Journal of Pure and Applied Agriculture (2023) 8(4): 48-68
ISSN (Print) 2617-8672, ISSN (Online) 2617-8680
http://jpaa.aiou.edu.pk/

Response of *Triticum aestivum* cultivars to salt stress with reference to different biochemical attributes

Humaira Gul[1], Hina Nazeer[1], Aqib Khan[2], Khushnood Ur Rehman[2]*, Ghulam Saddiq[3], Muhammad Iftikhar[4] and Zahid Ali Butt[5]

[1]Department of Botany, Abdul Wali Khan University, Mardan, Khyber Pakhtunkhwa, Pakistan
[2]Department of Botany, Islamia College Peshawar, Khyber Pakhtunkhwa, Pakistan
[3]Department of Physics, Islamia College Peshawar, Khyber Pakhtunkhwa, Pakistan
[4]Senior Scientific Officer, Land Resources Research Institute (LRRI), National Agricultural Research Centre (NARC), Islamabad, Pakistan
[5]Department of Botany, GC Women University Sialkot, Pakistan

*****Corresponding author: Khushnood Ur Rehman (drkhushnood@icp.edu.pk)

Abstract

Different abiotic stresses, especially salinity and drought predicted to be increase with global change. Most of the soil degradation caused through this salt stress has a major negative impact on crops growth and production, particularly in the world's arid and semi-arid regions. Wheat (*Triticum aestivum*) is an important food-crop in Pakistan and contributes 60 % of dietary supplement for human being. Production of this crop can be enhanced up-to marginal and moderately salt affected areas through using salt tolerant varieties of this crop. Present investigation highlighted this aim through using six local wheat varieties for screening their salt tolerance in terms of biochemical traits such as photosynthetic pigments, primary metabolites, secondary metabolites, antioxidant enzyme activities and endogenous hormones level of *Triticum aestivum* cultivars. The experiment was completely randomized design and six selected *Triticum aestivum* varieties, were irrigated with NaCl (0 and 150 mM). Salinity exposure causes degradation in chlorophyll and reduction in endogenous IAA level under high salt application. Furthermore, obtained results indicated that ratio of chlorophyll, total carotenoids, carbohydrates, proteins, lipids, phenols, as well as lycopene and proline, antioxidant, ascorbate-peroxidase, catalase, flavonoids, lipid-peroxidation, tannins, β-carotene, guaiacol-peroxidase endogenous ascorbic acid and salicylic acid increase with increasing salinity. Results of present research suggested that wheat variety F. Sarhad is more tolerant. Further, this variety must be tested in the fields of salt affected areas for better yield.

Keywords: Guaicol peroxidase, IAA, Lipid peroxidation, Proline, Salt tolerance, Total lipids, Wheat

To cite this article: Gul, H., Nazeer, H., Khan, A., Rehman, K. U., Saddiq, G., Iftikhar, M., & Butt, Z. A. (2023). Response of *Triticum aestivum* cultivars to salt stress with reference to different biochemical attributes. *Journal of Pure and Applied Agriculture, 8*(4), 48-68.

Introduction

Wheat is regarded as tufted annual grass with 0.4 to 1.2 meters length. Variation in grain morphology, inflorescence and plant size depends on the species and variety of this plant. On the basis of these variations, plants can be adapted to specific growth conditions. Wheat plants contain cylindrical, erect, and glabrous stems, having hollow internodes and solid nodes. On other hand, in bread wheat stem is hollow with solid nodes. In this plant the leaf structure is flat with 1-3 cm width and 20-38 cm length. Inflorescence of wheat is called head or ears, structurally it is long, thin, and slightly flattened spike (Ecocrop, 2011). Flour of wheat grains are used for making breads (steamed, leavened and flat), cake, couscous, noodles, beer, pasta and biscuits (Curtis & Halford, 2014). There is 11-14 % moisture content present in wheat flour (Batool et al., 2012). Durum wheat specific use is reflected in making pasta and macaroni products, semolina, bulgur and couscous (Caverzan et al., 2016).

When different soluble salts accumulate in soil, it is known as soil salinity/soil salinization (Bockheim & Gennadiyev, 2000). Ionic-stress and osmotic-stress in plant cell induced because of saline condition in soil/water that negatively affect different physiological processes in plant and finally cause reduction in growth and production of exposed plant. This salt stress also causes oxidative stress in cells after synthesizing high number of radicals of oxygen (Chawla et al., 2013). Darko et al., 2017) also regarded this salt-accumulation in soil/water as an important abiotic factor that negatively affects plant growth and yield of crops in the world. Negative impacts of salt-stress on plants are caused at biochemical and physiological level and create harmful effects on plant development and growth (Munns, 2002). Kausar et al. (2012; 2015) worked on sensitive genotypes of different crops and explained that development and growth of plants mainly depend on photosynthetic rate and salinity had negative effect on it. Hasanuzzaman et al., 2013; Zafar et al., 2015) also explained the harmful effects of salt stress on different biochemical and physiological characters and growth of plants. Different researchers worked on different strategies to nullify the harmful-effects of this particular stress, these strategies include application on leaves, regulation of hormones of salt-tolerance and running of breeding programs for production of salt-tolerant varieties of different crops. This investigation was based on examining different local varieties of wheat for salt tolerance. Investigation based on estimation of different biochemical attributes (photosynthetic pigments, primary and secondary metabolites, antioxidant enzymes, lipid peroxidation, endogenous hormonal level etc.) in different wheat varieties grown under salt stress.

Materials and Methods

To establish the experimental framework, we procured seeds of various Triticum aestivum (wheat) varieties, including F. Sarhad, Insaf, Lalma, Tatora, Bathoor, and Barsat, from the Agriculture Research Institute in Tarnab Peshawar, Khyber Pakhtunkhwa. Subsequently, we initiated the experiment by sowing these seeds in 36 individual pots, with each pot accommodating precisely five seeds. Our allocation of these pots adhered to a rigorous completely randomized design, subdivided into six sets, each comprising six pots. Within this setup, we implemented three distinct treatment groups: a control, signifying no saline influence (0 mM NaCl), and two experimental groups subjected to NaCl application at a concentration of 150 mM. To ensure robustness in our observations, we replicated each treatment three times. In preparation for sowing, we subjected the seeds to a meticulous surface sterilization process, employing a 0.1% mercuric chloride solution. Subsequently, filling with loamy soil, ensuring proper drainage via basal outlets. The experimental venue was the Botanical Garden, housed within the Department of Botany at Abdul Wali Khan University Mardan. This location provided a controlled yet natural environmental setting. Throughout the experiment's duration, we adhered to a precise irrigation regimen, consistently providing equal quantities of tap water to each pot, commencing from the initial stages and continuing until the seedlings attained the critical three-leaf stage. Subsequently, at the two-week mark post-germination, we commenced irrigation sessions, administered twice a week, with saline solutions of varying concentrations. Upon the culmination of the vegetative growth phase, we judiciously harvested fresh leaf samples from the plants. These samples were earmarked for in-depth analysis, targeting an array of biochemical parameters, antioxidative enzymes, and endogenous hormone profiles. This meticulous analysis allowed us to gain invaluable insights into the adaptive responses of the wheat plants to distinct saline treatments.

Chlorophyll analysis

It can be estimated using the Maclachlan and Zalik method (1963). This involves grinding the sample and mixing it with 3ml of 80% acetone, followed by centrifugation. The supernatant is combined, and optical densities are measured at specific wavelengths:

$$\text{Chlorophyll-a (mg/g)} = [(12.3)\, D_{663} - (0.86)\, D_{645} / d * 1000 * w)] * V$$
$$\text{Chlorophyll-b (mg/g)} = [(12.3)\, D_{645} - (0.86)\, D_{663} / d * 1000 * w)] * V$$
$$\text{Total-Chlorophyll} = \text{Chl-a} + \text{Chl-b}$$
$$\text{Carotenoids (mg/g)} = [(7.6)\, D_{480} - (1.49)\, D_{510} / D * 1000 * w)] * V$$

Carbohydrates in fresh leaves

We measured total sugars in fresh-leaf samples using the Anthron method. We crushed 0.55g of fresh material into 10ml water and centrifuged for 5 minutes at 3000rpm. For estimation, we mixed 0.1ml of supernatant with 1CC of phenol after incubation for 10 minutes. We added 5 ml of concentrated H2SO4 and incubated it for an hour before measuring OD at 485 nm.

Estimation of total proteins

Plant protein measurement was conducted by homogenizing 0.2 g of homogenate in 10 ml of ice-cold phosphate buffer. The resulting extract was incubated and centrifuged before adding Bradford reagent (Bradford, 1976). Concentration was estimated through a spectrophotometer.

Estimation of total lipids

Van Handel's method (1985) was used to measure lipid content. A 0.2 g leaf sample was dissolved in chloroform and methanol (2:1 v/v), and treated with NaCl, sulfuric acid, and vanillin reagent. Absorbance at 490 nm was recorded.

Proline estimation

To determine proline in plants, Bates et al. (1973) followed. In this study, 0.5 g leaves were ground with 10 ml sulphosalicylic acid, centrifuged, and mixed with acetic acid and ninhydrin reagent after heating and cooling.

Total phenols measurements

A protocol from Malick and Singh (1980) was followed to determine phenols in homogenate extracting and measuring, after adding Folin-Ciocalteau reagent and Na_2CO_3 to the extract.

Total flavonoids measurements

To measure flavonoids in a plant homogenate incubated, and mixed with NaNO2, $AlCl_3.H_2O$, and NaOH. The flavonoids were calculated with the help of the quercetin standard curve.

Total tannins measurements

Tannin content in plants was determined using Akindahunsi and Oyetayo's (2006) method. A 0.5 g leaf sample was homogenized in 100 ml of 70 % acetone for 6 hours, tannic acid stock solution was added and Na_2CO_3 and folin-phenol reagent were mixed in. The solution was measured at 510 nm against the reagent blank.

Lycopene and β carotene measurements

Lycopene and β-carotene were measured in an experimental plant following Nagata and Yamashita (1992). A leaf sample was crushed in methanol, incubated, and an extract was prepared. Optical density was taken, and the following formula was used:

$$\text{Lycopene (mg/100 ml)} = -0.0458*A_{663} + 0.372*A_{505} - 0.0806*A_{453}$$
$$\text{ß carotene (mg/100 ml)} = 0.216*A_{663} - 0.304*A_{505} + 0.452*A_{453}$$

Determination of total antioxidants

The protocol involves mixing a sample extract with Phosphate-Buffer, Potassium-Ferricyanide, and Trichloro acetic-acid. After centrifugation, the upper layer is collected and mixed with FeCl3 by following Shoib and Malik (2014).

Determination of lipid peroxidation

Lipid peroxidation in plants was determined by macerating plant material with trichloro acetic acid, followed by centrifugation. The resulting extract was mixed with Thio barbituric-acid and trichloro acetic-acid, heated, cooled, and centrifuged again. Finally, the optical density was recorded.

Catalase activity

We used Chandlee and Scandalios' protocol (1984) with slight modifications.

Determination of ascorbate peroxidases activity

Asada (1987) developed a method to measure ascorbate peroxidase in plants. The reaction mixture contained enzyme-extract, ascorbic acid, Potassium-Phosphate buffer, EDTA, and H_2O_2. Optical density was measured at 290 nm at 30-second intervals for 7 minutes.

Determination of guaiacol peroxidase activity

To measure guaiacol peroxidase activity in plants, an extract was prepared from fresh leaves and combined with enzyme, guaiacol, and a buffer containing EDTA and H2O2. Optical density was measured at 420 nm at 30-second intervals.

Evaluation of endogenous IAA level

Plant IAA was determined via Gordon and Weber's method (Gordon & Weber, 1951). 0.5 g leaf sample was ground, extracted in 10 ml D. H_2O. 2 ml Salkowski reagent was added to 1 ml supernatant, incubated for 30 min in the dark and optical density measured at 540 nm.

Evaluation of endogenous salicylic acid level

Salicylic acid in plant material was detected using a modified process by Warrier et al. (2013). The plant material was crushed in methanol and centrifuged. The supernatant was mixed with 0.1% ferric chloride, and the final volume was 3 ml. The violet mixture was recorded at 540 nm.

Evaluation of Endogenous Ascorbic acid Level

Mitsui and Ohta (1961) measured ascorbic acid in plants by homogenizing the material in TCA, then preparing a mixture of supernatant, Sodium Molybdate, H_2SO_4, and sodium phosphate. After incubation and centrifugation, the optical density was measured at 540 nm to estimate the amount of ascorbic acid.

Statistical analysis

Data for different factors was analyzed using One-way ANOVA and DMRT.

Results and Discussion

Chlorophyll contents

Photosynthesis, based on chlorophyll, is influenced by stress conditions. The level of chlorophyll provides crucial information about photosynthetic activity, making it a primary criterion for determining plant health under such circumstances (Xu et al., 2008). During present study, treatment of different wheat varieties with NaCl @ 150 mM exhibited reduction in leaf chlorophyll-a and chlorophyll-b content, total-chlorophyll and chlorophyll a and b ratio, and reduction percentages were recorded as 26.6%, 36.4%, 59.2%, 36.8%, 53.5% and 30.29% for chlorophyll a, 28.1 %, 30.8 %, 67.3 %, 44.9 %, 55.8 % and 47.9 % for chlorophyll b and 27.2 %, 34.4 %, 62.4 %, 40.2 %, 54.4 % and 36.24 % in total chlorophyll (Fig. 1-4). F. Sarhad, Insaf, Lalma, Tatora, Bathoor and Barsat respectively. F. Sarhad proved to be more tolerant over other varieties for this parameter. Parida and Das (2005) discussed that reduction in chlorophyll content under salinity stress condition is common observation leads to disordered chlorophyll causing chlorosis in different plant species. Reduction in photosynthetic pigments because of salt induction was observed in *Picea species* and

Rosmarinus officinalis by Croser et al. (2001); Kh et al. (2010) respectively. According to Doganlar et al. (2010) reduction in chlorophyll content under stress condition associated with reduction of its synthesis. Molazem et al. (2010) stated that reduced chlorophyll content under stress condition is due to ions accumulation in cell and disturbance in perceiving function related to opening and closing of stomates. Cha-um and Kirdmanee, (2009) worked on two tomato varieties raised in salinity stress, they observed that chlorophyll-b more affected than chlorophyll-a with improved ratio between chlorophyll a and b under applied stress.

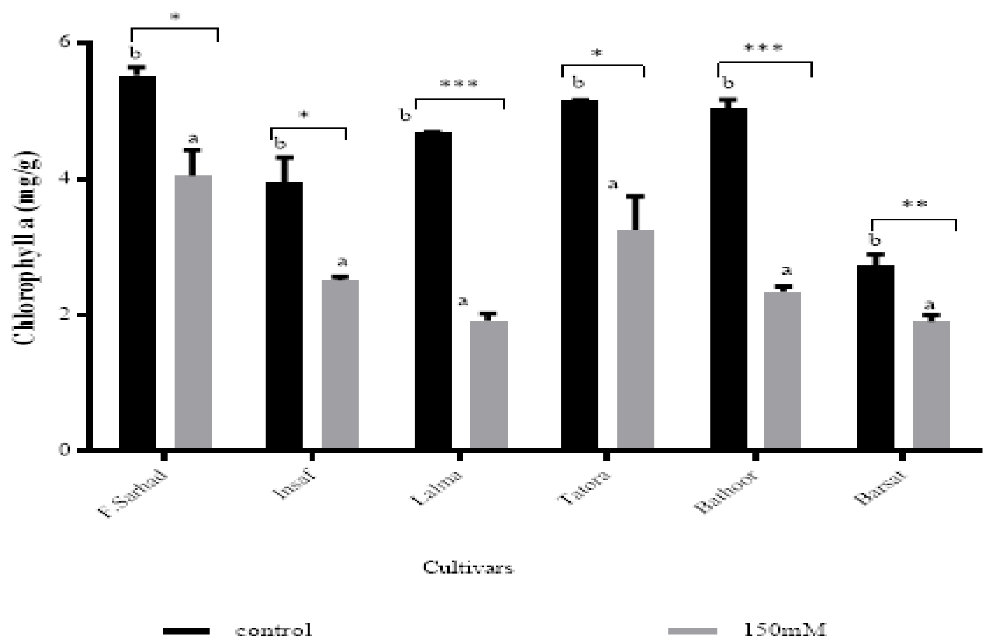

Fig. 1 Effect of salt stress on chlorophyll "a" of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

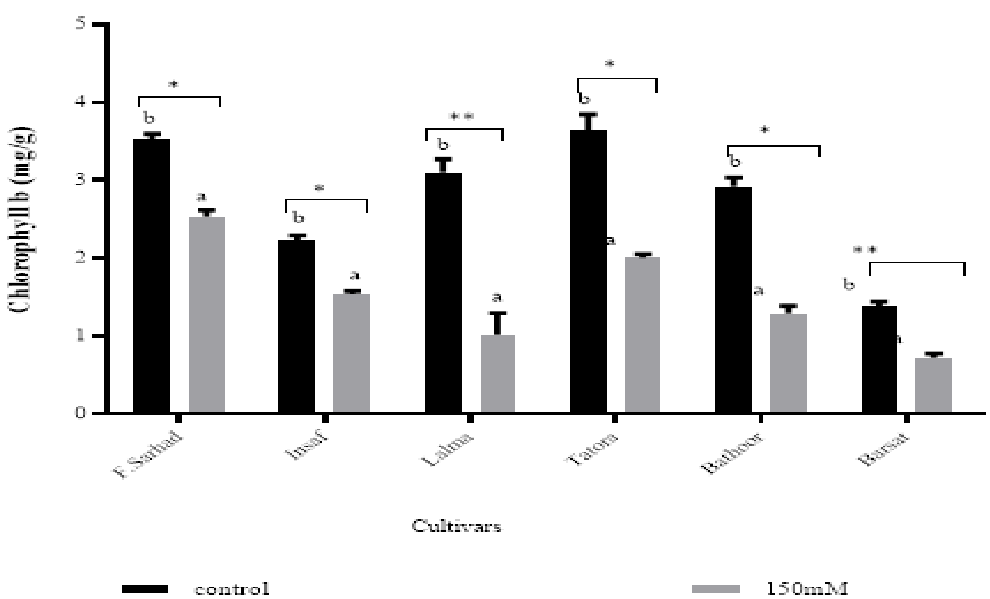

Fig. 2 Effect of salt stress on chlorophyll "b" of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

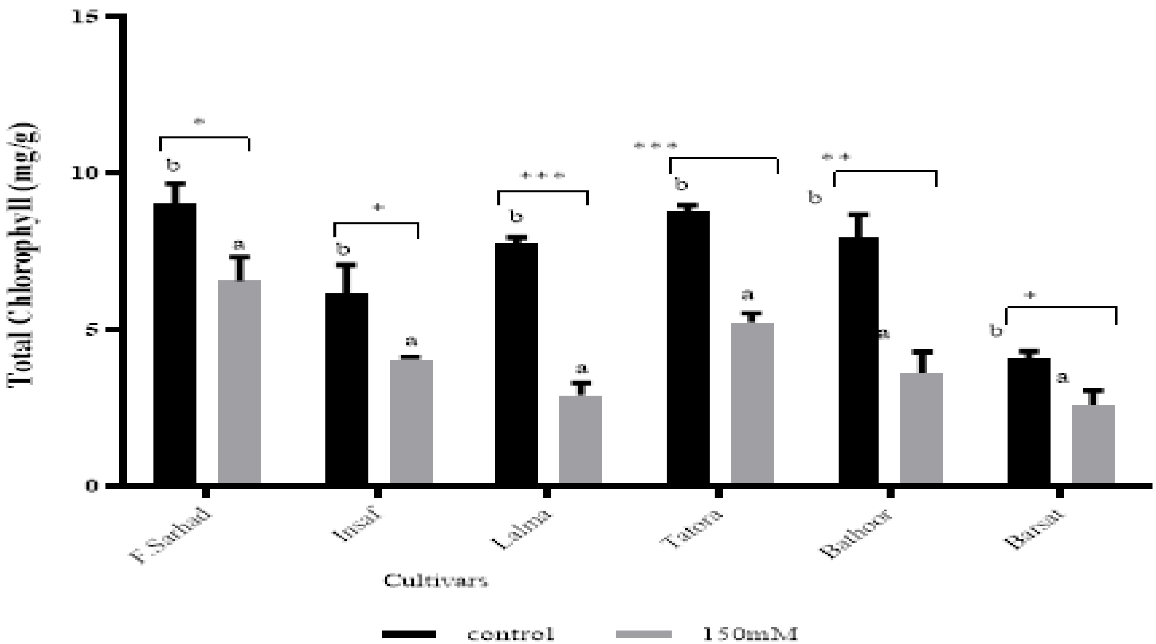

Fig. 3 Effect of salt stress on total chlorophyll of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

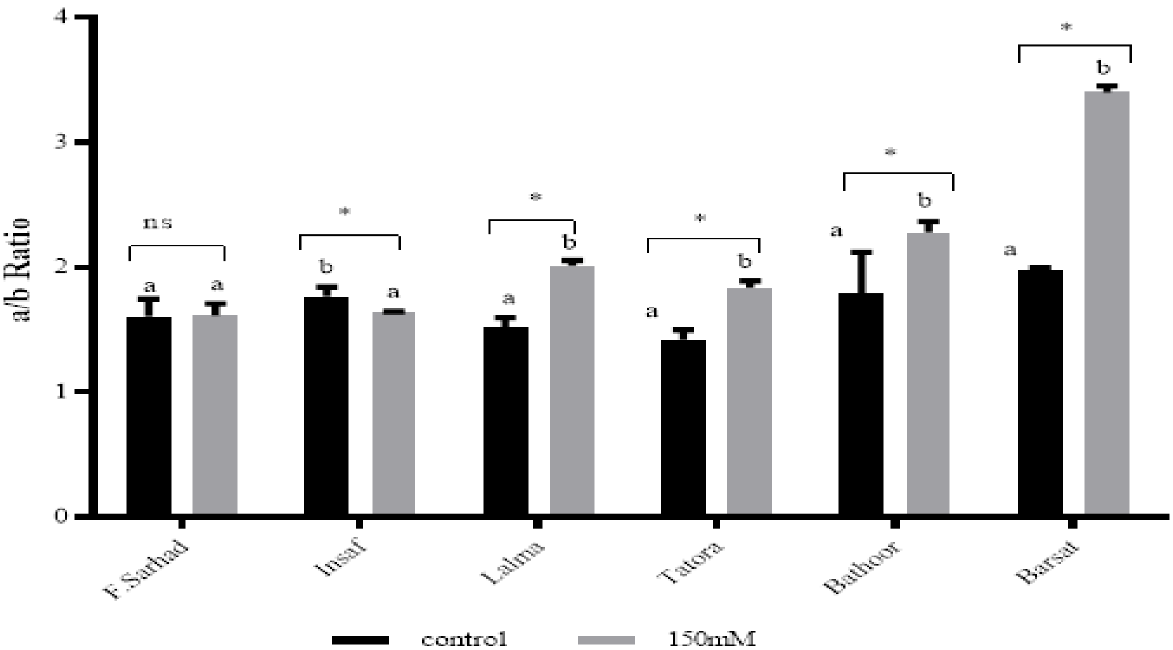

Fig. 4 Effect of salt stress on chlorophyll *a* to *b* ratio of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Carotenoids

In plants, carotenoids are regarded as important pigment, that work also as antioxidant agent (Edge et al., 1997), this pigment also perform a function of protection for lipid bilayer of plasma membrane from oxidative damage under abiotic stress specially salinity (Falk & Munné-Bosch, 2010). Presently, the application of NaCl @ 150 mM different wheat varieties exhibited significant promotion in carotenoid content and promotion percentages were recorded as 40.08 %, 44.9 %, 128.4 %, 50.2 %, 113.6 % and 58.9 %, respectively (Fig. 5). F. Sarhad proved to be more tolerant over other varieties for this parameter. Chlorophyll a, b and carotenoids are considered as main pigments involved in photosynthetic process and any change in these pigments content make changes in rate of photosynthesis. So, under any stress condition, sensitivity of crops can be measured through change in these pigments (Eryilmaz, 2006). In a study of Boughalleb and Denden, (2011), they observed high carotenoids level and provide better salt tolerance to *N. retusa* species. Chaum and Kirdmanee, (2009) stated that under stress condition, enhanced chloroplast number results in increase in carotenoids content of plant. This pigment is involved in prevention through the formation of singlet chlorophyll and through direct quenching of triplet chlorophyll.

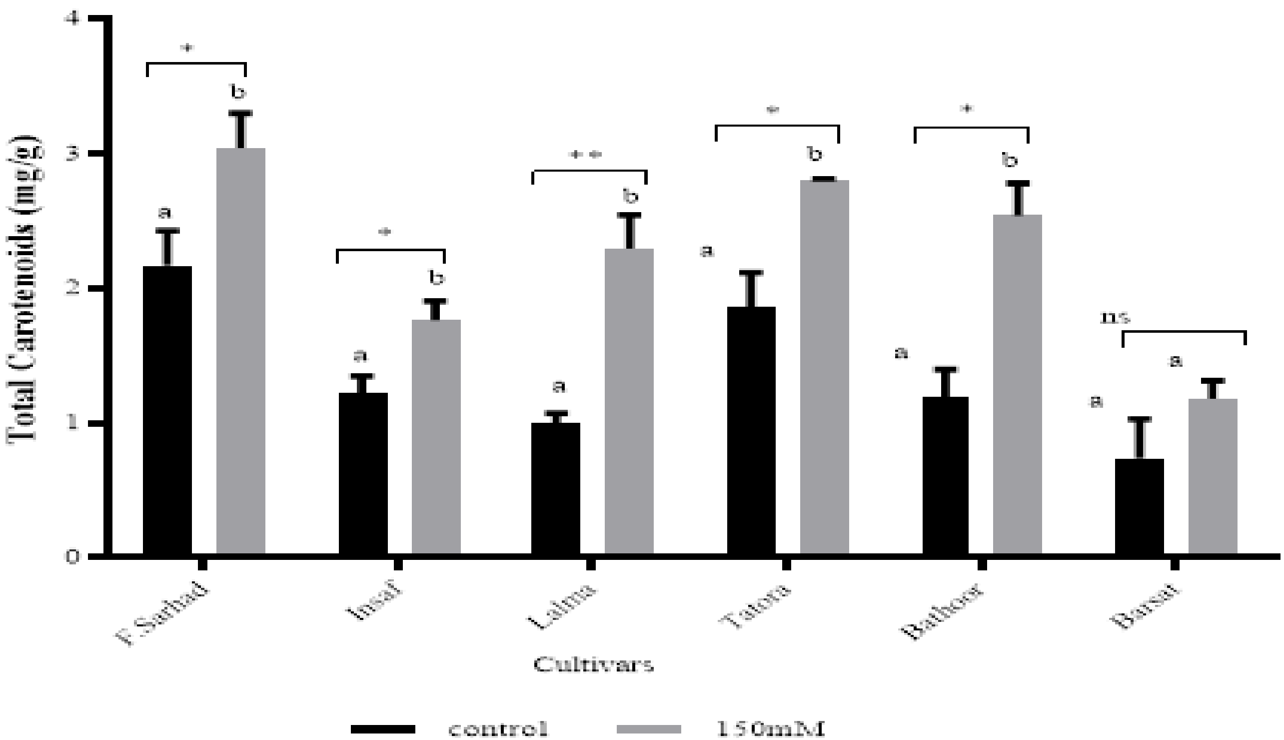

Fig. 5 Effect of salt stress on total carotenoids of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Carbohydrates

Generally, in plants different carbohydrates (e.g. fructose, glucose, trehalose, fructans etc.) accumulated under abiotic stress condition (Parida et al., 2004). The present study shown that application of NaCl @ 150 mM different wheat varieties exhibited non-significant increase in total carbohydrates and promotion percentages were recorded as 23.1 %, 14.4 %, 15.7 %, 12.4 %, 28.2 % and 13.5 % (Fig. 6). F. Sarhad proved to be more tolerant over other varieties for this parameter. Nemati et al. (2011) observed in their study, under saline stress condition different soluble carbohydrates accumulated in plants which help in maintaining turgor and provide osmotic adjustment in plants for normal growth. Hendawy and Khalid (2005) observed increased concentration of total sugars in *Salvia officinalis* after salinity treatment. Under stress conditions, partitioning in roots and for homeostasis is done through accumulation of different compatible solutes and other assimilates (Hajiboland et al., 2014).

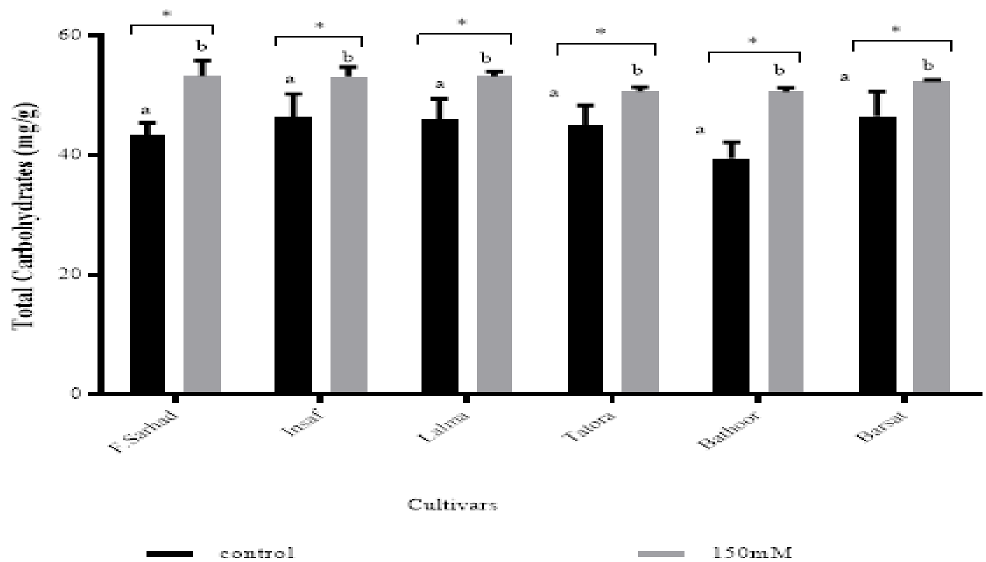

Fig. 6 Effect of salt stress on total carbohydrates of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Protein

Under stress conditions, in plants stress proteins are divided into two groups, the first category is salt stress proteins, and another group is stressing associated proteins (Mansour, 2000). Salt stress proteins accumulated in response to salt stress while another group (associated proteins) accumulated in other stresses also in plants. During the present investigation, application of NaCl @ 150mM different wheat varieties exhibited increase except F. Sarhad in total proteins and promotion percentages were recorded as 235.3 %, 100.4 %, 60.5 %, 84.6 % and 231.5 % (Fig. 7). Hayat et al. (2007) observed increased proteins synthesis in plants when grown in stressful conditions. Hussein et al., (2007) also observed the same results in plants under salinity stress and correlate it with enhanced level of different amino acids in plant tissues. In another study on *Vigna mungo* (L.), Kapoor and Srivastava (2010) observed enhanced levels of different proteins in plant tissues when raised under stress condition. According to Tester and Davenport (2003) plant-tolerance for any type of stress can be determined through its ability to solute accumulation in tissues of plant body.

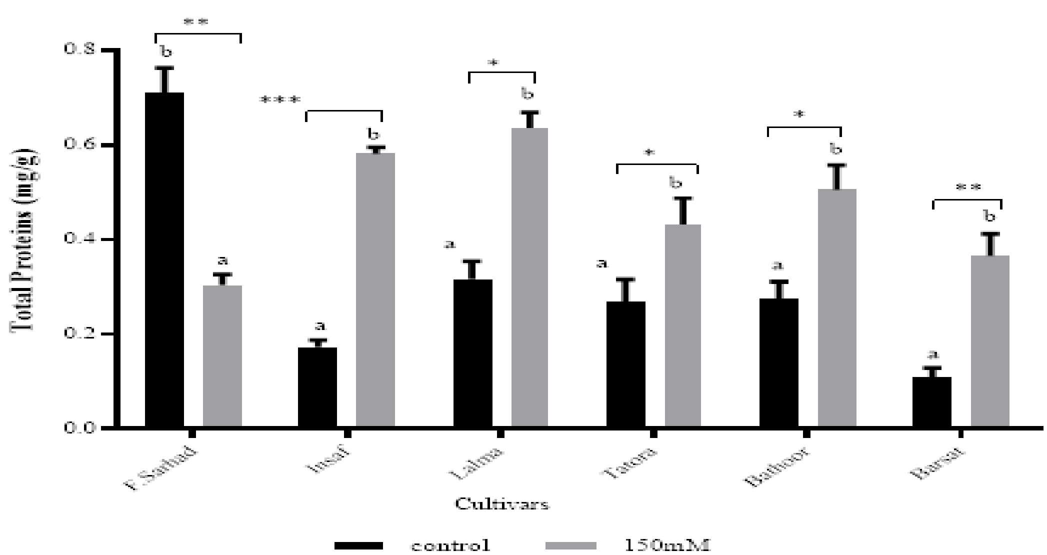

Fig. 7 Effect of salt stress on total proteins of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Proline

In plants under stress environment, proline is major compatible solute with soluble sugars that worked as osmo protectant and accumulates. It occurs in vascular plants with high quantities (Chelli-Chaabouni et al., 2010). Current study shown that application of NaCl @ 150 mM different wheat varieties exhibited increase in leaf proline content and promotion percentages were recorded as 757.1 %, 556.5 %, 125 %, 223.6 %, 137.8 % and 636.7 % (Fig. 8). F. Sarhad proved to be more tolerant over other varieties for this parameter. Sabir et al., (2011) explained that proline-accumulation in plants when exposed to abiotic stress condition provides protection to plant after maintaining water content through reducing water potential in leaves. In another study, Turkan and Demiral (2009) studied enhanced proline content in salt tolerant plant species as compared to salt sensitive species. According to Munns and Tester (2008) any change in plant's external potential cause accumulation of compatible metabolites. Under salinity stress, proline provides protection as well as work as cell osmolyte.

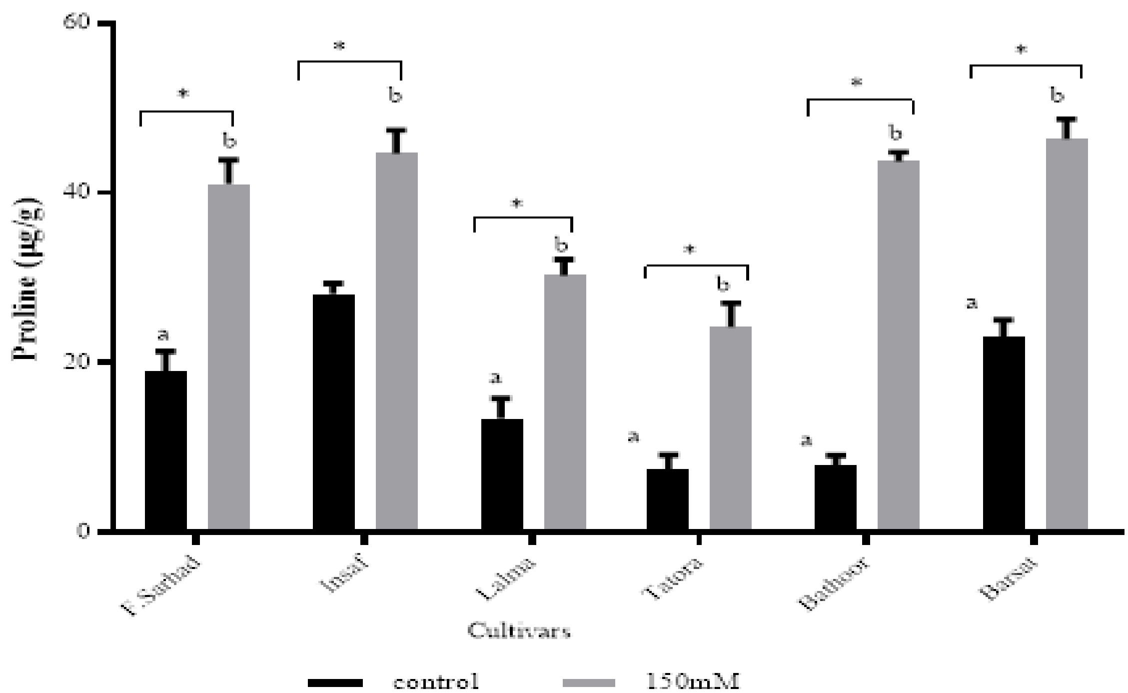

Fig. 8 Effect of salt stress on proline of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Lipid

Lipid content in plants played an essential role for permeability of cell membranes and is considered as important component of cells (Baybordi et al., 2010). Results of current research indicated; application of NaCl @ 150 mM different wheat varieties exhibited increase in lipid content and promotion percentages were recorded as 25.7 %, 76.7 %, 77.5 %, 107.9 %, 60.9 % and 42.3 % (Fig. 9). Tatora proved to be more tolerant over other varieties for this parameter. Yardanov et al., (2003) explained that stress cause disturbances in membrane components mainly proteins and lipids. Mano (2002) also suggested that damage of cell membrane lipids and proteins caused after exposure of plants to saline stress environment.

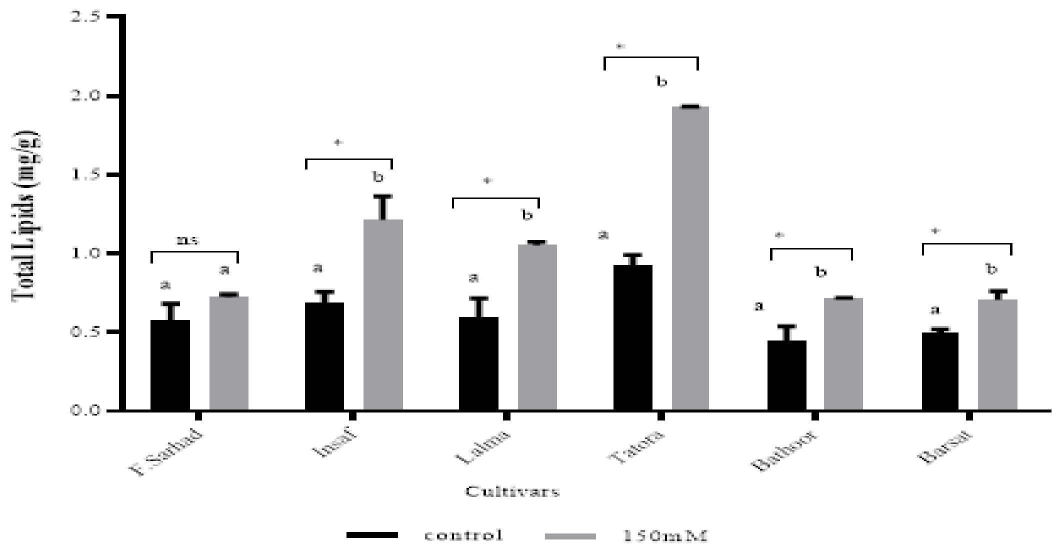

Fig. 8 Effect of salt stress on total lipids of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Phenols

In majority plant species under stress condition, ROS produced in plants and different phenolic compound play vital role as antioxidants which help in counteracting during such condition (Zheng and Wang, 2001). Salt stress cause promotion in the activity of total phenolic contents and antioxidants in plants. In present research results indicate, application of NaCl @ 150mM different wheat varieties exhibited increase in phenol content and promotion percentages were recorded as 71.1 %, 34.6 %, 42.4 %, 50.9 % and 24.8 % (Fig. 10). Ayaz et al. (2000) observed enhanced level of phenolic contents under stress condition because of altered metabolic processes. According to Mamdouh *et al.*, (2002) phenolic contents in plants provide diverse biological roles and accumulated in plants under stress condition. In the study of Singh (2018) he observed same results of phenols under salt stress. He explained that in salt-tolerant plants, phenolic content accumulation provide protection to cell-membrane damage as well as provide protective mechanism through scavenging the oxygen free radicals.

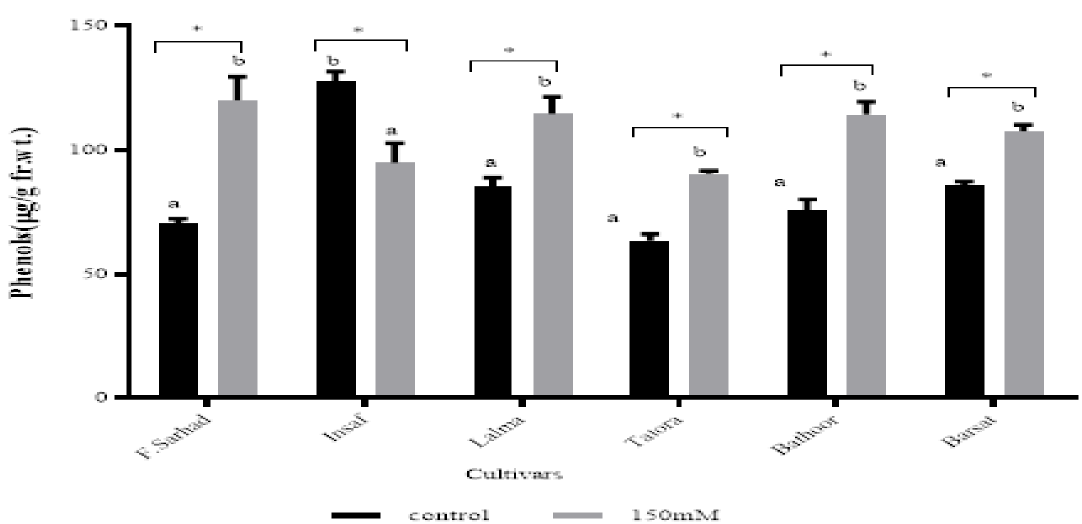

Fig. 10 Effect of salt stress on phenols of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Flavonoid

Flavonoids are phenolic compounds and considered as important components in plants participating with strong antioxidant activity under normal and stressed conditions (Jun et al., 2001). They are main and complex poly-phenolic sub-group, having wide spectrum of different biological-functions, including reduction in lipid-peroxidation (Di Ferdinando et al., 2012). Currently results indicate that application of NaCl @ 150 mM different wheat varieties exhibited increase in flavonoid content and promotion percentages were recorded as 101.7 %, 46.7 %, 114.9 %, 106.8 %, 57.1 % and 105.3 % (Fig. 11). Insaf proved to be more tolerant over other varieties for this parameter. Under different studies by Dixon & Paiva, (1995); Grace and Logan, (2000), they observed high level of flavonoid content under stress condition with induction of protective role in metabolism of plant for its normal growth under such condition. Nijveldt et al. (2001) stated that numerous flavonoids in plants inhibit the lipoxygenase enzyme activity, which involve in conversion of poly-unsaturated fatty-acids to those compounds having oxygen.

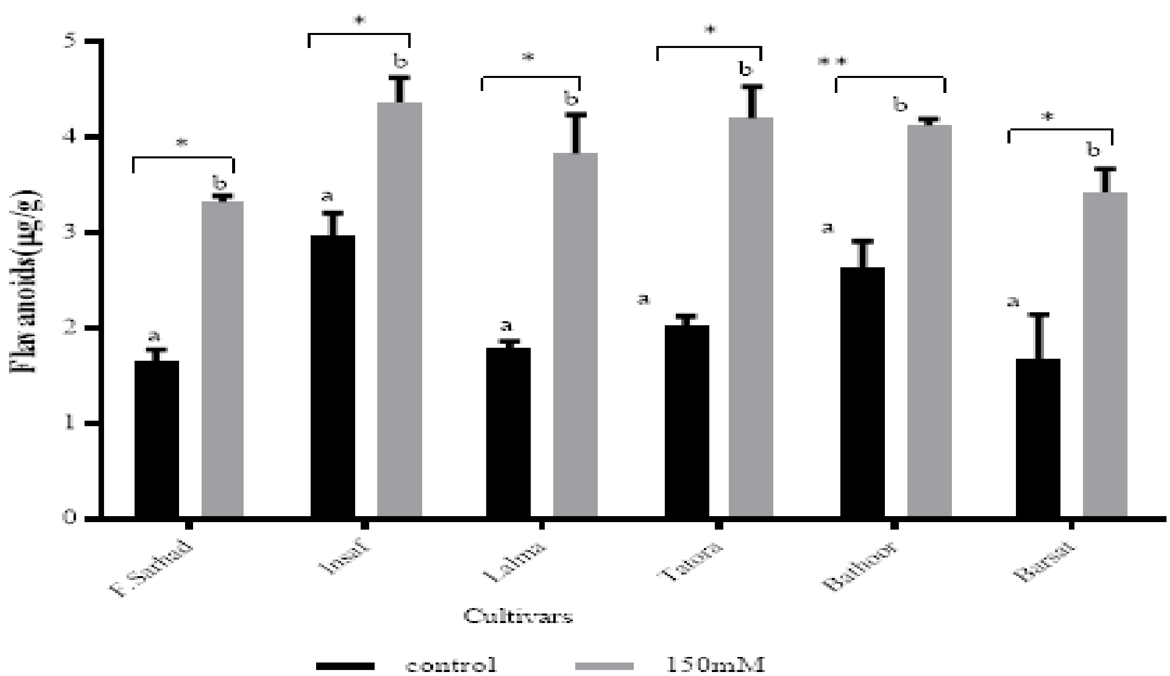

Fig. 11 Effect of salt stress on flavonoids of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Tannins

Among secondary-metabolites, flavonoids, phenolic-compounds, and tannins are the most-common from ecological point of view, widespread and important group. Concentration of tannin varies in species while level of tannin also varies with tissue and age within species (Schweitzer et al., 2008). In the present investigation, application of NaCl @ 150 mM different wheat varieties exhibited increase in tannin content and promotion percentages were recorded as 212.0 %, 106.9 %, 137.5 %, 86.5 %, 56.6 % and 53.8 % (Fig. 12). Insaf proved to be more tolerant over other varieties for this parameter. According to different studies (Mahipalaa et al., 2009; Tamir and Asefa, 2009), although increased tannin concentration provide tolerance to plant, on other hand, increased level of this metabolite with lignins, connected to increased level of fibers and produce negative effect on forage quality.

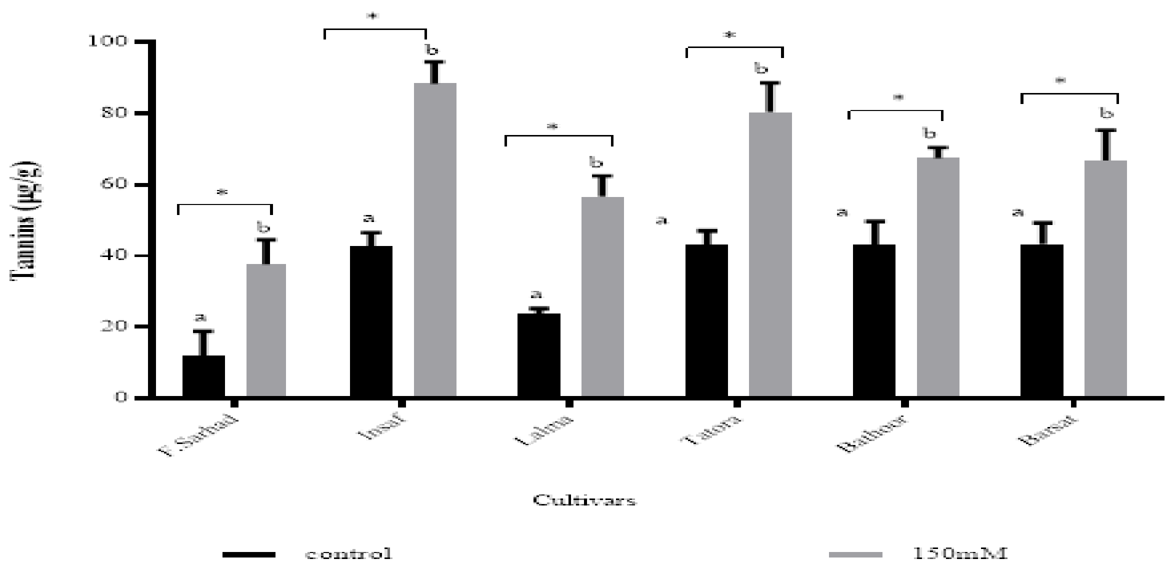

Fig. 12 Effect of salt stress on tannins of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Lycopene

Generally, in plants, lycopene and beta carotene considered as natural powerful antioxidant, worked as quencher with efficiency for singlet-oxygen with other common carotenoids under normal and stressed conditions (Di Mascio et al., 1989). Currently, results revealed that application of NaCl @ 150 mM different wheat varieties exhibited increase in lycopene content and promotion percentages were recorded as 245.8 %, 1372.5 %, 191.8 %, 115.1 %, 154.4 % and 396.9 % (Fig. 13). Pascale et al. (2001) studied tomato plants under saline stress condition and observed high carotenoids level in root region while enhanced level of lycopene in tomato fruits. When lycopene exposed to any oxidizing agent or free radicals, its color change and deactivation of this bioactive compound occur. After reaction with any oxidizing agent disruption of polyene chain occur and conjugated double bond affected as a result of addition or cleavage of bond in structure (Krinsky and Johnson, 2005). Prajapati et al. (2018) observed enhanced lycopene content in tomato when grown under salt stress.

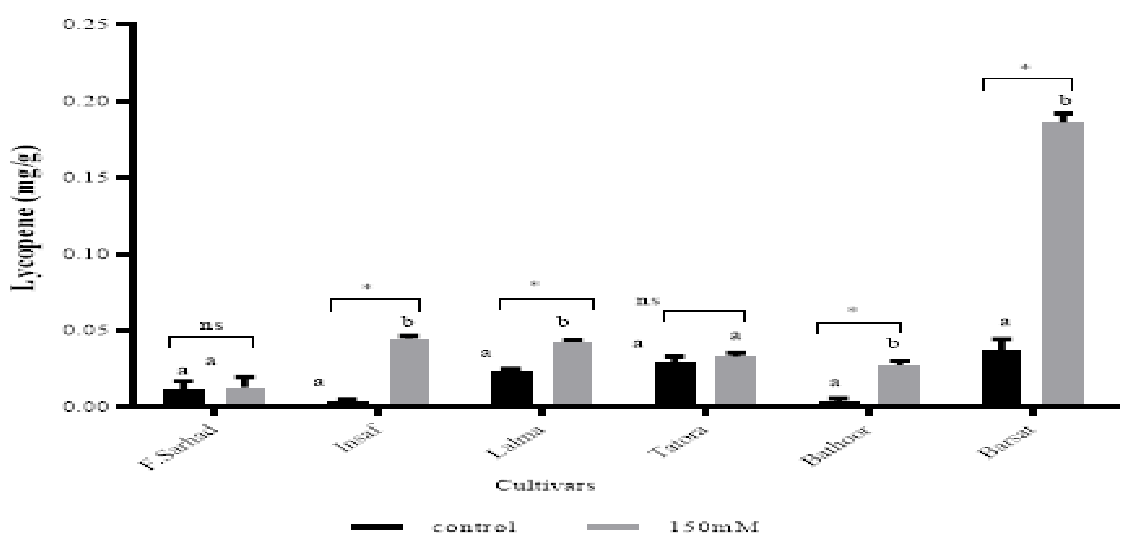

Fig. 13 Effect of salt stress on lycopene of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Beta carotene

Another bioactive compound in plants is β carotene, which acts as an antioxidant, produced in plants while growing in any stress factor. Its production protects the cell organelle from the negative effects of free radicals produced under such conditions (Story et al., 2010). Results of current study revealed that application of NaCl @ 150 mM different wheat varieties exhibited increase in beta carotene content and promotion percentages were recorded as 1313.6 %, 183.4 %, 894.3 %, 192.5 %, 270.2 % and 81.3 % (Fig. 14). Kim et al., (2008) studied different plant species under stress conditions and observed enhanced level of different carotenoids as 37% promotion in beta carotene while 80% increase in lutein content. Randome et al. (2017) observed increased concentration of β-carotene and lycopene under salt stress condition while, Raiola et al. (2014) reported, this bioactive compound as strong antioxidant and best quencher of $^1O_2$.

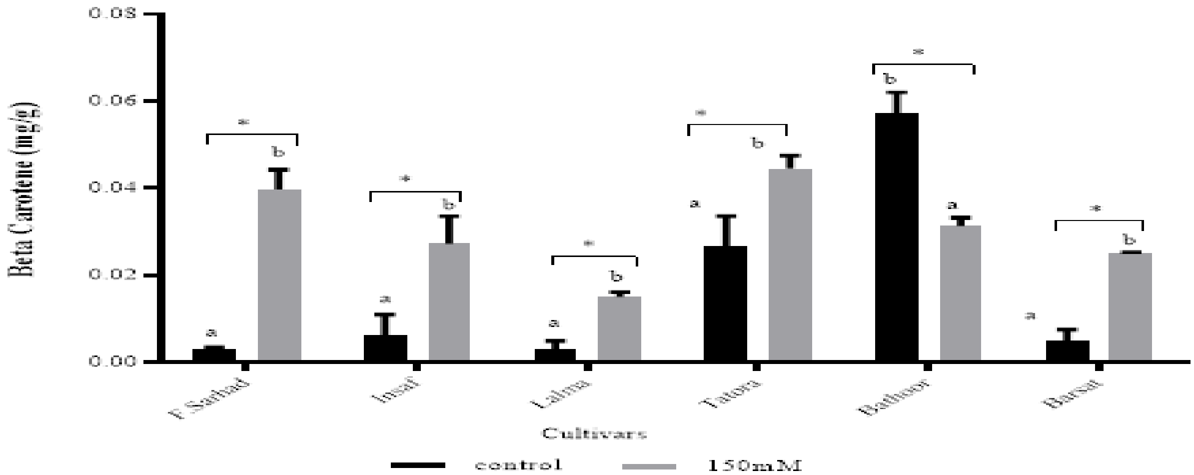

Fig. 14 Effect of salt stress on beta carotene of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Catalase

In most plant species under salinity stress condition, different antioxidants are involving in reduction of H_2O_2 and super oxide radicals (Kaymakanova & Stoeva, 2008). Results of present investigation showed that application of NaCl @ 150 mM different wheat varieties exhibited increase in catalase activity and promotion percentages were recorded as 30.9 %, 147.3 %, 49.6 %, 162.3 %, 96.8 % and 356.3 % (Fig. 15). Chawla et al. (2013) explained after their studies that enhanced activity of catalase under salinity stress considered as an adaptive method to nullify salinity stress damage in plants through reduction in hydrogen peroxide (H_2O_2) level and provide protection to plants under such environment. Enhanced level of catalase is reported in *Pisum sativum*. In another study, Khosravinejad et al. (2008) also observed high catalase activity in barley seedlings when raised in saline stress condition.

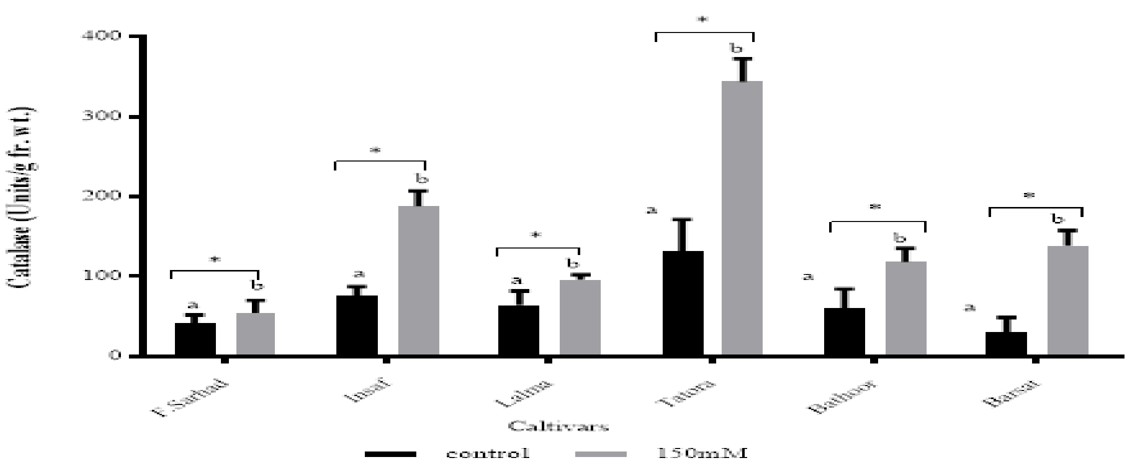

Fig. 15 Effect of salt stress on catalase of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Ascorbate peroxidase

In plant cells, ascorbate is an important substrate which is involved in reducing H_2O_2 detoxification. This substrate is used by ascorbate peroxidase enzyme to reduce H_2O_2 radical into water molecules (Noctor & Foyer, 1998). Currently, the application of NaCl @ 150 mM different wheat varieties exhibited increase in ascorbate peroxidase activity and promotion percentages were recorded as 114.5 %, 563.7 %, 66.2 %, 411.04 %, 249.5 % and 115.3 % (Fig. 16). Weisany et al. (2012) worked on soybeans under salinity stress condition, they observed high ascorbate peroxidase activity in leaf tissues because of enhanced level of hydrogen peroxide (H_2O_2). In another study, Mittova et al. (2004); Gharsallah et al., (2016) also observed the same results of high ascorbate peroxidase activity in plants under salinity stress condition. Increased ascorbate peroxidase activity provides salt tolerance capacity to plants under such conditions, further Mittova et al. (2004) in tomato while Bor et al. (2003) in wild beet, documented the same relation of ascorbate peroxidase and salinity tolerance of plant.

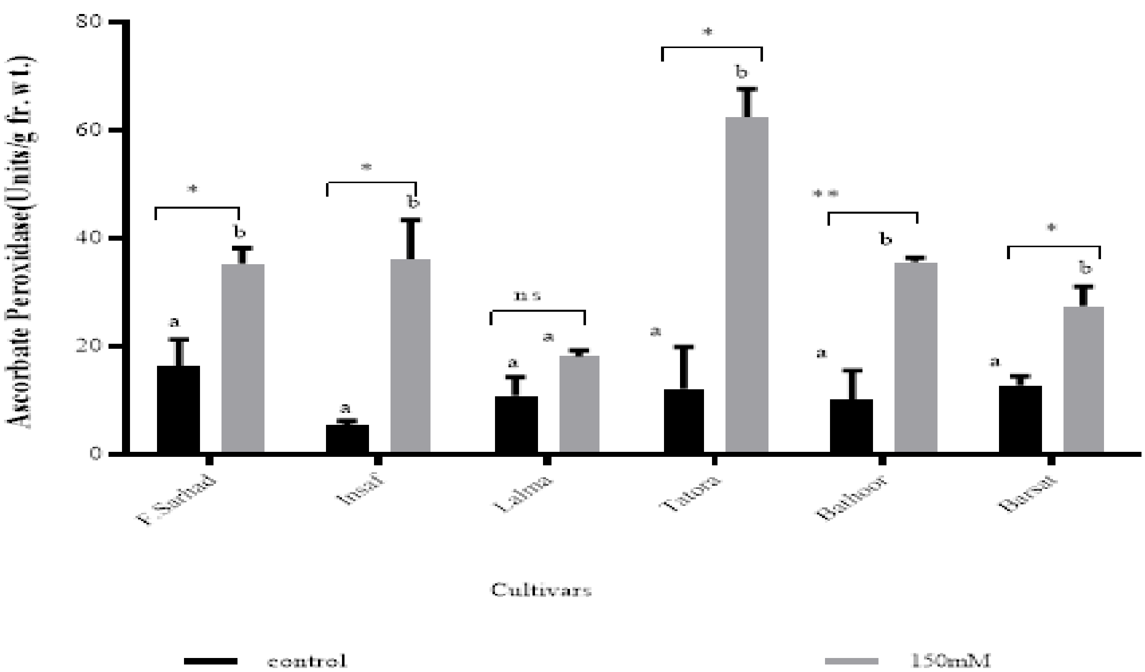

Fig. 16 Effect of salt stress on ascorbate peroxidase of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Guaiacol peroxidase

Under salinity, plant defense and biochemical mechanisms also consist of enzymatic and non-enzymatic antioxidants that help in detoxification of reactive-oxygen-species. According to Sharma, et al. (2012); Hasegawa et al. (2000), during different environmental-stresses including salinity-stress, plants produced antioxidant enzymes that combat the oxidative stress. During present research, application of NaCl @ 150 mM different wheat varieties exhibited increase in guaiacol peroxidase and promotion percentages were recorded as 41.6 %, 42.03 %, 64.9 %, 31.1 %, 249.5 % and 42.9 % (Fig. 17). Zare and Pakniyat (2012) observed high activity of guaiacol peroxidase (GPOX) enzyme in oilseed rape when raised under salinity stress. it is considered as common sigh of oxidative damage as a result of stress-response. In another study, Parida et al. (2004) also observed increased activity of guaiacol peroxidase (GPOX) in *B. parviflora* and in *L. stocksii* seedlings under salinity stress. Quan et al. (2008) considered this enzyme as a protectant in plants which belong to the family of isozymes and utilize lipid hydrogen peroxide to provide protection to cells from oxidative damage. Roumyana et al. (2016) studied sunflower under salinity stress and observed enhanced activity of guaiacol peroxidase.

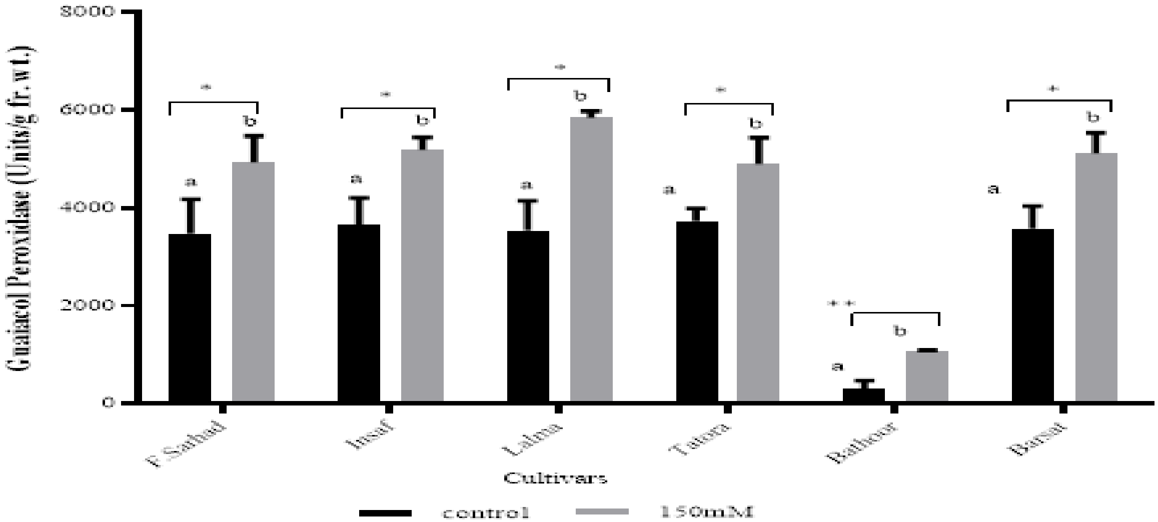

Fig. 17 Effect of salt stress on guaiacol peroxidase of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Total antioxidant enzymes

Different antioxidant enzymes act as significant components of plants under salt response mechanism in H2O2 and superoxide detoxification (Jaleel et al., 2007). Under stress conditions, plant respond through the development of non-enzymatic and enzymatic (e.g. catalase, different peroxidases, glutathione, and reductase) antioxidants, played a role of detoxification of cell from ROS and oxidative damage (Sairam and Tyagi, 2004). Presently, application of NaCl @ 150mM different wheat varieties exhibited increase in total antioxidants and promotion percentages were recorded as 83.7 %, 83.01 %, 278.2 %, 75.03 %, 190.1 % and 545.1 % (Fig. 18). The improved antioxidants activity observed as common phenomenon in leaves under different abiotic-stress condition (Pastori et al., 2000). Elhamid et al. (2014) worked on wheat (*Triticum aestivum*) varieties grown in salt-stress and observed enhanced activity of different antioxidant enzymes (e.g. superoxide-dismutase, catalase, peroxidase, polyphenol-oxidase, ascorbate-peroxidase, glutathione-reductase etc.). El-Shabrawi et al. (2010) documented that increase in antioxidant activity of plants helps the plant to improve salt-tolerance through reduction in oxidative damage. In another study, Bor et al. (2003) studied *Beta vulgaris* under salt stress and observed reduced level of lipid peroxidation while total antioxidant activity enhanced.

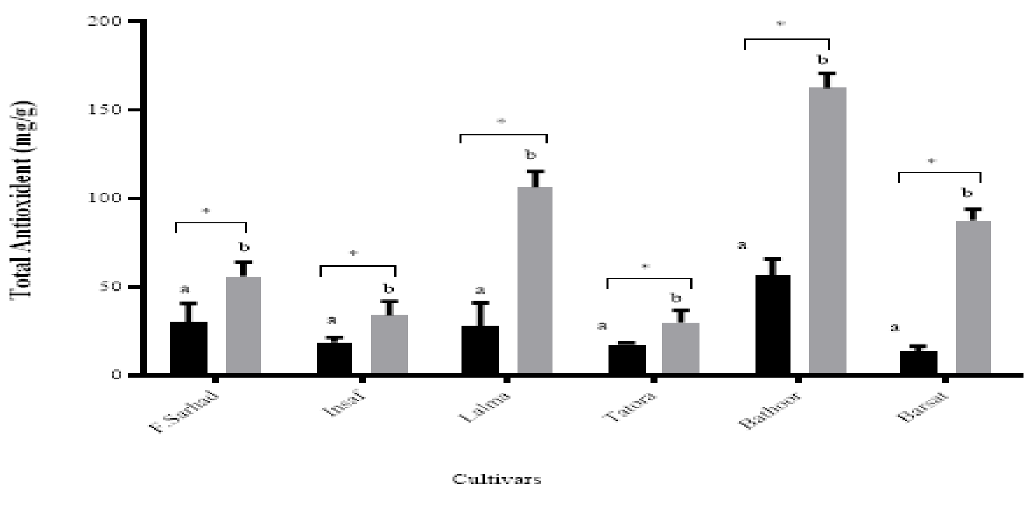

Fig. 18 Effect of salt stress on total antioxidant of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Lipid peroxidation

In plants, lipid peroxidation causes oxidative damage in plants, so this parameter is used as an indicator of oxidative damage in plants under different stresses (Khan & Panda, 2008). Current study revealed that application of NaCl @ 150 mM different wheat varieties exhibited increase in lipid peroxidation and promotion percentages were recorded as 862.1 %, 830.4 %, 309.6 %, 418.9 %, 500.9 % and 580.2 % (Fig. 19). Lipid-peroxidation is measured by MDA amount, which is produced as its reaction with TBA. As reported earlier by Mittova et al. (2004), they stated, under salt stress, high level of MDA observed in plants produced due to H_2O_2 lipoxygenase enzyme activity and which ultimately cause lipid peroxidation. In another study, Misra & Gupta (2006) observed increased values of lipid peroxidation in plants as a result of toxic-ions accumulation cause reactive-oxygen-species production during stress conditions. Bandeoğlu et al. (2004) explained that high MDa content in leaves reduces plant capacity of ROS scavenging with accumulation of hydrogen peroxide which further causes membrane damage in leaves.

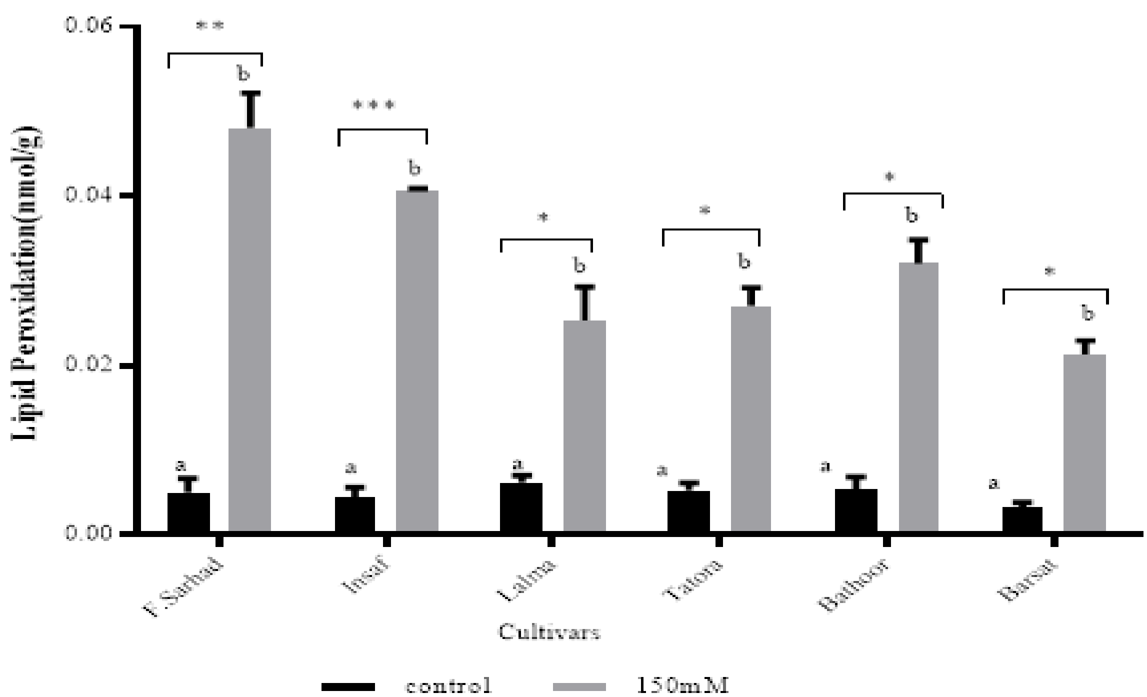

Fig. 19 Effect of salt stress on lipid peroxidation of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Indoleacetic acid

Indole -3- acetic acid (IAA) regulator had vital and significant role in plant (Wang et al., 2001). This growth regulator is the first identified plant-hormone; however, its genetic-level of biosynthesis is still not clear (Fahad et al., 2015). Results of current study demonstrated that application of NaCl @ 150mM different wheat varieties exhibited decrease in endogenous IAA level and reduction percentages were recorded as 29.02 %, 54.8 %, 38.5 %, 48.8 %, 48.04 % and 25.3 % (Fig. 20). IAA homeostasis strongly influenced through abiotic stresses (e.g. salinity, drought etc.), these agents modify IAA metabolism and distribution. Another factor is ROS (reactive-oxygen-species), produce during stress conditions and had strong influence on IAA response in plants (Schopfer et al., 2002). According to Sakhabutdinova et al. (2003), IAA level reduced in plant root system after exposure to saline stress condition. In another study, Sastry and Shekhawa (2001); Afzal et al., (2005) also observed low level of endogenous IAA level under salinity stress, while exogenous application of this hormone provide alleviation in seedlings growth under normal and stressed conditions. Ünyayar, (2002) studied *Funalia trogii* after treatment with salt and observed variation in IAA level of plants. Reduction in accumulation of IAA could be the reason of inhibition of its biosynthetic pathway or its transformation to inactive form and its degradation.

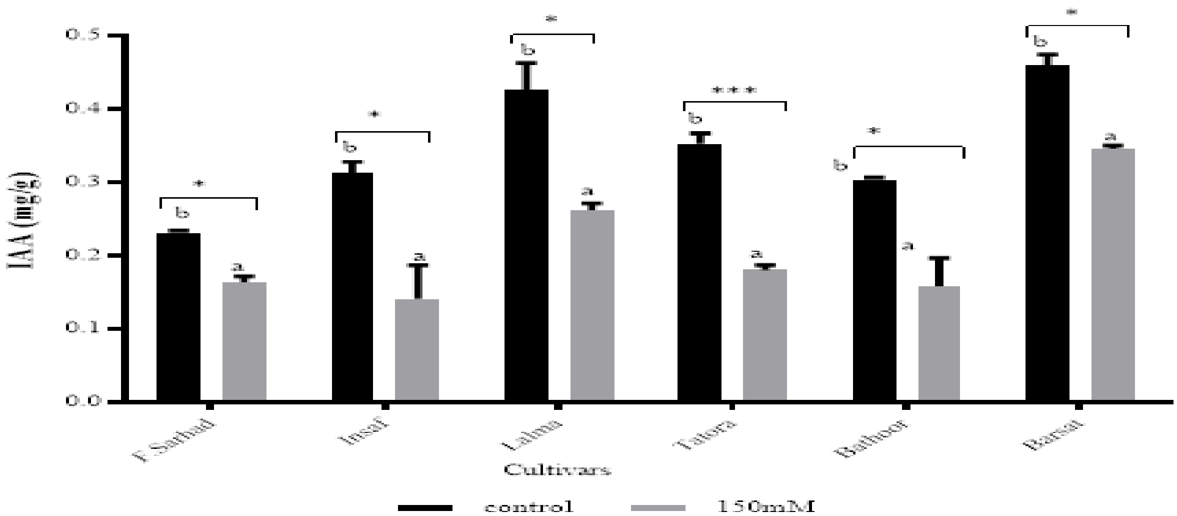

Fig. 20 Effect of salt stress on indoleacetic acid of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Salicylic acid

Salicylic acid (SA), used as growth regulator and considered a well-known phenolic compound (Agamy et al., 2013). This hormone also plays key role in seed germination, flower induction in plants, fruit yield, uptake of different minerals and their transport in pants, glycolysis, photosynthetic rate, transpiration rate, nodulation, senescence, stomatal-conductance, and thermo-tolerance (Khan et al., 2003). Present investigation exhibited that application of NaCl @ 150 mM different wheat varieties exhibited increase in endogenous salicylic acid level and promotion percentages were recorded as 108.4 %, 40.7 %, 105.9 %, 351.9 %, 90.6 % and 70.1 % (Fig. 21). Shakirova et al. (2003) worked on wheat (*Triticum aestivum*) seedlings and raised them under salinity stress, they observed high endogenous levels of salicylic acid in seedlings under such conditions. In another study, Fernández et al. (2012) studied sunflower in salt stress and observed enhanced endogenous salicylic-acid level in seedlings. Variation in response with reference to salicylic acid, under stress conditions based on organ, genetic age of plant, species and type of stress applied on plant. Sakhabutdinova et al. (2003) explained that this growth regulator improves different physiological processes (e.g. photosynthesis, uptake and transport of solutes, germination of seeds etc.), finally induce anatomical and structural changes in chloroplast and leaves and enhance plant growth.

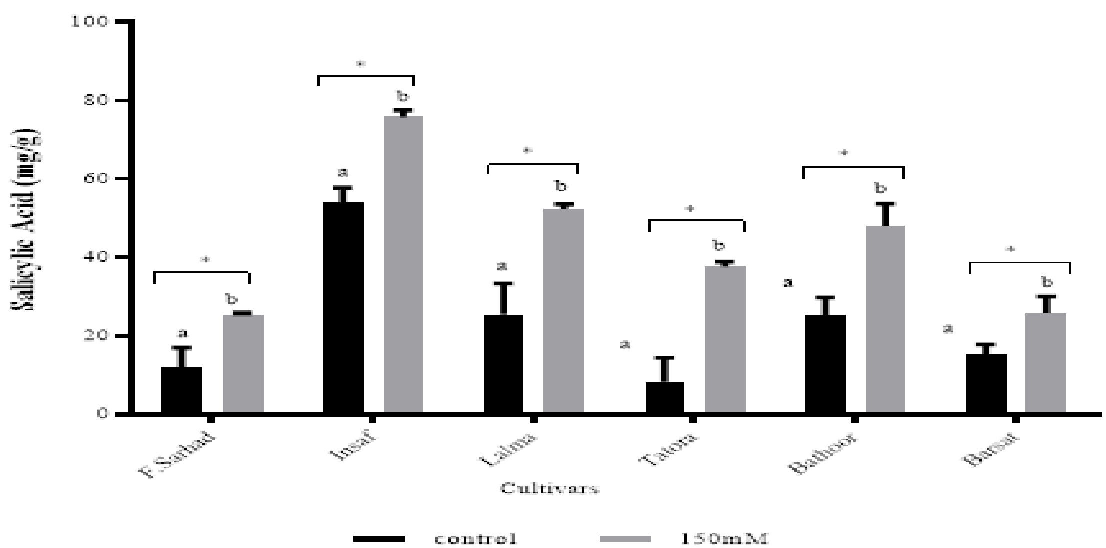

Fig. 21 Effect of salt stress on salicylic acid of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Ascorbic acid

Ascorbic acid (Vitamin C) act as non-enzymatic factor with carotenoids and vitamin E (alpha-tocopherol) (Sairam et al., 2002), exhibited key role in enhancing tolerance to plants under different stress conditions. This hormone had different functions e.g. cell-division, expansion etc. (Pignocchi & Foyer, 2003). During present research, application of NaCl @ 150 mM different wheat varieties exhibited increase in ascorbic acid level and promotion percentages were recorded as 153.6 %, 154.3 %, 95.2 %, 141.4 %, 162.4 % and 100.5 % (Fig. 22). In plants, under normal conditions, ascorbic acid has major functions in metabolism and other different processes (Chen & Gallie, 2004).

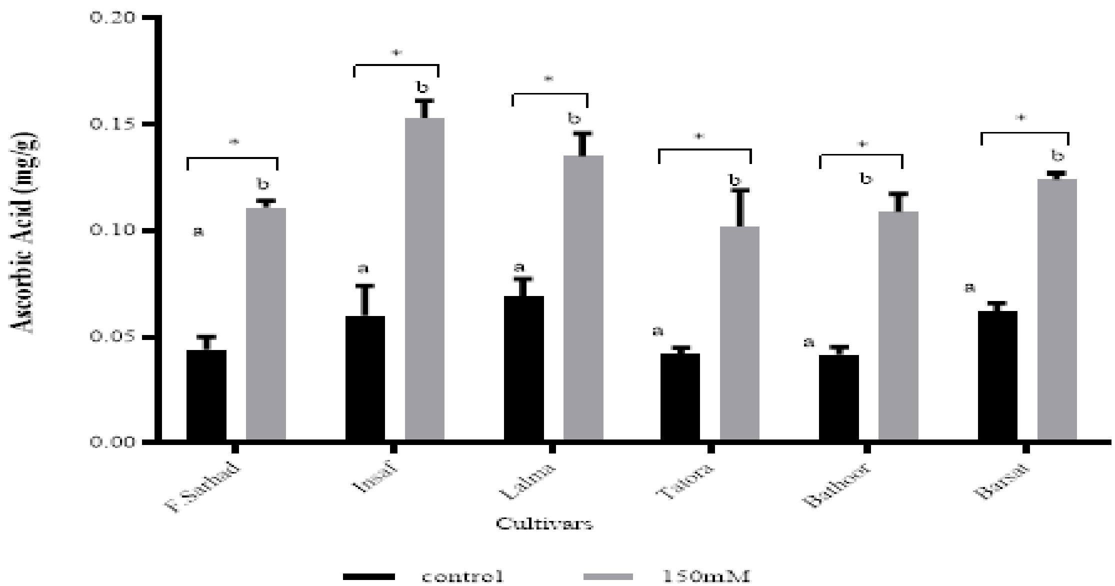

Fig. 221 Effect of salt stress on ascorbic acid of different varieties of wheat. Statistically, bars with alike letters do not differ from each other. Each bar represents Mean ± SD. ns = non-significant; * = significant at $p < 0.05$

Conclusion

Based on current research results it is concluded that application of salt on different wheat varieties reduce growth in terms of different biochemical attributes (primary and secondary metabolites), photosynthetic pigments, antioxidant enzymes, endogenous hormones. The present study suggested that F. Sarhad variety is more salt tolerant as compared to other varieties and suggested it to be used in moderate saline environment for better yield of this crop.

References

Afzal, I., Basra, S. A., & Iqbal, A. (2005). The effects of seed soaking with plant growth regulators on seedling vigor of wheat under salinity stress. *Journal of Stress Physiology & Biochemistry, 1*(1).

Agamy, R. A., Hafez, E. E., & Taha, T. H. (2013). Acquired resistant motivated by salicylic acid applications on salt stressed tomato (Lycopersicon esculentum Mill.). *The American-Eurasian Journal of Agricultural & Environmental Sciences, 13*, 50-57.

Ayaz, F. A., Kadioglu, A., & Turgut, R. (2000). Water stress effects on the content of low molecular weight carbohydrates and phenolic acids in Ctenanthe setosa (Rosc.) Eichler. *Canadian Journal of Plant Science, 80*(2), 373-378.

Akindahunsi, A. A., & Oyetayo, F. L. (2006). Nutrient and antinutrient distribution of edible mushroom, Pleurotus tuber-regium (fries) singer. *LWT-Food Science and Technology, 39*(5), 548-553.

Asada, K. (1987). Production and scavenging of active oxygen in photosynthesis. In Photoinhibition (pp. 227-287).

Boughalleb, F., & Denden, M. (2011). Physiological and biochemical changes of two halophytes, Nitraria retusa (Forssk.) and Atriplex halimus (L.) under increasing salinity. *Agricultural Journal, 6*(6), 327-339.

Bandeoğlu, E., Eyidoğan, F., Yücel, M., & Öktem, H. A. (2004). Antioxidant responses of shoots and roots of lentil to NaCl-salinity stress. *Plant Growth Regulation, 42*(1), 69-77.

Bybordi, A., Tabatabaei, S. J., & Ahmedov, A. (2010). Effects of salinity stress on fatty acids composition of Canola (*Brassica napus* L.). *Food and Agricultural Journal, 8*(1), 113-115.

Batool, S. A., Rauf, N., Tahir, S. S., & Kalsoom, R. (2012). Microbial and physico-chemical contamination in the wheat flour of the twin cities of Pakistan. *International Journal of Food Safety, 14*, 75-82.

Bor, M., Özdemir, F., & Türkan, I. (2003). The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet Beta vulgaris L. and wild beet Beta maritima L. *Plant Science*, *164*(1), 77-84.

Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil, 39*(1), 205-207.

Bockheim, J. G., & Gennadiyev, A. N. (2000). The role of soil-forming processes in the definition of taxa in Soil Taxonomy and the World Soil Reference Base. *Geoderma, 95*(1-2), 53-72.

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

Caverzan, A., Casassola, A., & Brammer, S. P. (2016). Antioxidant responses of wheat plants under stress. *Genetics and Molecular Biology*, *39*(1), 1-6.

Chawla, S., Jain, S., & Jain, V. (2013). Salinity induced oxidative stress and antioxidant system in salt-tolerant and salt-sensitive cultivars of rice (*Oryza sativa* L.). *Journal of Plant Biochemistry and Biotechnology, 22*(1), 27-34.

Croser, C., Renault, S., Franklin, J., & Zwiazek, J. (2001). The effect of salinity on the emergence and seedling growth of *Picea mariana*, *Picea glauca*, and *Pinus banksiana*. *Environmental Pollution*, *115*(1), 9-16.

Chandlee, J. M., & Scandalios, J. G. (1984). Analysis of variants affecting the catalase developmental program in maize scutellum. *Theoretical and Applied Genetics, 69*(1), 71-77.

Cha-Um, S., & Kirdmanee, C. (2009). Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. *Pakistan Journal of Botany*, *41*(1), 87-98.

Chelli-Chaabouni, A., Mosbah, A. B., Maalej, M., Gargouri, K., Gargouri-Bouzid, R., & Drira, N. (2010). In vitro salinity tolerance of two pistachio rootstocks: *Pistacia vera* L. and *P. atlantica* Desf. *Environmental and Experimental Botany, 69*(3), 302-312.

Chen, Z., & Gallie, D. R. (2004). The ascorbic acid redox state controls guard cell signaling and stomatal movement. *The Plant Cell, 16*(5), 1143-1162.

Curtis, T., & Halford, N.G. (2014). Food security: The challenge of increasing wheat yield and the importance of not compromising food safety. *Annals of Applied Biology, 164*(3), 354-372.

Darko, E., Gierczik, K., Hudák, O., Forgó, P., Pál, M., Türkösi, E., Kovács, V., Dulai, S., Majláth, I., Molnár, I., & Janda, T. (2017). Differing metabolic responses to salt stress in wheat-barley addition lines containing different 7H chromosomal fragments. *PloS One, 12*(3), e0174170.

Di Mascio, P., Kaiser, S., & Sies, H. (1989). Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Archives of Biochemistry and Biophysics, 274*(2), 532-538.

Di Ferdinando, M., Brunetti, C., Fini, A., & Tattini, M. (2012). Flavonoids as antioxidants in plants under abiotic stresses. In Abiotic Stress Responses in Plants (pp. 159-179). Springer, New York, NY.

Dixon, R. A., & Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. *The Plant Cell*, *7*(7), 1085.

Doganlar, Z.B., Demir, K., Basak, H., & Gul, I. (2010). Effects of salt stress on pigment and total soluble protein contents of three different tomato cultivars. *African Journal of Agricultural Research, 5*(15), 2056-2065.

Ecocrop. (2011). Ecocrop database. FAO.

El Far, M.M., & Taie, H.A. (2009). Antioxidant activities, total anthocyanins, phenolics and flavonoids contents of some sweetpotato genotypes under stress of different concentrations of sucrose and sorbitol. *Australian Journal of Basic and Applied Sciences, 3*(4), 3609-3616.

Elhamid, E.M.A., Sadak, M.S., & Tawfik, M.M. (2014). Alleviation of adverse effects of salt stress in wheat cultivars by foliar treatment with antioxidant 2-changes in some biochemical aspects, lipid peroxidation, antioxidant enzymes and amino acid contents. *Agricultural Sciences, 5*(13), 1269-1280.

El-Shabrawi, H., Kumar, B., Kaul, T., Reddy, M. K., Singla-Pareek, S. L., & Sopory, S. K. (2010). Redox homeostasis, antioxidant defense, and methylglyoxal detoxification as markers for salt tolerance in Pokkali rice. *Protoplasma, 245*(1-4), 85-96.

Edge, R., McGarvey, D. J., & Truscott, T. G. (1997). The carotenoids as antioxidants: A review. *Journal of Photochemistry and Photobiology B: Biology, 41*(3), 189-200.

Eryılmaz, F. (2006). The relationships between salt stress and anthocyanin content in higher plants. *Biotechnology & Biotechnological Equipment*, *20*(1), 47-52.

Falk, J., & Munné-Bosch, S. (2010). Tocochromanol functions in plants: Antioxidation and beyond. *Journal of Experimental Botany, 61*(6), 1549-1566.

Fahad, S., Hussain, S., Bano, A., Saud, S., Hassan, S., Shan, D., Khan, F.A., Khan, F., Chen, Y., Wu, C., & Tabassum, M.A. (2015). Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: Consequences for changing environment. *Environmental Science and Pollution Research*, *22*(7), 4907-4921.

Fernández, C., Alemano, S., Vigliocco, A., Andrade, A., & Abdala, G. (2012). Stress hormone levels associated with drought tolerance vs. sensitivity in sunflower (*Helianthus annuus* L.). In Phytohormones and Abiotic Stress Tolerance in Plants: 249-276.

Gharsallah, C., Fakhfakh, H., Grubb, D., & Gorsane, F. (2016). Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. *AoB Plants,* 8.

Grace, S. C., & Logan, B. A. (2000). Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 355*(1402), 1499-1510.

Gordon, S. A., & Weber, R. P. (1951). Colorimetric estimation of indoleacetic acid. *Plant physiology, 26*(1), 192.

Hajiboland, R., Norouzi, F., & Poschenrieder, C. (2014). Growth, physiological, biochemical and ionic responses of pistachio seedlings to mild and high salinity. *Trees, 28*(4), 1065-1078.

Hasanuzzaman, M., Nahar, K., & Fujita, M. (2013). Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In Ecophysiology and responses of plants under salt stress (pp. 25-87). Springer, New York, NY.

Hendawy, S. F., & Khalid, K. A. (2005). Response of sage (*Salvia officinalis* L.) plants to zinc application under different salinity levels. *Journal of Applied Sciences Research, 1*(2), 147-155.

Hayat, S., Ali, B., & Ahmad, A. (2007). Salicylic acid: biosynthesis, metabolism and physiological role in plants. In Salicylic acid: A plant hormone (pp. 1-14). Springer, Dordrecht.

Hussein, M. M., Balbaa, L. K., & Gaballah, M. S. (2007). Salicylic acid and salinity effects on growth of maize plants. *Research Journal of Agriculture and Biological Sciences*, *3*(4), 321-328.

Hasegawa, P. M., Bressan, R. A., Zhu, J. K., & Bohnert, H. J. (2000). Plant cellular and molecular responses to high salinity. *Annual Review of Plant Biology, 51*(1), 463-499.

Jun, W. J., Han, B. K., Yu, K. W., Kim, M. S., Chang, I. S., Kim, H. Y., & Cho, H. Y. (2001). Antioxidant effects of *Origanum majorana* L. on superoxide anion radicals. *Food Chemistry, 75*(4), 439-444.

Jaleel, C. A., Gopi, R., Manivannan, P., & Panneerselvam, R. (2007). Antioxidative potentials as a protective mechanism in *Catharanthus roseus* (L.) G. Don. plants under salinity stress. *Turkish Journal of Botany, 31*(3), 245-251.

Kausar, A., Ashraf, M. Y., Ali, I., Niaz, M., & Abbass, Q. A. I. S. E. R. (2012). Evaluation of sorghum varieties/lines for salt tolerance using physiological indices as a screening tool. *Pakistan Journal of Botany, 44*(1), 47-52.

Kausar, A., Khurshid, A., Ashraf, M. Y., Ghafoor, R., & Gull, M. (2015). Photosynthesis, transpiration, stomatal conductance potential and water uptake in selected rice cultivars (*Oryza sativa*) under various saline conditions. *Sylwan*, *159*(3), 258-265.

Khan, M. H., & Panda, S. K. (2008). Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. *Acta Physiologiae Plantarum, 30*(1), 81.

Kim, H. J., Fonseca, J. M., Choi, J. H., Kubota, C., & Kwon, D. Y. (2008). Salt in irrigation water affects the nutritional and visual properties of romaine lettuce (*Lactuca sativa* L.). *Journal of Agricultural and Food Chemistry, 56*(10), 3772-3776.

Kapoor, K., & Srivastava, A. (2010). Assessment of salinity tolerance of Vinga mungo var. Pu-19 using ex vitro and in vitro methods. *Asian Journal of Biotechnology, 2*(2), 73-85.

Khan, W., Prithiviraj, B., & Smith, D. L. (2003). Photosynthetic responses of corn and soybean to foliar application of salicylates. *Journal of Plant Physiology, 160*(5), 485-492.

Kh, K., Mohseni, R., & Saboora, A. (2010). Biochemical changes of Rosmarinus officinalis under salt stress. *Journal of Stress Physiology & Biochemistry, 6*(3), 114-122.

Khosravinejad, F., Heydari, R., & Farboodnia, T. (2008). Antioxidant responses of two barley varieties to saline stress. *Pakistan Journal of Biological Science, 11*, 905-909.

Krinsky, N. I., & Johnson, E. J. (2005). Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine*, *26*(6), 459-516.

Kaymakanova, M., & Stoeva, N. (2008). Physiological reaction of bean plants (*Phaseolus vulg.* L.) to salt stress. *General and Applied Plant Physiology, Special*, *34*, 3-4.

Maclachlan, S., & Zalik, S. (1963). Plastid structure, chlorophyll concentration, and free amino acid composition of a chlorophyll mutant of barley. *Canadian Journal of Botany, 41*(7), 1053-1062.

Misra, N., & Gupta, A.K. (2006). Effect of salinity and different nitrogen sources on the activity of antioxidant enzymes and indole alkaloid content in *Catharanthus roseus* seedlings. *Journal of Plant Physiology, 163*(1), 11-18.

Mittova, V., Guy, M., Tal, M., & Volokita, M. (2004). Salinity up-regulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii*. *Journal of Experimental Botany, 55*(399), 1105-1113.

Mahipala, M.K., Krebs, G.L., McCafferty, P., & Gunaratne, L.H.P. (2009). Chemical composition, biological effects of tannin and in vitro nutritive value of selected browse species grown in the West Australian Mediterranean environment. *Animal Feed Science and Technology, 153*(3-4), 203-215.

Mamdouh, N.A., Younis, M.E., Shihaby, O.A., & Elbastawisy, Z. (2002). Kinetin regulation of growth and secondary treated Vigna sinensis and *Zea mays*. *Acta Physiologiae Plantarum*, *24*(1), 19-27.

Mansour, M.M.F. (2000). Nitrogen containing compounds and adaptation of plants to salinity stress. *Biologia Plantarum*, *43*(4), 491-500.

Mano, J. I. (2002). Early events in environmental stresses in plants. Induction mechanisms of oxidative stress. Oxidative Stress in Plants, 217-245.

Mitsui, A., & Ohta, T. (1961). Photooxidative consumption and photoreductive formation of ascorbic acid in green leaves. *Plant and Cell Physiology, 2*(1), 31-44.

Malik, C.P., & Singh, M.B. (1980). Plant enzymology and histo-enzymology.

Molazem, D., Qurbanov, E.M., & Dunyamaliyev, S.A. (2010). Role of proline, Na and chlorophyll content in salt tolerance of corn (*Zea mays* L.). *American-Eurasian Journal of Agricultural & Environmental Science, 9*(3), 319-324.

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, Cell & Environment, 25*(2), 239-250.

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology, 59*, 651-681.

Nemati, I., Moradi, F., Gholizadeh, S., Esmaeili, M. A., & Bihamta, M. R. (2011). The effect of salinity stress on ions and soluble sugars distribution in leaves, leaf sheaths and roots of rice (Oryza sativa L.) seedlings. *Plant, Soil and Environment*, *57*(1), 26-33.

Nagata, M., & Yamashita, I. (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *Nippon Shokuhin Kogyo Gakkaishi*, *39*(10), 925-928.

Nijveldt, R. J., Van Nood, E. L. S., Van Hoorn, D. E., Boelens, P. G., Van Norren, K., & Van Leeuwen, P. A. (2001). Flavonoids: A review of probable mechanisms of action and potential applications. *The American Journal of Clinical Nutrition, 74*(4), 418-425.

Noctor, G., & Foyer, C. H. (1998). Ascorbate and glutathione: Keeping active oxygen under control. *Annual Review of Plant Biology, 49*(1), 249-279.

Parida, A. K., & Das, A. B. (2005). Salt tolerance and salinity effects on plants: A review. *Ecotoxicology and Environmental Safety, 60*(3), 324-349.

Parida, A. K., Das, A. B., & Mohanty, P. (2004). Investigations on the antioxidative defence responses to NaCl stress in a mangrove, *Bruguiera parviflora*: Differential regulations of isoforms of some antioxidative enzymes. *Plant Growth Regulation, 42*(3), 213-226.

Pascale, S. D., Maggio, A., Fogliano, V., Ambrosino, P., & Ritieni, A. (2001). Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. *The Journal of Horticultural Science and Biotechnology, 76*(4), 447-453.

Pastori, G. M., Mullineaux, P. M., & Foyer, C. H. (2000). Post-transcriptional regulation prevents accumulation of glutathione reductase protein and activity in the bundle sheath cells of maize. *Plant Physiology, 122*(3), 667-676.

Prajapati, K. S., Pandey, P. P., & Suman, M. (2018). Impact of Gibberellic acid under salinity stress on Tomato (*Lycopersicon esculentum* L.). *Journal of Pharmacognosy and Phytochemistry, 7*(5), 2324-2328.

Pignocchi, C., & Foyer, C. H. (2003). Apoplastic ascorbate metabolism and its role in the regulation of cell signalling. *Current Opinion in Plant Biology, 6*(4), 379-389.

Quan, L. J., Zhang, B., Shi, W. W., & Li, H. Y. (2008). Hydrogen peroxide in plants: A versatile molecule of the reactive oxygen species network. *Journal of Integrative Plant Biology, 50*(1), 2-18.

Raiola, A., Rigano, M. M., Calafiore, R., Frusciante, L., & Barone, A. (2014). Enhancing the health-promoting effects of tomato fruit for biofortified food. Mediators of inflammation, 2014.

Randome, I., Basu, S., & Pereira, A. (2017). Effect of different stress treatments on mature green tomatoes (Solanum lycopersicum) to enhance fruit quality. *African Journal of Food, Agriculture, Nutrition and Development, 17*(4), 12547-12556.

Roumyana, V. I., Shtereva, L., Stancheva, I., & Geveva, M. (2016). Salt stress response of sunflower breeding lines developed after wide hybridization. *Türk Tarım ve Doğa Bilimleri Dergisi, 3*(3), 197-204.

Sairam, R. K., & Tyagi, A. (2004). Physiological and molecular biology of salinity stress tolerance in deficient and cultivated genotypes of chickpea. *Plant Growth Regulation*, *57*(10).

Sairam, R. K., Rao, K. V., & Srivastava, G. C. (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Science, 163*(5), 1037-1046.

Sakhabutdinova, A. R., Fatkhutdinova, D. R., Bezrukova, M. V., & Shakirova, F. M. (2003). Salicylic acid prevents the damaging action of stress factors on wheat plants. *Bulgarian Journal of Plant Physiology, 21*, 314-319.

Sastry, E. V., & Shekhawat, K. S. (2001). Alleviatory effect of GA3 on the effects of salinity at seedling stage in wheat (*Triticum aestivum*). *Indian Journal of Agricultural Research*, *35*(4), 226-231.

Schopfer, P., Liszkay, A., Bechtold, M., Frahry, G., & Wagner, A. (2002). Evidence that hydroxyl radicals mediate auxin-induced extension growth. *Planta, 214*(6), 821-828.

Shakirova, F. M., Sakhabutdinova, A. R., Bezrukova, M. V., Fatkhutdinova, R. A., & Fatkhutdinova, D. R. (2003). Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Science, 164*(3), 317-322.

Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*, Article ID 217037. https://doi.org/10.1155/2012/217037

Story, E. N., Kopec, R. E., Schwartz, S. J., & Harris, G. K. (2010). An update on the health effects of tomato lycopene. *Annual Review of Food Science and Technology*, *1*, 189-210.

Schweitzer, J. A., Madritch, M. D., Bailey, J. K., LeRoy, C. J., Fischer, D. G., Rehill, B. J., ... & Whitham, T. G. (2008). From genes to ecosystems: the genetic basis of condensed tannins and their role in nutrient regulation in a Populus model system. *Ecosystems*, *11*(6), 1005-1020.

Shoaib, A. B., & Malik, S. A. (2014). Evaluation of antioxidant and antibacterial activity of methanolic extracts of Gentiana kurroo royle. *Saudi Journal of Biological Sciences, 21*(5), 493-498.

Singh, A. K. (2018). The physiology of salt tolerance in four genotypes of chickpea during germination.

Sabir, P., Ashraf, M., & Akram, N. A. (2011). Accession variation for salt tolerance in proso millet (*Panicum miliaceum* L.) using leaf proline content and activities of some key antioxidant enzymes. *Journal of Agronomy and Crop Science, 197*(5), 340-347.

Türkan, I., & Demiral, T. (2009). Recent developments in understanding salinity tolerance. *Environmental and Experimental Botany, 67*(1), 2-9.

Tamir, B., & Asefa, G. (2009). Effects of different forms of Acacia saligna leaves inclusion on feed intake, digestibility and body weight gain in lambs fed grass hay basal diet. *Animal Feed Science and Technology, 153*(1-2), 39-47.

Tester, M., & Davenport, R. (2003). Na+ tolerance and Na+ transport in higher plants. *Annals of Botany, 91*(5), 503-527.

Ünyayar, S. (2002). Changes in abscisic acid and indole-3-acetic acid concentrations in *Funalia trogii* (Berk.) Bondartsev & Singer and *Phanerochaete chrysosporium* Burds. ME446 subjected to salt stress. *Turkish Journal of Botany, 26*(1), 1-4.

Van Handel, E. M. I. L. E. (1985). Rapid determination of glycogen and sugars in mosquitoes. *Journal of the American Mosquito Control Association, 1*(3), 299-301.

Warrier, R. R., Paul, M., & Vineetha, M. V. (2013). Estimation of salicylic acid in Eucalyptus leaves using spectrophotometric methods. *Genetics and Plant Physiology, 3*(1-2), 90-97.

Weisany, W., Sohrabi, Y., Heidari, G., Siosemardeh, A., & Ghassemi-Golezani, K. (2012). Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). *Plant Omics, 5*(2), 60.

Wang, Y., Mopper, S., & Hasenstein, K. H. (2001). Effects of salinity on endogenous ABA, IAA, JA, and SA in Iris hexagona. *Journal of Chemical Ecology, 27*(2), 327-342.

Xu, X., Xu, H., Wang, Y., Wang, X., Qiu, Y., & Xu, B. (2008). The effect of salt stress on the chlorophyll level of the main sand-binding plants in the shelterbelt along the Tarim Desert Highway. *Chinese Science Bulletin, 53*(2), 109-111.

Yardanov, I., Velikova, V., & Tsonev, T. (2003). Plant responses to drought and stress tolerance. *Bulgarian Journal of Plant Physiology*, (Special Issue), 187-206.

Zafar, S. A. R. A., Ashraf, M. Y., Niaz, M., Kausar, A., & Hussain, J. (2015). Evaluation of wheat genotypes for salinity tolerance using physiological indices as a screening tool. *Pakistan Journal of Botany, 47*(2), 397-405.

Zare, S., & Pakniyat, H. (2012). Changes in activities of antioxidant enzymes in oilseed rape in response to salinity stress. *International Journal of Agriculture and Crop Sciences, 4*(7), 398-403.

Zheng, W., & Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry, 49*(11), 5165-5170.