 (control + NAA) treatment of the two varieties, while in Pahari, the CAT activity of T12 (control + NAA) was compared with T6 (60 mM + NAA), T11 (control) and T4 (40 mM). + NAA) at $P < 0.05$ in the nutritional phase. The lowest CAT activity of T9 (100 mM + NAA), T1 (20 mM), T3 (40 mM), T5 (60mM) and T10

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(100mM + NAA) was reported in two varieties, at P<0.05, indicating positive NAA effect of enhancing CAT activity under induced salt stress.

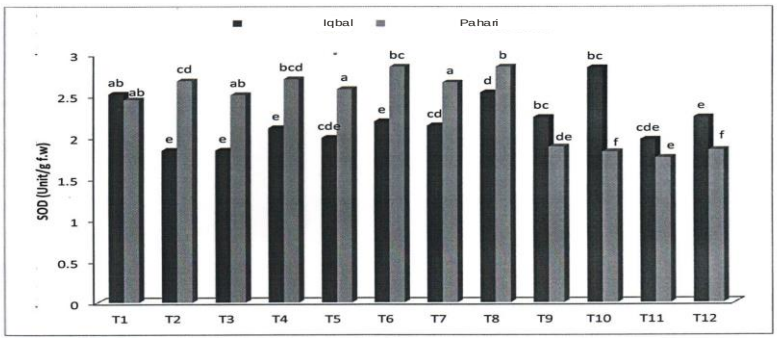

Figure 9. *Superoxide dismutase (unit/g f/w) of the selected maize varities i.e Iqbal and Pahari at vegetative stage under induced soil salinity of l00mM, 80mM, 60mM, 40mM, 20mM with nephthyl acetic acid foliar spray. Tl= (l00mM NaCl concentration) T2= (l00mM + NAA) T3= (80mM NaCl concentration) T4= (80mM + NAA) T5= (60mM NaCl concentration) T6= (60mM + NAA) T7= (40mM NaCl concentration) T8= (40mM + NAA) T9= (20mM NaCl concentration) T10= (20mM + NAA) Tl1= (control) T12= (Control + NAA)*

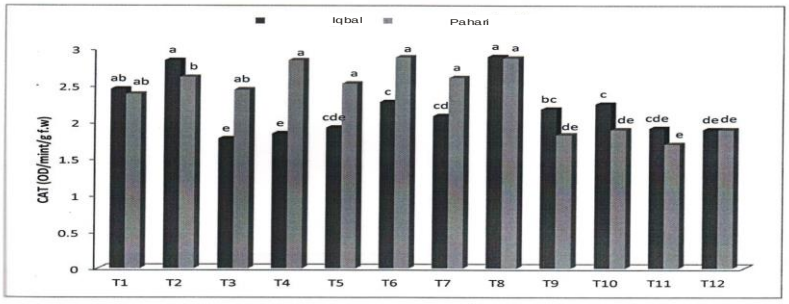

Figure 10. *Catalase (OD/mint/g f.w) of the selected maize varities i.e. Iqbal and Pahari at vegetative stage under induced soil salinity of l00mM, 80mM, 60mM, 40mM, 20mM with nephthyl acetic acid foliar spray. Tl= l00mM NaCl concentration) T2= (l00mM + NAA) T3= (80mM NaCl concentration) T4= (80mM + NAA) T5= 60mM NaCl concentration) T6= (60mM + NAA) T7= (40mM NaCl concentration) T8= (40mM + NAA) T9= 20mM NaCl concentration) T10= (20mM + NAA) Tl1= (control) T12= (Control + NAA)*

Discussion

Effect of nephthyl acetic acid foliar spray on the physiological character of maize under salt stress

The importance of this study shows significant changes in all physiological indicators of all maize genotypes. For both genotypes, all treatments showed significant changes in chlorophyll content under control and salinity conditions. During the vegetative growth phase, an increase in salt stress resulted in an increase in Pahari's chlorophyll "a", chlorophyll "b", chlorophyll "a/b" ratio and total chlorophyll content, and a decrease in registered Iqbal (*Figure 1*). The application of nephtyl acetic acid (NAA) showed a significant increase in both Iqbal and Pahari in both conditions (saline and control). Current surveys show a significant increase in the levels of chlorophyll "a"

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(*Figure 1*) and chlorophyll "b" (*Figure 2*) as the salinity of the two germplasm stages decreases. These results are consistent with the views of Jamil et al. (2012). They believe that the content of chlorophyll a (Chl a), chlorophyll b (Chl b) and total carotenoids in salt-stressed plants are significantly reduced, depending on the NaCl concentration. Salinity has a greater effect on chlorophyll "a" than chlorophyll "b". In Basmati 385, chlorophyll "a" is disturbed by salinity. In the current study, chlorophyll content was significantly reduced under salt stress because chlorophyll content was sensitive to salt experience, and salt stress caused a decrease in chlorophyll content (Ashraf et al., 2004; Elgamaal and Maswada, 2013).

Current studies indicate that chlorophyll content "a" (*Figure 1*), chlorophyll "b" (*Figure 2*), chlorophyll a/b ratio (*Figure 3*) and total chlorophyll content (*Figure 2*) are significant ($P < 0.05$) with all consent. The increase in duration of salt stress during the somatic cell phase. In all treatments, GAs (gibberellin 5 mM) showed the best result with increased chlorophyll content, and GA (gibberellic acid 10 mM) also showed a significant increase with increased chlorophyll content. These results are similar to those of Aldesuquy and Gaber (1993), who reported the use of gibberellic acid to increase plant growth and pigment content. Carotenoids act as unique pigments and activate the defense system, but the effects of SA are not significant under stress-free conditions. Current studies have shown a significant increase in carotenoid content (*Figure 5*) in both materials (*Figure 5*) ($P < 0.05$), increased salinity in the vegetative growth phase and decreased carotenoid content as saline conditions decrease. At the somatic stage, the extreme increase in carotenoid content is a consideration when added Iqbal variety in (*Figure 5*) initially increased but decreased at the minimum NaCl treatment, while the maximum increase in carotenoid content observed when Pahari was added (*Figure 5*). Increasing carotenoid levels under salt stress can express a tolerant genotype because it may be a mechanism to escape stress (*Figure 5*). Carotenoids successfully eliminated singlet oxygen from primary photochemical reactions, so there is a close relationship between leaf carotenoid content and leaf genotype production of tomato genotypes under salt stress (Juan et al., 2005; Sami et al., 2016).

The current results show that under salt stress, most of the treatments increased significantly, while under salt stress, the increase of Iqbal variety increased significantly, while Pahari increased significantly under the maximum and minimum salt stress. Under salinity and control conditions, all treatments showed the lowest proline content in Iqbal, but the greatest increase in proline content under minimal saline conditions (*Figure 7*). NAA foliar application in Iqbal showed its effect in both conditions, whereas, in foliar sprays, no effect on Pahari was observed under both conditions of vegetative phase ($P < 0.05$). These results are consistent (Cha-um et al., 2009; Tůmová et al., 2018). Salt-tolerant plant species may survive salt stress conditions using other defense mechanisms such as ionic homeostasis, anti-oxidation and hormonal systems (Sami et al., 2016; Zhang et al., 2006). Therefore, the evaluation of many parameters in salt-stressed plants will lead to the identification of some valid criteria for the classification of plants for salt tolerance.

Sugars are compatible solutes which accumulate in plant tissues that are exposed to abiotic stresses, such as water deficit, or dangerous salt stress. Addition of solutes especially proline, glycine-betaine, and sugar is a common observation under stress condition (Al-Temimi et al., 2013; Qasim et al., 2003; Tůmová et al., 2018). All treatments showed a significant increase in sugar content under both salinity and control conditions in all two genotypes Iqbal and Pahari, but initially sugar content was

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increased. Present investigation revealed that sugar content (*Figure 6*) significantly ($P< 0.05$) decreased but increased with the increase in the duration of water stress at vegetative stages in all accessions. At vegetative stage maximum increase in sugar content was observed in Pahari (*Figure 6*). Our result is similar to the work of those (Al-Temimi et al., 2013; Shah and Bano, 2012; Kareem et al., 2017), who suggested that salinity increased sugar contents, protein, proline and superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APOX) activities. Gemes et al. (2008) have described that SA application increased the soluble sugar content of tomato plants exposed to salt stress. Sugars are compatible solutes which accumulate in plant tissues that are exposed to abiotic stresses, such as water deficit, or dangerous salt stress (Morsy et al., 2007; Kareem et al., 2017).

The effect of the current examination showed that all treatments of Iqbal increased significantly under saline conditions, while Pahari was added, some treatments began decrease significantly, under minimal saline conditions and then increased (*Figure 8*). All treatments resulted in a significant increase in sugar content of Iqbal and Pahari under salinity compared to the control. Application of NAA foliar spray in Pahari did not show its effect in both conditions (salinity and control), whereas, in Iqbal, the foliar spray was significant for both conditions of the plant growth period (salinity and control) ($P < (0.05)$. Our results are compared with Jamil et al., 2012. By increasing the salt concentration, a significant decrease in the protein content of rice plants under stress was observed. Khan and Srivastava (1998) and Shanker et al. (2014) also reported a decrease in chlorophyll and protein content with increasing NaCl.

The current analysis showed that all treatments of the Iqbal population were significantly increased under saline conditions, while some treatments initially decreased significantly and then decreased under minimal saline conditions when Pahari was added (*Figure 9*). All treatments resulted in a significant increase in peroxidase (POD) (*Figure 8*), superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APOX), levels of Iqbal and Pahari under saline conditions compared to the control. Application of NAA foliar application in Iqbal did not affect both conditions (salinity and control), while foliar application in Pahari had significant effects on both vegetative conditions (salinity and control) ($P < 05$). According to Garratt et al. (2002) plants containing high concentrations of antioxidants showed considerable resistance to oxidative damage caused by reactive oxygen species.

Conclusion

The study was meant to evaluate the outcome of nephthyl acetic acid on chlorophyll content, protein, proline, sugar, and carotenoid along with certain enzyme activates (POD, SOD, CAT, and APOX). It was concluded that both varieties namely Iqbal and Pahari showed a different level of salt tolerance. All of the antioxidative enzyme activities (POD, SOD, CAT, and APOX), sugar, proline and protein contents reached maximum values in treatment T2, T4, T10, and T12 under saline condition Pahari. On physiological basis variety Pahari was found the most tolerant to the saline condition and responsive to the exogenous supply of NNA while variety Iqbal was more sensitive to salt stress. However, further studies into the best method of application of the bio-regulator to achieve optimum effect should be encouraged and the possiblity to combine treatment of bio-regulators to improve plant productivity should also be considered.

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Recommendations

The salinity stress is a major salt stress limiting factor affecting crop yield, much research has been conducted to develop plants with improved salt tolerance. Salinity stress affects many aspects of plant physiology, making it difficult to conduct comprehensive research. Instead, the plant's response is broken down into a trait that is assumed to be involved in the overall tolerance of the plant to salinity.

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