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Differential response of parent and advanced mutant lines of wheat (*Triticum aestivum* L. cv. *Tabasi*) genotypes in antioxidant activity to salinity stress at seedling stage

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Key words: Antioxidant Enzymes, Salinity, Mutant Line, Lipid Peroxidation, Wheat.

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Abstract

Effect of sodium chloride salinity was studied in advanced mutant lines *T-65-7-1* and *T-67-60* (tolerant) and their wild type *Tabasi* (moderately tolerant) wheat genotypes under control and level of salinity (EC 6 ds m^{-1}) at seedling stage. Salinity treatment decreased the contents of chlorophyll (CHL), carotenoids (CAR), α-Tocopherol (TOC) as well as enzyme activities including catalase (CAT), superoxide dismutase (SOD), ascorbic acid (AA), ascorbate peroxidase (APOX), and glutathione reductase (GR), whereas increased the contents of superoxide ion (O_2^-), hydrogen peroxide (H_2O_2), thiobarbituric acid reactive material (TBARM) (measuring of lipid peroxidation) in wild type *Tabasi* cultivar. Salinity tolerant mutant line *T-67-60* showed more enzymatic activities (CAT, SOD, ASA, APOX, GR) as well as CHL, CAR and TOC contents than *T-65-7-1* mutant line and *Tabasi* cultivar. Interestingly, the contents of O_2^-, H_2O_2 and TBARM in *T-67-60* line showed less increased than *T-65-7-1* line and *Tabasi* cultivar by salinity treatment. Base on these results the tolerance of *T-67-60* mutant line to salinity, justified by intensified enzyme activities to control oxidative damage via quench ROS levels.

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Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops grown world-wide and covers the largest area of the world (Faostat, 2014). Genetic diversity is the most important base in order to improve breeding program. Therefore, it is necessary generate variation within existing crops by using different methods such as mutagenesis (Flowers, 2004). The best way to improve crops to salt stress is genetic modification for salinity tolerance (Genc *et al.,* 2007). Induced mutation by gamma ray irradiation is one way to increase genetic variability of plants (Hanafiah *et al.,* 2010). Induced mutation breeding could change one or more important traits and could therefore play an important role in isolating interesting traits for plant breeding (Predieri, 2001). These induced mutations can upgrade of some important traits to increase tolerance or resistance to abiotic and biotic stresses use in major crops such as barley, rice, cotton, peanuts and wheat (Maluszynski and Kasha, 2002). Numerous environmental factors affect biochemical processes of organisms and reduce their ability to grow and reproduce (Mekail and Sharafaddin, 2009).Various environmental stresses, including biotic and abiotic stresses are produced reactive oxygen species (Navabpour *et al.,* 2003). These ROS are associated with a number of physiological and metabolic disorders in plants. Various intracellular components, such as chloroplasts, mitochondria and peroxisomes are usual locations for produced reactive oxygen species. These molecules are all over cells that produced during metabolism but plants have the ability to control their levels. The excessive level of this ROS can cause serious damage to the cells (Foyer and Noctor, 2003). ROS can strongly react with biomolecules such as lipids, proteins, pigments and nucleic acids, that can cause lipid peroxidation, protein denaturation and oxidation of sulfhydryl groups (-SH), discoloration or loss of pigments such as chlorophyll and other pigments and mutation in DNA. That disrupts the normal metabolism of the plant and leads to cell death ultimately (Noctor and Foyer, 1998; Ashraf, 2009). Plants have mechanisms of different enzymatic and non-enzymatic with high efficiency for against with oxidative stress, that can to adjust the amount free radicals. Enzymatic mechanisms include the enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate-glutathione cycle enzymes (such as ascorbate peroxidase (APX), glutathione reductase (GR), and enzymes of glutathione synthesis) and peroxidase. Non-enzymatic mechanisms in plants are also including ascorbate, glutathione, alpha-tocopherol and carotenoids and other compounds (including flavonoids, polyphenols, and mannitol) (Blokhin *et al.,* 2003; Sairam and Tyagi, 2004). In this article, measurement of superoxide ion, hydrogen peroxide and thiobarbituric acid reactive material evaluated under control and salt-stress treatments. In addition, enzymatic and non-enzymatic antioxidant defense system discussed in advanced mutant lines *T-65-7-1* and *T-67-60* and their parental wild type (*Tabasi* cultivar) under normal and salt stress conditions.

Materials and methods

Growth conditions

The seeds sixth generation of mutant lines *T-67-60* and *T-65-7-1* produced by irradiation of wheat cultivar *Tabasi* with a dose of 150 Gy cobalt-60 gamma source, and wheat cultivar *Tabasi* were obtained from Agricultural, Medical and Industrial Research School, Nuclear Science and Technology Research Institute, Karaj, Iran. Before sowing, seeds were surface sterilized with 1% sodium hypochlorite solution for 10 min, and washed thoroughly with sterilized distilled water.Then seeds were surface sterilized in 70% ethanol for 5 min, followed by a thorough washing in distilled water. These seeds were sown on sheets of Whatman No.1 filter paper moistened with 10 ml of distilled water. Petri dishes were placed in an incubator at 25℃ under dark conditions for five days.

Seedling establishment and salt treatments

The seedlings of three genotypes were placed on the nylon net (insect proofing type) in holds of Styrofoam sheets (1.25 cm thick for making seedling foaling over nutrient solution), and transferred to plastic tanks with outside

measurements of (49 cm length, 20 cm width, and 17 cm height with 14 L capacity), containing 12 liters Yoshida nutrient solution. The composition of the nutrient solution in 1 L work solution was: 0.114 gr Ammonium nitrate (NH_4No_3), 0.044 gr Sodium phosphate, monobasic monohydrate ($NaH_2PO_4.H_2O$), 0.089 gr Potassium sulfate (K_2SO_4), 0.146 gr Calcium chloride, dehydrate ($CaCl_2.2H_2O$), 0.405 gr Magnesium sulfate, 7-hydrate ($MgSO_4.7H_2O$), 0.001 gr Manganous chloride, 4-hydrate ($MnCl_3.4H_2O$), 92×10^{-6} gr Ammonium molybdate, 4-hydrate [$(NH_4)_6Mo_7O_{24}.4H_2O$], 43×10^{-6} gr Zinc sulfate, 7-hydrate ($ZnSO_4.7H_2O$), 11×10^{-4} gr Boric acid (H_3BO_3), 38×10^{-6} gr Cupric sulfate, 5-hydrate ($CuSO_4.5H_2O$), 96×10^{-4} gr Ferric chloride, 6-hydrate ($FeCl_3.6H_2O$), and 14×10^{-3} gr Citric acid, monohydrate ($C_6H_8O_7.H_2O$) described by Yoshida *et al.,* (1976). Each 6 tanks contained mutant lines *T-67-60*, *T-65-7-1* and *Tabasi* cultivar germinated seeds.

The seedlings in 3 tanks were treated with 46.08 gr NaCl per 12 liter (EC 6 ds/m), which was added to the nutrient solution. Other 3 tanks untreated used as control. The pH of the nutrient solution was measured by a pH meter and adjusted to 5.5–5.8 by adding KOH or HCL daily. The nutrient solution aerated by aquarium pump. Then tanks placed in an environmentally controlled growth-phytotron chamber with a photoperiod of 16/8 h light/dark regime at 25/20 ℃. The nutrient solution was changed every 5 days till 10 days of growth from start of the growth period. After 10 days of treatment leaves were harvested from seedlings, immediately frozen in liquid nitrogen, and stored at -80℃ until required for analysis. This experiment based on factorial completely randomized design with three replications was carried out under greenhouse conditions in Department of Agronomy and Plant Breeding, Gorgon university of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Experimental design and data analysis

The experimental design was a completely randomized with three replications treatments included factorial combination of three genotypes (*Tabasi* cultivar and two advanced mutant lines *T-67-60* and *T-65-7-1*) and two salinity levels (control and EC 6 ds/m). The data were analyzed statistically with SPSS-19 software. Means were statistically compared by least significance difference test (LSD) at $P < 0.01$ level.

Measurements

Extraction and Determination of enzymatic activities

Frozen leaf samples (1gr) were homogenized in 10 ml of chilled 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% (w/v) polyvinylpyrolidone (PVP) in mortar and pestle under cool condition. The homogenate was centrifuged at 15000 g for 10 min at 4 ℃ and supernatant was used for enzyme activity assay.

Catalase (CAT) activity was determined in terms of decrease in absorbance due to decomposition of H_2O_2 at 240 nm using an extinction coefficient of 39.4 mM^{-1} cm^{-1} (Aebi, 1984). Three ml reaction mixture containing 1.5 ml of 100 mM potassium phosphate buffer (pH 7.0), 0.5 ml of 75 mM H_2O_2, 0.05 ml enzyme extraction and distilled water to make up the volume to 3 ml. One unit of enzyme activity is defined as 1 n mol H_2O_2 decomposed mg^{-1} protein min^{-1}.

Glutathione reductase (GR) activity was assayed by measuring the decrease in absorbance due to oxidation of NADPH at 340 nm using an extinction coefficient of 6.2 $mM^{-1}cm^{-1}$ (Schaedle and Bassham, 1977). Reaction mixture (2 mL) contained 50 mM potassium phosphate buffer (pH 7.8) containing 2 mM EDTA, 0.15 mM NADPH, 0.5 mM oxidized glutathione (GSSG) and enzyme extract (200 µl). The reaction was initiated by adding NADPH. Corrections were made for the background absorbance at 340 nm, without NADPH. One unit of enzyme activity is defined as 1 n mol NADPH oxidized mg^{-1} protein min^{-1}.

Super oxide dismutase (SOD) activity was assayed by Giannopolitis and Reis, (1977). Reaction mixture (3 ml) contained 50 mM potassium phosphate buffer (pH 7.8), 1.3 µM riboflavin, 0.1 mM EDTA, 13 mM

methionine, 63 µM nitroblue tetrazolium chloride (NBT), 0.05 M sodium carbonate (pH 10.2) and enzyme extract (100 µl). The reaction mixture was illuminated for 20 min at light intensity of 4000 lux. The photo reduction of NBT (formation of purple formazone) was measured at 560 nm. One unit of SOD activity is defined as the amount of enzyme, which is required to cause 50% inhibition in the reduction of NBT.

Ascorbate peroxidase was assayed by recording the decrease in absorbance at 290 nm due to a decrease in ascorbic acid content (Nakano and Asada, 1981). Reaction mixture (3 cm^3) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA, and 1.5 mM H_2O_2 and 0.1 cm^3 enzyme extract. The reaction was started with the addition of H_2O_2. Absorbance was measured at 290 nm. Change in absorbance was monitored at 290 nm and activity was calculated from there action rate using an extinction coefficient of 2.8$mM^{-1}cm^{-1}$. The activity was expressed as mM ascorbic acid oxidized/min/mg protein.

Determination of non-enzymatic activities

For measurement of AsA, leaves samples of 0.5 gr were homogenized with a cold mortar and pestle using 10 ml ice-cooled 5% trichloroacetic acid (TCA). The homogenate was filtered through four layers of miracloth and centrifuged at 14,000 g for 10 min at 4 °C. The supernatant was used for the AsA assays. The absorbance of the color solution is read at 534 nm in spectrophotometer (model M36, Beckman, CA, USA). AsA was determined using the methods of Arakawa *et al.,* (1981). A standard curve in the range 0 - 25 n mol AsA was used.

Chlorophyll and Carotenoid content were estimated as described below. Leaves samples were frozen in liquid nitrogen, and after grinding, leaf samples (0.5 gr) were extracted with 10 ml of 80% acetone. Extract was then centrifuged at 1500 g for 10 min. Absorbance's were read at 663.6, 646.6 and 440.5 nm on a UV-visible spectrophotometer (model M36, Beckman, CA, USA). Concentrations of total chlorophyll and total carotenoid were calculated by the following equations Porre *et al.,* (1989) and Holm, (1954), respectively:

$$\text{Chl a} = 12.25\ A_{663.6} - 2.55\ A_{646.6}\ (\mu g/ml)$$
$$\text{Chl b} = 20.31\ A_{646.6} - 4.91\ A_{636.6}\ (\mu g/ml)$$
$$\text{Chl a + b} = 17.76\ A_{646.6} + 7.34\ A_{663.6}\ (\mu g/ml)$$
$$\text{Car} = 4.69\ A_{440.5} - 0.267\ \text{Chl a+ b}\ (\mu g/ml)$$

Alpha tocopherol content was measured as described by Munné-Bosch *et al.,* (1999) with some modifications. Leaves samples were frozen in liquid nitrogen, and after grinding, leaf samples (0.5 gr) were extracted with 5 ml of methanol containing 1% of ascorbate. With the addition of 4 mL of hexane, the extract was vortex for one minute and then centrifuged at 1500 g for 10 min. The upper layer hexane was removed and dried carefully with a partial vacuum chamber. Then 2 mL of methanol was added to the extract and, the homogenate was transferred into a HPLC. α-Tocopherol was separated by oltrasfer column (OD = 5µm), purified in concentration 1.5 ml per minute, and its concentration was determined by using excitation wavelength of 295 nm and an output of 328 nm in the UV detector (Model 280).

Lipid peroxidation was estimated as thiobarbituric acid reactive material (TBARM) described by Hagege *et al.,* (1990), with some modifications. Frozen leaf samples (0.5 gr) were homogenized in 10 cm^3 of 0.1 % trichloracetic acid (TCA), and the homogenate was centrifuged at 15000 g for 15 min. To 1.0 cm^3 aliquot of the supernatant 4.0 cm^3 of 0.5 % thiobarbituric acid (TBA) in 20 % TCA was added. The mixture was heated at 95 °C for 30 min in the laboratory electric oven and then mixture was quickly cooled in an ice bath. After centrifugation at 10000 g for 10 min the absorbance of the supernatant was recorded at 532 nm, and the amount of nonspecific absorption at 600 nm read and subtracted from this value. The blank was 0.25 % TBA in 10 % TCA. The TBARM content was calculated according to its coefficient of absorbance 155 mM^{-1} cm^{-1}.

Determination of reactive oxygen species

superoxide ion and hydrogen peroxide

O_2^- was measured as described by Elstner and Heupel, (1976), with some modifications. One gr of frozen leaf sample was homogenized with 3 ml of 65 mM potassium phosphate buffer (pH 7.8) and centrifuged at 5000 g for 10 min. The incubation mixture contained 0.9 ml of 65 mM phosphate buffer (pH 7.8), 0.1 ml of 10 mM hydroxylamine hydrochloride and 1 ml of supernatant. After incubation at 25℃ for 20 min, 1 ml of 17 mM sulphanilamide and 1 ml of 7 mM α-naphthylamine were added to the incubation mixture. After reaction at 25℃ for 20 min, ethyl ether in the same volume was added and centrifuged at 1500 g for 5 min. The absorbance in the aqueous solution was read at 530 nm. A standard curve with NO_2^- was used to calculate the production rate of O_2^- from the chemical reaction of NO_2^- and hydroxyl amine. The content of H_2O_2 was measured by monitoring the A_{415} of the titanium-peroxide complex following the method described by Brennan and Frenkel, (1977). Absorbance values were calibrated to a standard curve generated with known concentrations of H_2O_2. (H_2O_2 kit Orozco-Cardenas *et al.,* (2001)).

Results

Total Chlorophyll, Carotenoid and α-Tocopherol

Total CHL, CAR and TOC contents under control condition were higher in *T-67-60* than in other genotypes. Though *Tabasi* cultivar had lower CHL, CAR and TOC under normal condition, CHL, CAR and TOC decreased under salinity in all the genotypes. The interaction effect of cultivar × salt treatments was highly significant ($p < 0.01$) for CHL, CAR and TOC contents (Table 1).Salinity tolerant genotype *T-67-60* and *T-65-7-1* had higher CHL, CAR and TOC under salt stress as well as less declines in compared to *Tabasi*. However, the decrease under salt stress was also higher in *Tabasi*, while *T-67-60* maintained comparatively higher CHL, CAR and TOC content than *T-65-7-1* and *Tabasi* (Table 1 and Fig. 1).

Superoxide ion, hydrogen peroxide and thiobarbituric acid reactive material

The content of O_2^- and H_2O_2 increased under salinity stress in all the genotypes. Tabasi followed by T-65-7-1 showed higher H_2O_2 content in control and saline treated plants than T-67-60 genotype. Lipid peroxidation estimated as thiobarbituric acid reactive material (TBARM) also increased with salinity in all the genotypes, and similar to H_2O_2 accumulation TBARM content was highest in Tabasi cultivar in both control and salt treated plants and it was minimize in T-67-60, while T-65-7-1 showed middle response. The interaction effect of cultivar × salt treatments was highly significant ($p < 0.01$) for hydrogen peroxide and TBARM, although non-significant different for Superoxide ion (Table 1 and Fig. 2).

Table 1. Analysis of variance table for the effect of salinity on studied traits in advanced mutant lines T-65-7-1, T-67-60 and Tabasi cultivar.

Mean Squares	Chlorophyll(mg/g fresh weight)	1.466**	1.456**	0.210**	0.011
	Carotenoid(ug/mg chlorophyll)	151.475**	102.245**	10.333**	0.681
	α-Tocopherol(nmol/g fresh weight)	296.435**	140.728**	18.839**	2.087
	Superoxide ion(nmol/min/g fresh weight)	9.399**	74.664**	1.933ns	0.681
	Peroxide hydrogen(umol/g fresh weight)	117.671**	93.982**	8.565**	0.759
	TBARM(umol/g fresh weight)	16.513**	65.170**	0.999**	0.129
	Catalase activity(umol/min/mg protein)	31.170**	52.736**	3.061*	0.684
	Superoxide dismutase(u/mg protein)	192.193**	217.361**	59.014**	1.045
	Glutathion reductase activity(nmol/min/mg protein)	410.248**	173.725**	28.833**	2.170
	Ascorbic proxidase activity(nmol/min/mg protein)	99.149**	137.227**	2.477ns	2.546
	Ascorbic acid(umol/g fresh weight)	0.057*	0.271**	0.008ns	0.010
df		2	1	2	12
C.V		Genotype	Salinity	G*S	Error

*, ** Significant at the 5% and 1% levels of probability respectively and n.s (non-significant).

Catalase, superoxide dismutase, glutathione reductase, ascorbate peroxidase and ascorbic acid

Total SOD, CAT, GR, APX, and ASA activity increased under salt stress in all the genotypes. Salinity tolerant genotype *T67-60* and *T65-7-1* had higher activity in control and stressed plants than *Tabasi* cultivar. Their activity was highest in *T-67-60* in control and salt stressed plants, and *Tabasi* showed the lowest level. The interaction effect of cultivar × salt treatments was highly significant ($p < 0.01$) for SOD and GR, but CAT had a significant different by the probability level of 5%, although non-significant different for APX, and ASA activity (Table 1 and Fig. 3).

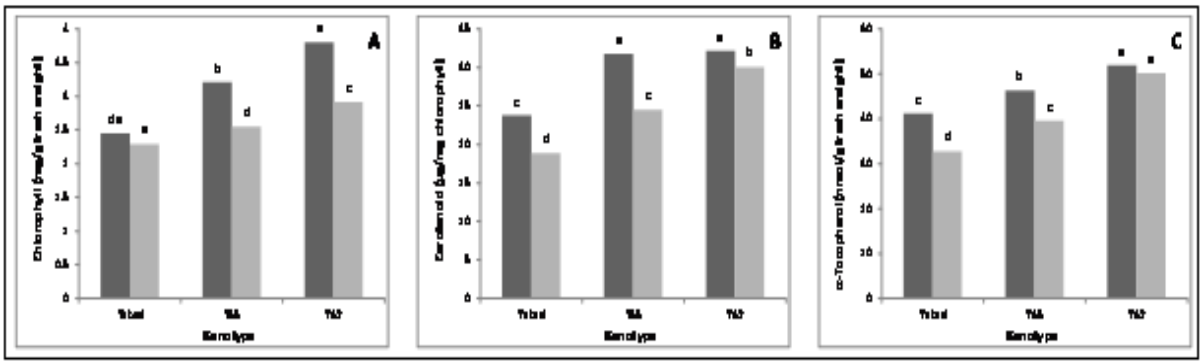

Fig. 1. Effect of salinity stress on total chlorophyll content (A), carotenoid content (B) and α-Tocopherol content (C) in tolerant and wild type wheat genotypes. LSD for cultivars and treatments were significant at $P = 0.01$. ■ Control; □ Salinity.

Discussion

Oxygen is necessary for life whereas its reduction leads to the formation of reactive oxygen species (ROS) (Asada, 1999). These reactive oxygen species are produced in low concentrations under normal growth conditions (Polle, 2001), and induced a partial adaptation in plants to resistance biotic and abiotic stresses, also act as signaling molecules involved in regulating gene expression and protein synthesis (Larkindale *et al.,* 2007; Pitzschke *et al.,* 2006), but are over produced under environmental stress conditions (Laloi *et al.,* 2004) and toxic at high concentrations, and caused oxidative stress and cell damage (Golden *et al.,* 2002). Salinity treatments caused significant increase in, O_2^-, H_2O_2 and TBARM (a measure of lipid peroxidation), which were higher in *Tabasi* cultivar than *T-67-60* and *T-65-7-1*. TBARM is regarded as a marker for evaluation of lipid peroxidation or damage to plasmalemma and organelle membranes that increases with environmental stresses. Lipid peroxidation is linked to the activity of antioxidant enzymes. Increase in lipid peroxidation during salt stress has been reported (Esfandiari *et al.,* 2011; Sairam *et al.,* 2002). Higher H_2O_2 accumulation and lipid peroxidation has been reported in salt stress sensitive rice varieties (Dionisio-Sese and Tobita, 1998) and wheat (Rao *et al.,* 2013; Rahman *et al.,* 2014). Salinity tolerant wheat genotype *T-67-60* showed significantly higher decline in O_2^-, H_2O_2 and lipid peroxidation than *T-65-7-1* and wild type genotypes *Tabasi* under salt stress that reflects their tolerant nature and increasing the activity of antioxidant systems. Lower O_2^-, H_2O_2 accumulation and lipid peroxidation have been reported in salinity tolerant genotype of wheat (Sairam *et al.,* 2005; Sairam *et al.,* 2002; Sairam and Srivastava, 2002). Increased O_2, H_2O_2 accumulation and TBARM due to salinity stress resulted in significant decrease in CH, CAR and TOC. CH, CAR, TOC and also enzymatic and non-enzymatic scavenging system activity were higher in tolerant genotype *T-67-60* and *T-65-7-1* than *Tabasi* cultivar. Koca *et al.,* (2007) and Li, (2009) indicated that salt tolerant cultivars accumulated less MDA in compared with salt sensitive cultivars at high salinity levels. Decrease contents of MDA is an important indicator of stress tolerance as shown in some earlier studies e.g., in salt tolerant cultivars of tobacco (Ruiz *et al.,*

2005), sorghum (Brankova *et al.,* 2005) and barley (Liang *et al.,* 2003). This reduction in the amount of MDA may be due to reduced production of ROS hence decreased oxidative stress. Salinity sensitive wheat genotype *Tabasi* cultivar showed significantly higher decline in CHL, CAR and TOC than tolerant genotypes *T-67-60* and *T-65-7-1* advanced mutant lines under salt stress that reflects their tolerant nature. Salinity induced decrease in Chl (Astorga *et al.,* 2010; Kazemi *et al.,* 2012), CAR (Sairam *et al.,* 2005; Sairam *et al.,* 2002; Parida and Das, 2005) have been reported earlier. CHL contents have been suggested as one of the parameters of salt tolerance in crop plants (Srivasta *et al.,* 1998; Hernandez *et al.,* 1995). Less decrease chlorophyll in tolerant genotype due to higher antioxidants activity in response to salt stress (Sairam and Srivastava, 2002). Rapid and large accumulation of ROS such as hydrogen peroxide is associated with degradation of chlorophyll (Farouk, 2011).

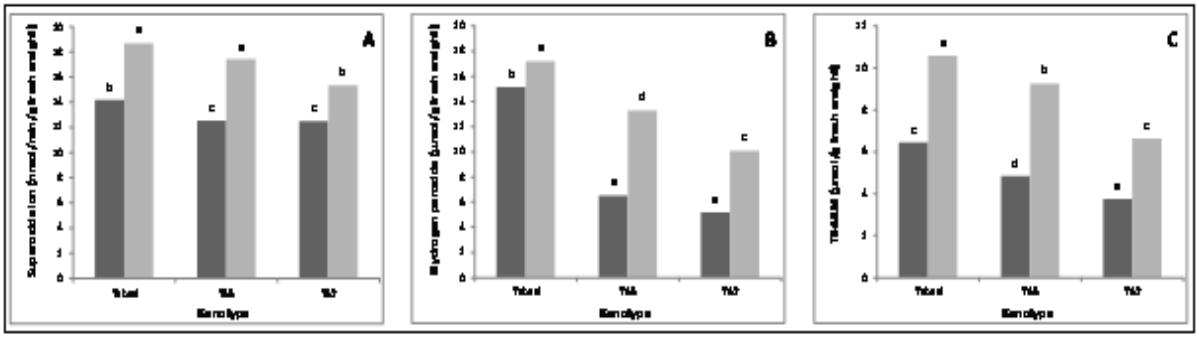

Fig. 2. Effect of salinity stress on superoxide ion content (A), hydrogen peroxide (B) and thiobarbituric acid reactive material (C) in tolerant and wild type wheat genotypes. LSD for cultivars and treatments were significant at $P = 0.01$. ■ Control; □ Salinity.

A negative correlation was observed in CHL, CAR and TOC production and leaf MDA contents in all wheat cultivars under salt stress, indicating that low lipid peroxidation resulted in increased CHL, CAR and TOC production. A positive relationship was reported between anti-oxidant enzymes and conserving rate of chlorophyll (Yang *et al.,* 2006). Tolerance to salt stress is partly correlated with tocopherol content (Munne-Bosch and Alegre, 2002; Fryer, 1992). Tocopherol can react with singlet oxygen, produced by photosensitized pigments such as chlorophyll (Nicholas, 2005). Tocopherol increased total chlorophyll either through the stimulation of its biosynthesis and/or delay of its degradation. However, this increase might be attributed to efficient scavenging of ROS by antioxidant enzymes and antioxidants; that would have destroyed the chlorophyll pigments (Farouk, 2011). Tocopherol Under the corresponding salinity levels, significantly decreased TBARS, membrane permeability and hydrogen peroxide (Farouk, 2011). Alpha-tocopherol is one of the most effective singlet-oxygen quenchers (Fryer, 1992), and decreased generation of ROS (Mohammed, 2011). Carotenoids can quench singlet oxygen in a similar manner to tocopherols. They are also able to react directly with superoxide and other free radicals (Young and Lowe, 2001; Krinsky and Yeum, 2003). The carotenoids have essential roles in photosynthesis, and could prevent lipid peroxidation chain reactions. It is noteworthy that the effect of carotenoid deficiency appears to be much more severe than tocopherol deficiency. This might suggest that carotenoids are more effective than tocopherols as chloroplast antioxidants, or they can substitute effectively in tocopherol-deficient plants (Nicholas, 2005). It has also been proposed that carotenoids can interact with tocopherols and ascorbate (Krinsky and Yeum, 2003; Wrona *et al.,* 2004). Ascorbate is an efficient quencher of a number of different ROS (Navabpour *et al.,* 2003). Synergistic interactions between carotenoids and tocopherol in protecting against lipid peroxidation in vivo and in vitro have been observed (Mortensen *et al.,* 1998; Yeum *et al.,* 2004). It should also be noted that carotenoids can

potentially act as pro-oxidants in the presence of high oxygen concentrations, although this may not occur in vivo (Young and Lowe, 2001; Krinsky and Yeum, 2003). It will be interesting to determine the extent to which tocopherol can affect signals generated in chloroplasts by singlet oxygen or the lipid peroxidation product malondialdehyde, both of which affect the expression of specific sets of genes (Almeras *et al.,* 2003; Weber *et al.,* 2004). Plants possess a number of antioxidant enzymes such as SOD, CAT (Asada, 2006; Caruso *et al.,* 2008), APX, GR and ascorbic acid (Halliwell, 2006; DalCorso *et al.,* 2008) that protect plant cells them from ROS cytotoxic effects. Environmental stresses induced increase in these antioxidant enzymes and metabolites has been reported by various workers (Rao *et al.,* 2013; Liu *et al.,* 2011), while higher activity has been reported in tolerant cultivars than the susceptible ones (Raza *et al.,* 2006; Ashraf and Foolad, 2007), suggesting that higher antioxidant enzymes activity have a role in imparting tolerance to these cultivars against environmental stresses. In this background the higher SOD, CAT, APX, GR and ASA activity was observed in *T-67-60*, both in control and salt stress treated plants, while *T-65-7-1* showed the middle response and *Tabasi* cultivar was the lowest. SOD, CAT, APX, ASA and GR activity increased significantly under salinity stress at seedling stage in the three genotypes and more activity was observed at *T-67-60*. Salinity induced increase in SOD activity (Atiq-ur-Raham *et al.,* 2014; Tan *et al.,* 2010), CAT activity (Yıldıztugay *et al.,* 2011; Weisany *et al.,* 2012), APX activity (Cai-Hong *et al.,* 2005; Amor *et al.,* 2006), ASA activity (Athar *et al.,* 2008; Zeid *et al.,* 2009), and GR activity (Sekmen esen *et al.,* 2012; Ozkur *et al.,* 2009).

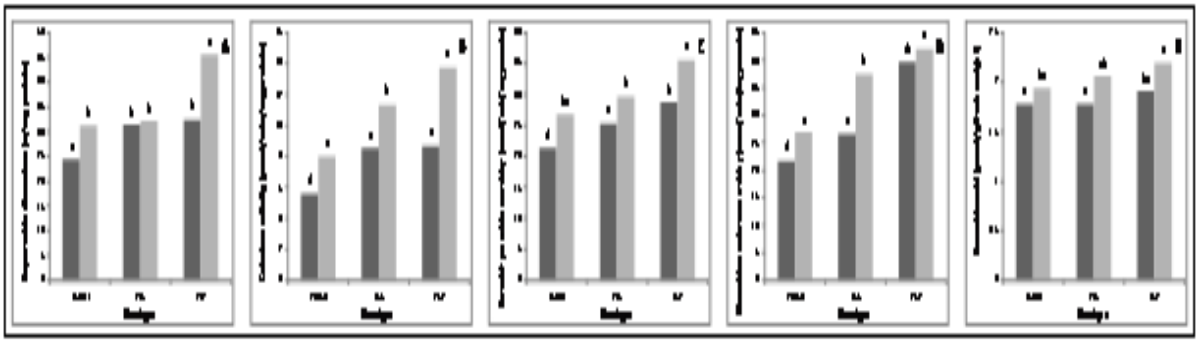

Fig. 3. Effect of salinity stress on the activities of superoxide dismutase (A), Catalase (B), ascorbate peroxidase (C) glutathione reductase (D) and ascorbic acid (E) in tolerant and wild type wheat genotypes. LSD for cultivars and treatments were significant at $P = 0.01$. ■ Control; □ Salinity.

Salinity tolerant wheat genotype *T-67-60* and *T-65-7-1* showed significantly higher in activities of these enzymes than *Tabasi* cultivar under salt stress that reflects their tolerant nature. Higher increase in the activity of CAT (Perveen *et al.,* 2011; Rahman *et al.,* 2014), SOD (De Azevedo Neto *et al.,* 2006; Noreen and Ashraf, 2009), APX (Sekmen *et al.,* 2007; Rao *et al.,* 2013), ASA (Athar *et al.,* 2008; Alamgir Hossain *et al.,* 2013) and GR (Meloni *et al.,* 2003; Sairam *et al.,* 2005) in salt tolerant genotype than in salt sensitive genotype has also been observed. The increase in H_2O_2 scavenging capacity of *T-67-60* could be one of the reasons of its tolerance to salinity stress. SOD directly converts superoxide to hydrogen peroxide and oxygen (Liu *et al.,* 2007; Zhao *et al.,* 2006). Then, the produced hydrogen peroxide is scavenged by CAT (Young *et al.,* 2008; Guo *et al.,* 2006), APX (Foyer and Noctor, 2005; Najami *et al.,* 2008), ASA (Asada, 2006; Ashraf, 2009) and GR (Sairam and Srivastava, 2002; Ben Amor *et al.,* 2006). Increases in activities of these enzymes cause decrease in oxidative damage were closely related. The tolerance of *T-67-60* to salinity as a result of increased activities of antioxidants (SOD, CAT, ASA, APX and GR) preventing toxic accumulation of ROS. Reduced these antioxidant enzymes and metabolites activity follows by the accumulation of O_2^- and H_2O_2 in leaf cells cause increase TBARM content in *Tabasi*

cultivar.

Conclusion

Advanced mutant line *T-67-60* has much better hydrogen peroxide scavenging mechanism as manifested by increase in enzymatic mechanism activities in salinity level, resulting in lower H_2O_2 content, lipid peroxidation, and higher contents chlorophyll, carotenoid and α-Tocopherol. Thus salt stress tolerance of *T-67-60* as indicated by lower H_2O_2 content and lipid peroxidation (TBARM content), and higher chlorophyll and carotenoid content under salt stress was mainly due to constitutively higher activity as well as salinity induced increase in SOD, CAT, APOX, GR and ASA than *T-65-7-1* and *Tabasi* cultivar. The results showed that in *Tabasi* cultivar, scavenging systems are very limiting, and the genotype is thus ill equipped to face salt stress as it fails to respond, resulting in higher H_2O_2 content, lipid peroxidation (TBARM content) and lower chlorophyll and carotenoid content under salt stress. From the above results it can be concluded that salt induced increase in antioxidant enzymes activities are important for providing protection against ROS. Therefore genetic changes obtained by wild type irradiation cause induced increase in antioxidant defense system activity in response to oxidative stress and higher the level of tolerance mutant lines.

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Abbreviations

O_2^- superoxide ion; H_2O_2 hydrogen peroxide; CAT catalase; SOD superoxide dismutase; GR glutathione reductase; ASA ascorbic acid; APOX ascorbate peroxidase; CHL chlorophyll; CAR carotenoid; Toc α-Tocopherol; ROS reactive oxygen species; TBARM thiobarbituric acid reactive material; EC electrical conductivity.

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