

*Full Length Research Paper*

## Antioxidant activity and lipid peroxidation of selected wheat cultivars under salt stress

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Accepted 9 May, 2012

Antioxidants and dietary fibers are compact sources that are recommended for healthy diets in whole grain foods. The antioxidant activity, total phenolic content (TPC) and lipid peroxidation was measured among 15 cultivars of wheat which are commonly used in Pakistan and the effect of salt stress was also evaluated. The results revealed that different concentrations of wheat (25, 50, 75, 150 and 300 µg/ml) in control and under salt stress (that is, electrical conductivity, 2 EC, 4 EC, 8 EC and 16 EC) showed antioxidant activity. The inhibitory concentration at 50% (IC<sub>50</sub>) values of different cultivars of wheat ranged from 22.9 to 27.01 µg/ml. On the basis of comparison of scavenging percentage of non-stress and stressed wheat groups at different doses of salt, we have concluded that LU26-CTR, PAS-90, BARS, NARC, WAFAQ, LISANI, SEHAR, MEHRAJ, GA-02 and SHAFaq are salt tolerant varieties. The change of phenolic content suggests that wheat uses antioxidant properties of phenolics as a mechanism of salt stress. Whereas, the lipid peroxidation data has indicated that LU26-CTR, PAS-90, BARS, NARC, FSD-08, PIRSBak-09, SEHAR, SH-03 and GA-02 are salt resistant varieties as they showed a less percentage increase in lipid peroxidation (malondialdehyde (MDA) content) compared to control at different doses of salt.

**Key words:** Antioxidant activity, phenolic content, salt stress, lipid peroxidation, wheat cultivars.

### INTRODUCTION

Wheat (*Triticum aestivum* L.) is a complete diet and has been used as a staple food by mankind since the late Stone Age (ca. 6700 BC) (Hind, 1931). All the basic nutrients such as vitamins, proteins, minerals, energy and balanced amount of bioactive compounds are contained in wheat grain. Abiotic factor which includes drought, temperature, excessive chemicals and soil salinity hinders its productivity by creating the free radicals and ultimately lessens the whole grain yield. About 7% of the world's total land area is affected by salt, equaling to similar percentage of its arable land (Ghassemi et al., 1995). Salt stress causes a number of changes in plant

metabolism like ion toxicity, osmotic stress and production of reactive oxygen species (ROS) (Mittler, 2002). The generation of ROS is limited or scavenged by an antioxidant system including antioxidant compounds (ascorbate, salicylate, glutathione, tocopherols etc.) and antioxidant enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) (Foyer and Noctor, 2003). Soil salinity and sodicity problems are common in arid and semi-arid regions, where rainfall is insufficient to leach salts and excess sodium ions out of the rhizosphere. All these salts that affect soils are distributed throughout the world (Szabolcs, 1989). The excess salt taken up by the plants is stored in older leaves: continued transport of salt into transpiration stream over long period results in very high concentration of Na<sup>+</sup>Cl<sup>-</sup> as a result the leaves die. This injury is probably caused by overloading the vacuolar capacity to

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compartmentalize toxic salt species. Alternatively, these toxic salt species might build up in cell wall and cause dehydration (Munns, 2005).

Wheat is one of the major important crops that have the property of antioxidation against the oxidation of important bio-molecules such as membrane lipids, proteins and DNA. It inhibits the human low-density lipoprotein (LDL) cholesterol per oxidation (Yu et al., 2005), super oxide anion ( $O_2^-$ ) (Ivana et al., 2011). Free radicals, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) ( $ABTS^+$ ) are also inhibited by wheat bran extracts (Zhou et al., 2004) as well as phospholipid liposomes hydrogen peroxide (Martínez-Tomé et al., 2004). Wheat genotypes, environmental or growing conditions such as average daily solar radiation, number of hours and growing locations are the significant factors that affect the scavenging percentage of antioxidants present in the whole wheat grain. Antioxidant capacity is contributed by a diverse array of bioactive compounds. These phytochemicals include phenolic acids, phytic acids, carotenoids, tocopherols, tocotrienols, phytosterols and flavonoids. Antioxidant properties vary with respect to wheat varieties (Yu et al., 2002; Zieliski and Kozłowska, 2000) and percentage of bioactive compounds such as carotenoids (Abdel-Aal et al., 2007), phenolic acids (Abdel-Aal et al., 2001), anthocyanins (Abdel-Aal, 2006) and tocopherols (Zhou et al., 2004). It is a well known fact that the salinity has the most important role in decreasing wheat growth and performance.

The DPPH assay has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods (Porto et al., 2000; Irina et al., 2002). The other analytical methods which measure the radical scavenging activity of antioxidants are superoxide anion radical ( $O_2^-$ ), the hydroxyl radical ( $\cdot OH$ ), or the peroxy radical ( $ROO\cdot$ ). There are other methods which determine the resistance of lipid or lipid emulsions to oxidation in the presence of the antioxidant being tested. The malondialdehyde (MDA) or thiobarbituric acid-reactive-substances (TBARS) assays have been used extensively since the 1950's to estimate the peroxidation of lipids in membrane and biological systems (Miller et al., 2000).

It has been shown that under stress conditions, MDA accumulation takes place in plants due to membrane lipid peroxidation. It is an effective means of assessing oxidative stress induced membrane damage (Shao et al., 2005) and cell membrane stability has been used as an efficient criterion to discriminate among crop cultivars with respect to degree of salt tolerance (Meloni et al., 2003; Sairam et al., 2005). To our knowledge, this is the first report on the antioxidant and lipid peroxidation among different cultivars of wheat from Pakistan. As the wheat has significant role in world food security, the research about salt stress tolerance mechanisms especially reactive oxygen scavenging system in wheat

could not be ignored. Different plant species and genotypes within a species respond differently to salt stress at different growth stages. The investigation was therefore, aimed to evaluate the antioxidant activity and lipid peroxidation among fifteen important wheat varieties against different salt concentrations to screen out the potential wheat cultivars for better performance under salt stress conditions and the development of functional wheat grains with natural healthy food products.

## MATERIALS AND METHODS

### Chemicals and reagents

DPPH radical (Sigma), Folin-Ciocalteu reagent (2 N) and 2-thiobarbituric acid were purchased from Sigma and Aldrich Chemicals (St, Louis, MO, USA). Ethanol (95%), trichloroacetic acid (TCA) and sodium carbonate were purchased from Bio-Chemical (Lahore). All the chemicals and reagents were of analytical grade.

### Plant growth conditions

The plant material (varieties) was selected on the base of their frequent cultivation in the area. The selected wheat varieties that is, LU-26CTR, PASBAN-90, BARS-2009, NARC-2009, FSD-2008, PIRSBK-90, WAFAQ-2001, LASANI-2008, SEHAR-2006, FAREED-2006, SH-2003, BAHAWALPUR-2000, GA-2002, MERAJ-2008 and SHFAQ-2006 were collected from different research stations of Pakistan, authenticated and grown in the experimental field of University of the Poonch Rawalakot Azad Kashmir Pakistan. The leaves from all varieties grown in pots at comparable conditions were taken for aqueous extraction. The experiment was laid out in two factors factorial randomized complete block design (RCBD) design with three replications. Equal amounts of soil, sand and farm yard manure was mixed and equal amount was used to fill the pots. Four doses of the table salt (NaCl) were applied to the wheat varieties. The doses included 2, 4, 8 and 16 desi Siemen's per meter (ds/m) against control (no salt applied) in the soil. The doses of salt were applied at jointing stage and the electrical conductivity (EC) was calculated according to the prescribed method of USDA (1954).

### Preparation of wheat extracts

The preparation of wheat extract was carried out by the method inspired from the study of Sabir and Rocha (2008). The leaves at full maturity (10 g) were ground and soaked in boiling water (250 ml) for 15 min, allowed to cool and filtered using Whatman filter paper. The obtained residue was further extracted twice and finally the whole extract was concentrated. The extract weight and percentage yield were found to be 1.2 to 1.8%, respectively. The serial dilutions of the extract were made to obtain the desired concentrations of wheat extract for experiment.

### Antioxidant activity by DPPH radical scavenging

The antioxidant activities of the wheat extracts were measured using the stable DPPH radical according to the method of Hatano et al. (1988). Briefly, 0.25 mM solution of DPPH radical (0.5 ml) was added to the sample solution in ethanol (1 ml) at different concentrations (25, 50, 100, 150 and 300  $\mu g/ml$ ). The mixture was

shaken vigorously and left to stand for 30 min in the dark, and the absorbance was measured at 517 nm. The capacity to scavenge the DPPH radical was calculated using the following equation:

$$(\%) = [(A_0 - A_1) / A_0] \times 100$$

Where,  $A_0$  is the absorbance of the control reaction and  $A_1$  is the absorbance of the sample itself. The inhibitory concentration at 50% ( $IC_{50}$ ) values (extract concentration that cause 50% scavenging of DPPH radical) were determined from the graph of scavenging percentage against the extract concentrations (25, 50, 100, 150 and 300  $\mu\text{g/ml}$ ) in slide write software. All determinations were carried out in triplicate.

#### Determination of total phenolics

The total phenolic content (TPC) was determined by adding 0.5 ml of the aqueous extract to 2.5 ml, 10% Folin-Ciocalteu reagent (v/v) and 2 ml of 7.5% sodium carbonate. The reaction mixture was incubated at 45°C for 40 min and the absorbance was measured at 765 nm in the spectrophotometer. Gallic acid was used as a standard phenol (Singleton et al., 1999). The mean of three readings was used and the TPC was expressed as milligrams of gallic acid equivalents/g extract.

#### Estimation of lipid peroxidation

Lipid peroxidation was measured as MDA in the leaves and was analyzed following Cakmak and Horst (1991) method. This method is based on the reaction with thiobarbituric acid. Fresh leaves (1.0 g) were ground properly in 20 ml of 0.1% TCA solution and centrifuged for 10 min at 12000 g. One (1) ml of the supernatant was reacted with 4 ml of 20% TCA solution comprising 0.6% thiobarbituric acid and then it was heated for 30 min at 95°C in a water bath and then immediately cooled on ice. After centrifugation for 10 min at 12000 g, the absorbance of the supernatant was read at 532 and 600 nm on a UV-VIS Spectrophotometer (UV-1201, SHIMADZU). The contents of MDA were worked out using the extinction coefficient of 155  $\text{mM}^{-1} \text{cm}^{-1}$  using the formula:

$$\text{MDA level (nmol)} = \Delta A(532 - 600) \text{ nm} / 1.56 \times 10^5$$

The percentage increase in lipid peroxidation was calculated to find out the resistant and salt sensitive cultivars.

#### Statistical analysis

The results were expressed as means  $\pm$  standard deviation. The data was analyzed by one way analysis of variance (ANOVA) and different group means were compared by Duncan's multiple range test where necessary.  $P < 0.05$  was considered significant in all cases. The software Package Statistica was used for analysis of data.

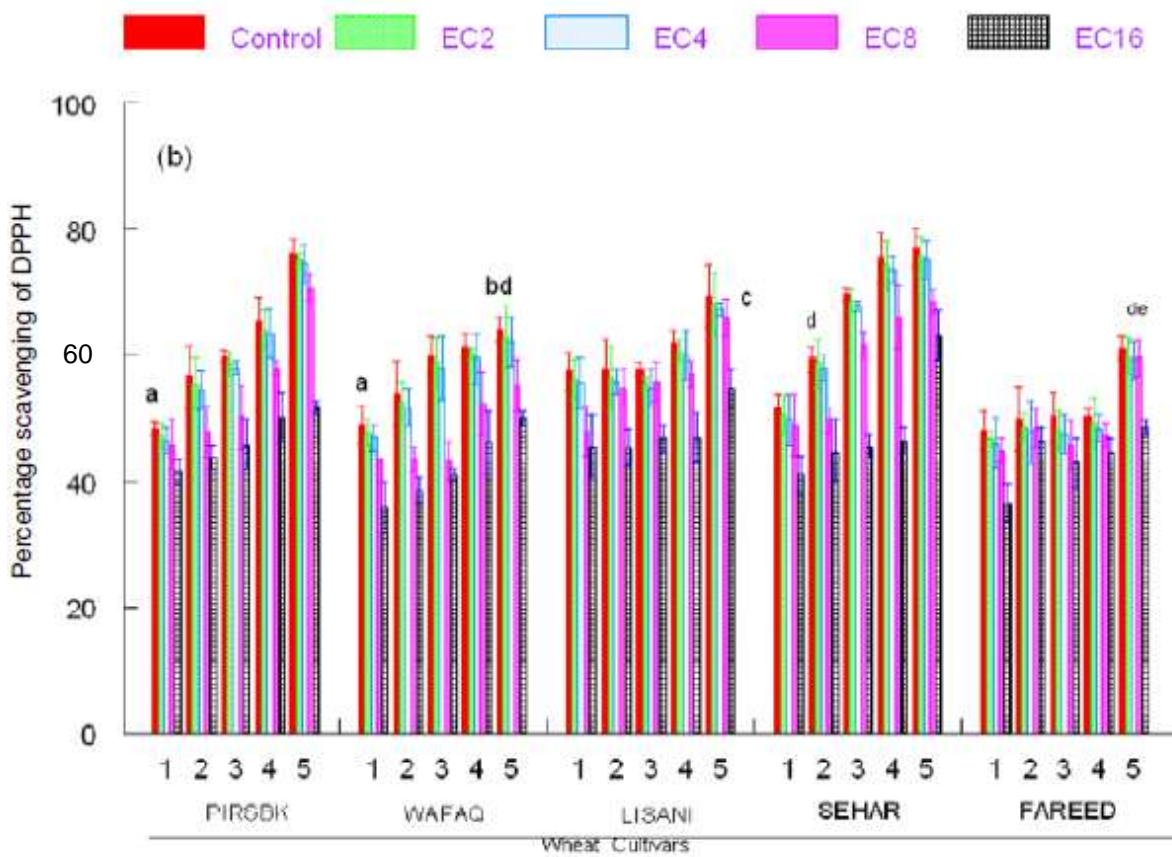
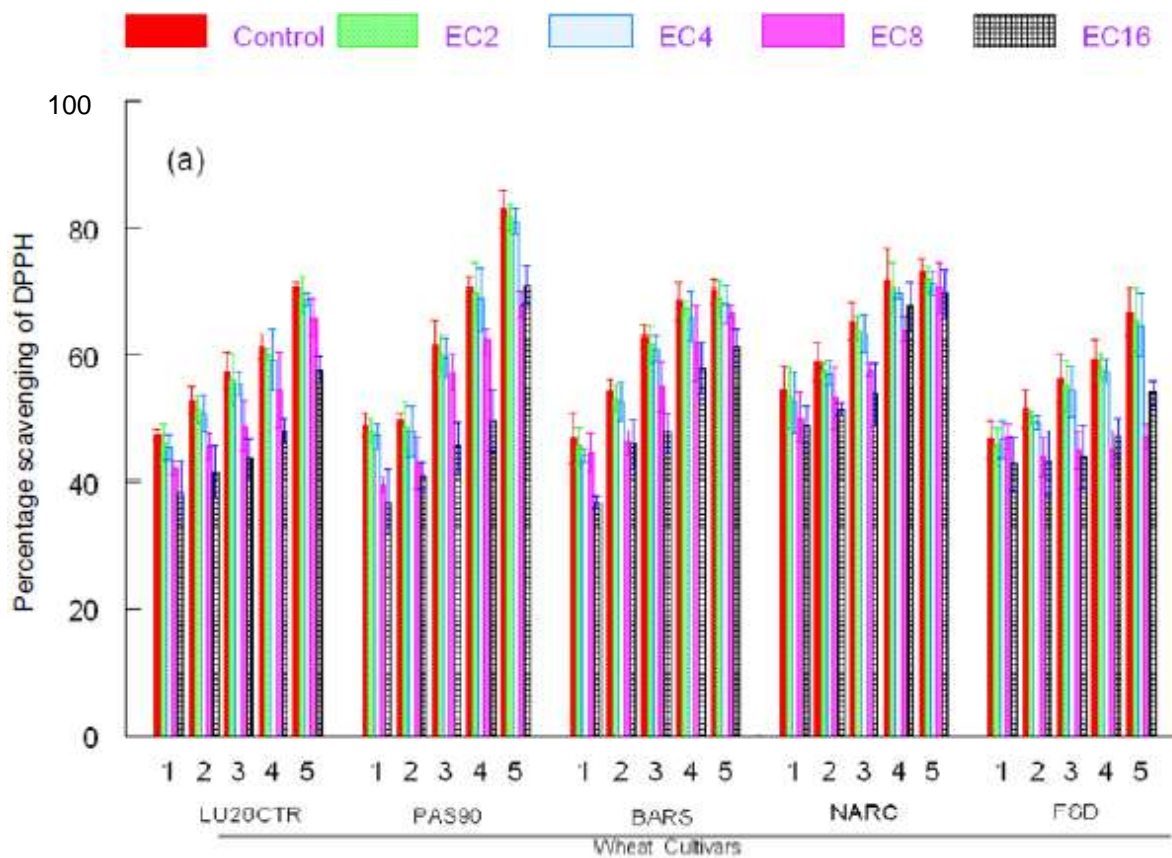
## RESULTS

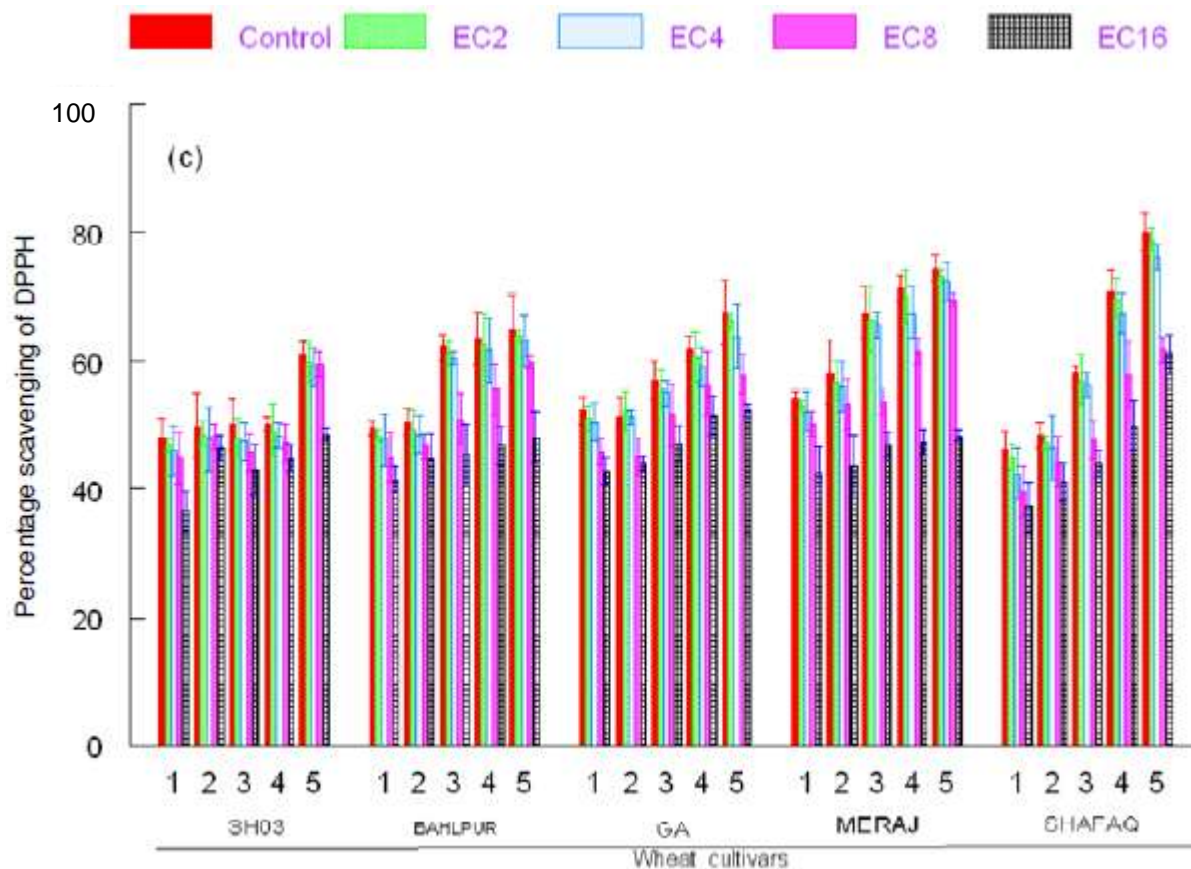
The antioxidant potential of cultivars LU-26-CTR, PAS-90, BARS, NARC and FSD is shown in Figure 1(a). The results revealed that the control of each variety showed higher scavenging percentage that was gradually

decreased by increasing the salt concentrations. Among different varieties the PAS-90 showed the highest scavenging percentage of  $82.98 \pm 3.1\%$  which was significantly reduced to  $67.98 \pm 2.1\%$  at salt stress of 8 EC and  $71.2 \pm 3.1\%$  at 16 EC. The antioxidant potential of PIRSBK, WAFAQ, LISANI, SEHAR and FAREED is shown in Figure 1(b). There was a concentration dependent scavenging for each variety. PIRSBK and SEHAR showed the highest antioxidant activity by discoloring the DPPH radical to  $76.29 \pm 2.1$  and  $76.99 \pm 3.1\%$  at the highest concentration (300  $\mu\text{g/ml}$ ). The wheat cultivars WAFAQ, LISANI and SEHAR were found to be salt tolerant as they showed higher percentage of scavenging at maximum dose of salt. The results of antioxidant activity and salt stress of SH-03, BAHLPUR, GA, MERAJ and SHAFQA are shown in Figure 1(c). Figure 2 shows the  $IC_{50}$  values (extract concentration that cause 50% scavenging) among different varieties of wheat. Among different cultivars NARC, MERAJ, GA and LISANI showed the highest antioxidant potential ( $IC_{50}$  values, 22.9, 23.07, 23.1 and 23.9  $\mu\text{g/ml}$ ) compared to LU-26 CTR, FSD, FAREED and SHAFQA (26.4, 26.7, 26, 27.1  $\mu\text{g/ml}$ , respectively). The lower the  $IC_{50}$  value, the higher is the antioxidant activity as the extract demonstrated 50% scavenging at the lower concentration.

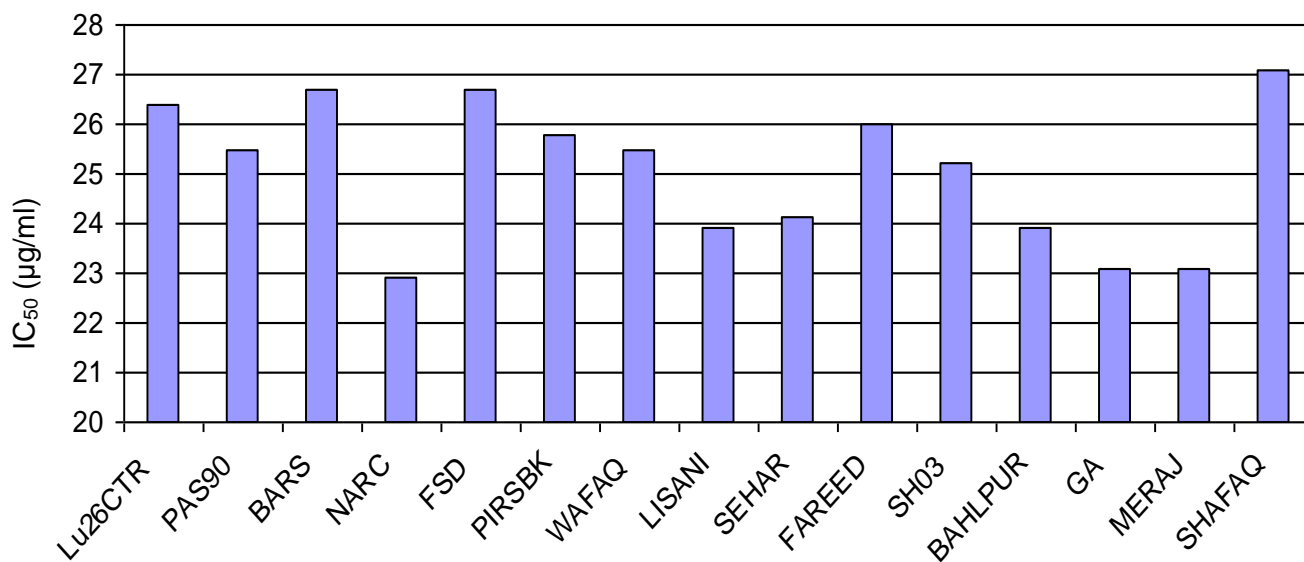
TPC measured by Folin-Ciocalteu reagent method ranged from 32.85 to 51.34 mg/g of gallic acid equivalent of aqueous wheat leaf extract (Figure 3). Treatment with different doses of salt caused a concentration dependent decrease in phenolic content. However, this decrease was not significant ( $P > 0.05$ ). The cultivars SEHAR, PIRSBK, PASBAN and SHAFQA relatively contained high quantity of phenolic content compared to other cultivars (Figure 3). To measure total phenolic content, 150 measurements were taken for all the wheat varieties and for different salt concentrations. Figure 4 shows that there was a strong positive correlation between total phenolic contents and antioxidant activity ( $r = 0.98$ ).

Lipid peroxidation as MDA content was found to be different among different cultivars. Among different cultivars (control), MDA level varied from 71 to 124 nmol/g of fresh weight of leaves. The minimum MDA was detected in WAFAQ (71 nmol/g) followed by MERAJ (77 nmol/g), SHAFQA (87 nmol/g) whereas, the maximum MDA was found in LU-26 CTR at control level (without stress) (data not shown). Lipid peroxidation showed increasing trend with increasing doses of salt. The tolerance of genotypes to salt stress is reflected by lower lipid peroxidation. Under salt stress of 16 EC maximum increase in lipid peroxidation was observed. Under NaCl stress conditions an increase in lipid peroxidation was found to be higher in WAFAQ (55.9%), LISANI (42.26%), FAREED (50.88%) and MERAJ (52.47%). Thus, these genotypes were found to be salt sensitive. Whereas, PASBAN (29.3%), LU-26 CTR (32%), NARC (33.37%), FSD (34.68%), SEHAR (30.72%) and SH03 (27.9%) showed decreased levels of lipid peroxidation and were

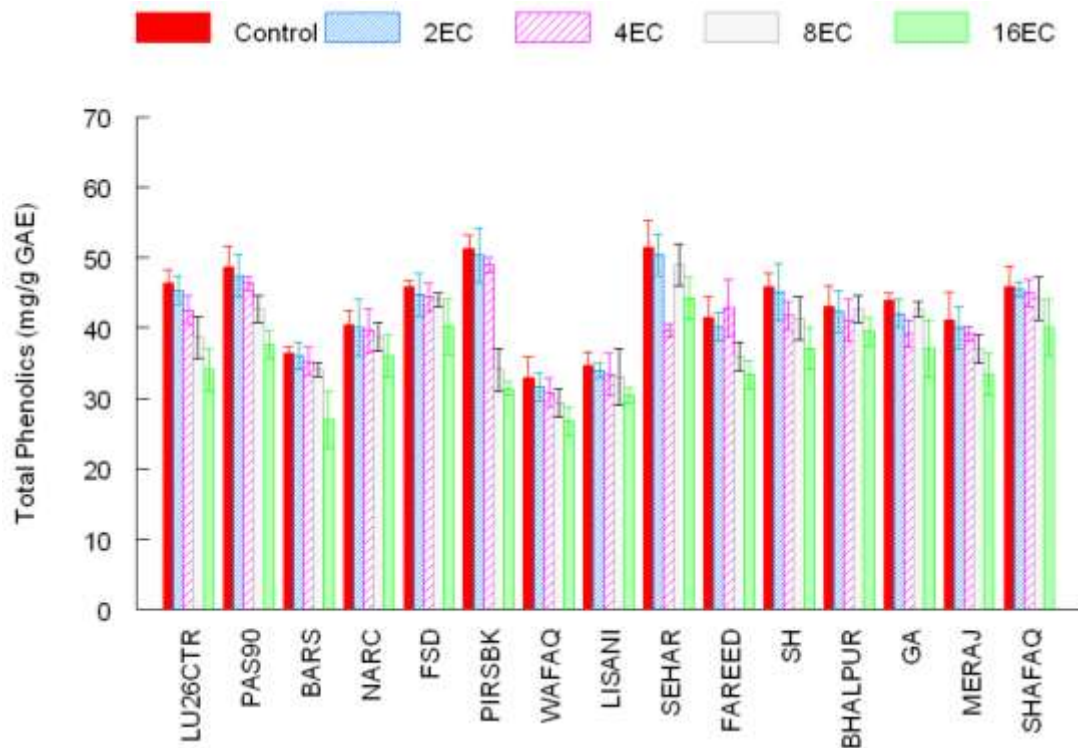




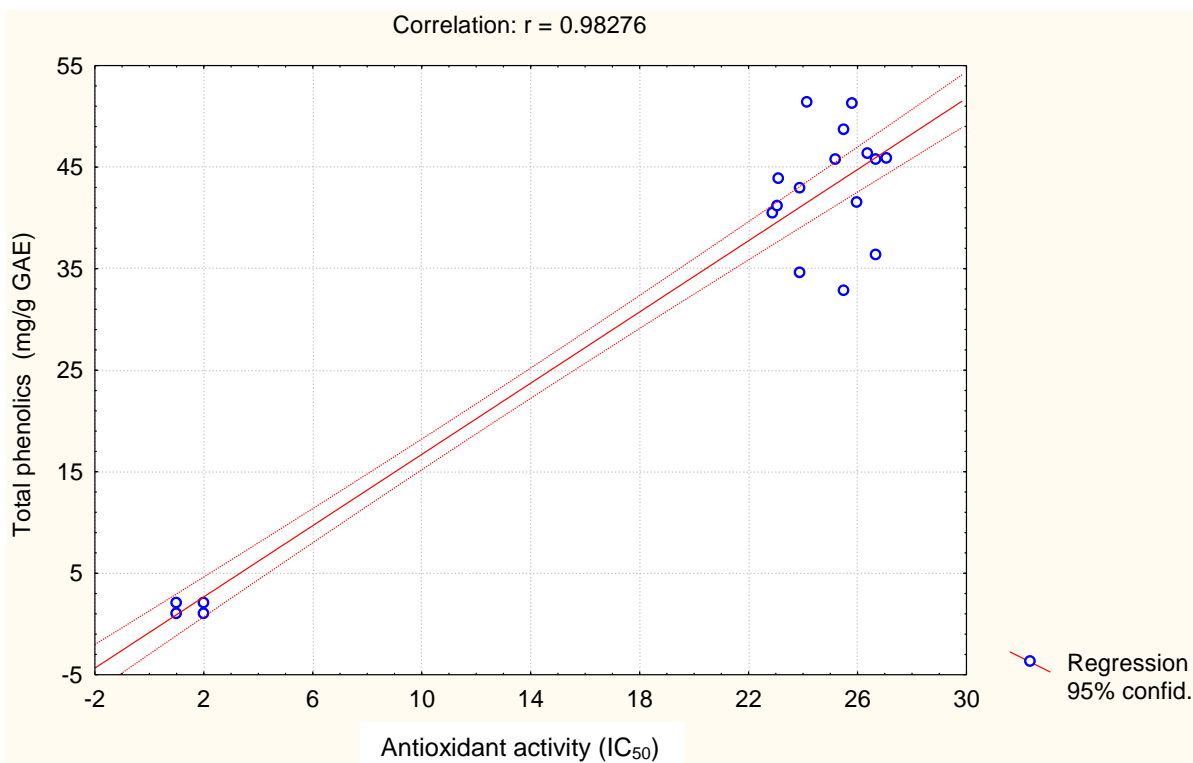
**Figure 1.** Antioxidant activity of aqueous extract of wheat leaves and the effect of salt stress on the antioxidant activity (a,b,c). DPPH radical scavenging activity of fifteen cultivars of wheat. Where, 1,2,3,4 and 5 represent the concentrations of 25, 50, 100, 150 and 300 µg/ml respectively. Values are mean ± SD (n=3). EC is the electrical conductivity. Values of concentrations in Figures 1(a) and (c) are significantly different (P < 0.05) from each other by DMR test, whereas, bars with similar letters are non-significantly different (P > 0.05) by DMR test in Figure 1(b).



**Figure 2.** Calculated IC<sub>50</sub> values (extract concentration that cause 50% scavenging) for wheat cultivars on DPPH radical scavenging.



**Figure 3.** TPC among non stress and stressed cultivars of wheat. Values are mean  $\pm$  SD (n=3). The stressed cultivars are non-significantly different ( $P > 0.05$ ) from control (non stress) by DMR test. EC is the electrical conductivity.



**Figure 4.** The relationship of antioxidant activity and TPC among different cultivars of wheat ( $r = 0.98$ ).

found to be salt tolerant (Figure 5).

## DISCUSSION

The whole wheat grains contain a variety of bioactive compounds that could contribute to health benefits of whole wheat foods reducing certain types of cancer and risk of coronary heart diseases (El-Sayed et al., 2008). Several authors have reported that the antioxidant properties of wheat or wheat products are significantly influenced by the genotype and/or the environment in which wheat is grown. To evaluate the whole wheat grains in relation to their health benefits and in the identification of potential constituents for the development of grain based functional diet the total antioxidant scavenging capacity and TPC have extensively been used. The wheat samples were categorized into five main groups based on different salt stresses and further divided into five sub groups on the base of increasing extract concentrations ranging from 25, 50, 100, 150 and 300 µg/ml. Significant differences were observed in the main groups (0 EC, 2 EC, 4 EC, 8 EC and 16 EC) and sub groups of different wheat extract application. The varieties, SHAFQA and MERAJ showed the highest antioxidant activity of  $67.66 \pm 5.1$  and  $74.33 \pm 2.4\%$  at 300 µg/ml. The varieties GA, MERAJ and SHAFQA showed significantly higher ability to tolerate the salt stress. The results revealed that the salt doses of 2 EC and 4 EC have very less effect on the antioxidant activity of wheat. However, the higher dose of salt (16 EC) resulted in a significant ( $P < 0.05$ ) and pronounced decrease in the antioxidant activity of wheat cultivars.

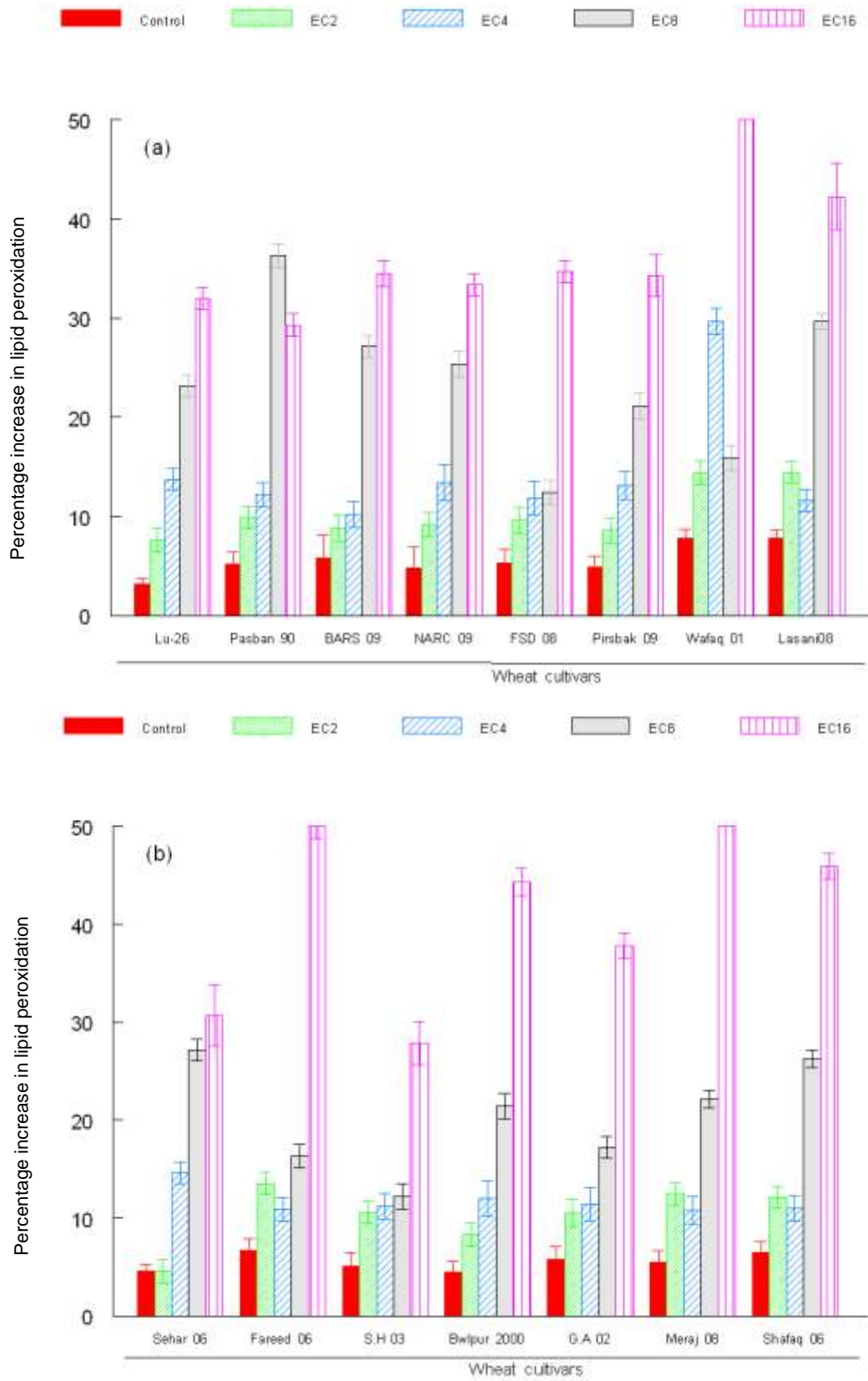
The salt stress produces a wide range of free radicals in whole plant and disturbs the most of its biochemical and metabolic reactions and ultimately results in low grain yield or the death of whole plant in severe conditions (Gara et al., 2003). It is the antioxidant potential of the plant that protects the plant against the free radicals. The scavenging capacity percentage decreases in the order of 2 EC > 4 EC > 8 EC > 16 EC, respectively. The result indicated a linear trend between increasing amount of wheat extracts and increasing scavenging capacities of the different varieties. It showed the clear cut dose dependent behavior of the wheat against the free radicals and oxidative insults thus, resulting to a wide range of variation among the different wheat cultivars. It was also observed that the lowest concentration of wheat extract (25 µg/ml) did not show variation among the scavenging percentage because the phytonutrients present at this concentration in the mixture were very low and the free radicals generated by salt were at its maximum level. However, at the highest extract concentration (300 µg/ml), the scavenging percentage as well as the antioxidant defense of wheat plant increases. Although the limited work has been done on the antioxidant activity of wheat leaves whereas most of

such studies are on the antioxidant activity of wheat grain. Our results are in agreement to the results of Sunil et al. (2006) where wheat grass has indicated the antioxidant activity on DPPH, ferric reducing antioxidant power (FRAP) and ABTS assays.

A strong positive correlation between TPCs and antioxidant activity ( $r = 0.98$ ) indicates that antioxidant activity is highly contributed by phenolics. Such strong correlation between total phenolics and antioxidant activity of wheat has already been reported (Trust et al., 2005). The phytochemicals responsible for the antioxidant activity are mainly due to phenolic acids and flavonoid compounds (Cao et al., 1997). The potential beneficial effects of the high antioxidant activity and protections of cells from free radical attack seem clear (Halliwell, 1994). In the flour of whole wheat grain, the bran germ fraction contributes 83% of TPC, 79% of total flavonoid content, 78% of total zeaxanthin, 51% of total luteins and 42% of total  $\beta$ -cryptoxanthin (Adom et al., 2005). Therefore, the whole wheat grain may help to reduce the risk of chronic diseases and produce greater health benefits when consumed as a regular part of diet (Liu, 2007). Insignificant differences of antioxidant capacity were found among some varieties compared. The results of our studies have shown that phenolic contents were decreased with different doses of salts in a dose dependent manner. Such decrease in phenolic content was already reported in wheat leaves (Muhammad et al., 2010).

The results of the DPPH• free radical-scavenging assay suggest that compounds within the extracts are capable of scavenging free radicals via electron- or hydrogen-donating mechanisms and thus should be able to prevent the initiation of deleterious free radical mediated chain reactions in susceptible matrices, for example, biological membranes. Wheat contains a large number of bioactive compounds which may possess the scavenging capacity against DPPH radicals and  $\text{Na}^+$  and  $\text{Cl}^-$ . However, percentage capacity to scavenge these free radicals by wheat antioxidants varies with a significant difference depending upon their synergic effect and concentration of the bioactive compounds in different wheat varieties. DPPH scavenging capacity was found to be different in their pearling fractions of eight Canadian wheat cultivars (El-Sayed et al., 2008). The study concluded that pearling is an effective milling technique to get wheat varieties extract enriched in antioxidants and total phenolic compounds by maximizing wheat for health benefits. Our results indicated that certain wheat varieties such as SEHAR-2006, PASBAN-90, NARC-09, BARS-09 and LU-26CTR would be highly potential antioxidant candidates based on their DPPH scavenging capacity and TPCs from leaves extracts.

An increase in salt stress induces oxidative stress in plant tissues (Hernandez et al., 1994). Lipid peroxidation requires active or uptake and involves the production of superoxide radical ( $\text{O}_2^{\cdot-}$ ). The other highly reactive



**Figure 5.** The percentage increase in lipid peroxidation (MDA) among different cultivars of wheat. Figure 5(a) and (b) show increase in lipid peroxidation at different doses of salt (2 EC, 4 EC, 8 EC, 16 EC). EC is the electrical conductivity.



chemical species are involving singlet oxygen ( $^1O_2$ ), hydroxyl free radical ( $\cdot OH$ ) and hydrogen peroxide ( $H_2O_2$ ) which initiate lipid peroxidation (Fridovich, 1986). Lower lipid peroxidation and higher membrane stability (lower ion leaching) have also been reported in tolerant genotypes of wheat (Kraus et al., 1995) and rice (Tijen and Ismail, 2005). On the basis of lipid peroxidation, Lu-26 CTR, NARC, FSD, SEHAR and SH03 were found to be salt tolerant genotypes, whereas, WAFAQ, LASANI, FAREED and MERAJ were found to be salt sensitive genotypes. A perusal of the results show that the higher free radical scavenging activity in resistant genotypes Lu26-CTR, PAS-90, NARC and SH03 are also associated with lowest lipid peroxidation and membrane thermo stability at the highest dose of salt (16 EC). However, further biochemical and molecular studies are in progress to indicate the salt resistant genotypes among fifteen cultivars of wheat.

## Conclusion

The examined wheat varieties exhibited a significant difference in their contents of total phenolics as well as their scavenging capacities against free radical. Certain wheat varieties, SEHAR-2006, LU-26 CTR, NARC-2009 and BARS-2009 were found to contain high concentration of bioactive compounds and antioxidant potential. In addition, some other varieties SHFAQ-06, LASANI-08 and GA-2002 also exhibit high radical scavenging activities and can be effectively utilized as a source of natural antioxidants. On the basis of results of lipid peroxidation, LU-26 CTR, NARC, FSD, SEHAR and SH03 were found to be salt tolerant genotypes. However, further detailed studies are required to evaluate the effect of salt stress on antioxidant enzymes and individual phenolic compounds in wheat leaves.

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