African Journal of Microbiology Research Vol. 6(3), pp. 617-623, 23 January, 2012
Available online at http://www.academicjournals.org/AJMR
ISSN 1996-0808 ©2012 Academic Journals
DOI: 10.5897/AJMR11.1167

Full Length Research Paper

Relationship between drought stress and some antioxidant enzymes with cell membrane and chlorophyll stability in wheat lines

Parisa Sharifi[1]*, Reza Amirnia[1], Eslam Majidi[2], Hashem Hadi[1], Mozafar roustaii[3], Babak Nakhoda[2] Hadi Mohammad Alipoor[4], and Foad Moradi[2]

[1]Department of Agronomy and Plant Breeding Department, University of Urmia, Urmia, Iran.
[2]Biotechnology Research Institute, Karaj, Iran.
[3]Rainfed Research Center, Maragheh, Iran.
[4]Tehran University, Tehran, Iran.

Accepted 14 December, 2011

In drought stress conditions, the imbalance between energy intake and consumption by photosynthetic organ causes the production of reactive oxygen species (ROS) and inability of the plant to control them, which eventually led to stress in the cell membranes and incidence of symptoms caused by oxidative damage. Antioxidant enzymes are considered as the fastest units that fight against reactive oxygen species. In this study, the changes in catalase and peroxidase activities in two levels of drought stress conditions (drought stress and control) and its effect on cell membrane and chlorophyll stability in the tolerant (UnKnown 11, HomaandOhadi), semi-tolerant (Sabalan and Rasad) and sensitive (SARA-PBWYT-85-86-22-5 and SHARK-4-0YC-0YC-0YC-5YC-0YC) lines of wheat were analyzed in a factorial experiment based on randomized complete blocks with three replicates. The drought stress caused increase in the peroxidase enzyme activity of Unknown 11, Ohadi and Rasad lines. Although, no increase in peroxidase enzyme activity was shown, Homa still had the highest rate of enzyme activity in drought and control conditions. The activity of catalase enzyme in stress condition in all investigated lines remained stable or decreased and there was no specific relation between the activity of the enzyme and drought resistant. The highest index of chlorophyll stability in the stress condition was perceived in lines Homa،Ohadi and Unknown 11. Also, the most stabilized and the most inconstant cell membrane in stress condition was related to lines Rasad, Homa and SARA-PBWYT-85-86-22-5. In drought condition, there was a positive correlation between peroxidase enzyme activity, yield and stability of chlorophyll b, whereas we did not perceive any positive correlation between catalase enzyme activity and yield. Moreover, considering the existence of a negative correlation between peroxidase enzyme activity and cell membrane stability, it can be concluded that more activity of peroxidase enzyme in drought stress condition leads to increased cell membrane and chlorophyll stability, and it is related to the drought resistance of different lines.

Key words: Wheat lines, catalase, peroxidase, cell membrane stability, chlorophyll stability index.

INTRODUCTION

Drought is the most significant factor restricting plant production on majority of agricultural fields of the world (Tas and Tas, 2007). During drought stress, improper adjustment of photosystem II and thermodynamic damages disrupt the flow of electrons and lead to production of free radicals. Oxidative damages results from incomplete detoxification of reactive oxygen species. Damages caused by oxidative stress includes; inactivation of enzymes, lipid peroxidation, protein degradation and destruction of DNA strands, reduction in

*Corresponding author. E-mail: Khayatneghad@yahoo.com. Tel: 0914-491-1206

chlorophyll content and pigments color (Friso et al., 2004; Gechevet al., 2006; Baruah et al., 2009).

Detoxification of reactive oxygen species in plants, includes enzymatic mechanisms (such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POX), glutathione reductase (GR) and mono dehydroascorbatereductase (MDAR) as well as non-enzymatic mechanisms (such as, flavonoids, anthocyanins, carotenoids and ascorbic acid, AA) (Frisoet al., 2004; Ramachandra et al., 2004). In enzymatic antioxidant systems, catalase (EC1.11.1.6) is an oxidoreductase, located in peroxysomes and considered as an important enzyme to counter hydrogen peroxide produced in stress conditions, so that at drought stress conditions new isomorphs of it are released and the rate of former isomorphs increases (Srivalli et al., 2003; Khana-chopra and Selote, 2007). Peroxidase (EC1.11.1.6) as another enzymatic antioxidant systems, is an oxidoreductase that has one homogeneous -b as a prostatic group and catalysis oxidation of the proton giver compounds with H_2O_2 and consequently cause H_2O_2 to breakdown (Jiang and Zhang, 2004). In most studies, under drought condition increase in activity of this enzyme was reported (Srivalli et al., 2003; Jiang and Zhang, 2004). This research was conducted to study the effect of drought stress on activity of some antioxidant enzymes and to measure the role of these enzymes in decreasing oxidative stress damages in chlorophyll and cell membrane due to drought in sensitive and resistant lines of wheat.

METHODS AND MATERIALS

In this study, seven wheat lines including tolerant lines (Unknown 11, Homa and Ohadi), semi-tolerant lines (Sabalan, Rasad) and sensitive lines (SARA-PBWYT-85-86-22-5 and SHARK-4-0YC-0YC-0YC-5YC-0YC) were evaluated in a factorial experiment based on randomized complete blocks with three replicates in rain-fed condition at Marageh Agricultural Research Station (2009-2010). The seeds were sown on the 22nd of November 2009, in 5 rows 20 cm apart with the density of 200 seeds m^{-2}. The needed nitrogen for wheat based on field experiment results was 60 kg net nitrogen per hectare from urea source added to the soil in fall (Feyziasl and Valizadeh, 2001, 2003). The needed phosphorous was supplied on the basis of soil test and phosphorous deficit from critical level in soil (9 milligram per hectare) (Feyziasl et al., 2004). Irrigation of control treatments were routinely done until the end of growth period while irrigation of drought stress treatment was interrupted at flowering stage. After removal of the marginal effects, that is, after the pollination stage, 10 competing plants were randomly selected, and then samples were immediately separated into flag leaf and spike and separately wrapped in aluminum foil and were immediately put in liquid nitrogen. Then samples were dried in freeze dryer (-120°C) and were kept in a -40°C freezer until measurement. In order to measure the yield, 10 plants were selected randomly after maturity and yield per plant was obtained from the average of these plants yields.

Leaf membrane damage

Leaf membrane damage was determined by recording electrolyte leakage (EL) as described by Valentovic et al. (2006) with few modifications. Plant material (0.5 g) washed with deionized water was placed in tubes with 20 ml of deionized water and incubated for 24 h at 25°C. Subsequently, the electrical conductivity of the solution (L1) was measured. Samples were then autoclaved at 120°C for 20 min and the final conductivity (L2) was measured after equilibration at 25°C. The EL was defined as follows:

$$EL\ (\%) = (L1/L2) \times 100$$

Chlorophyll content and stability index

Leaf samples were selected randomly from the plants and homogenized in a mortar in acetone. The extract was centrifuged at 5000 g for 5 min. Absorbance of the supernatant was recorded at 663 and 645 nm, spectrophotometrically. Chlorophyll (Chl) content was determined following the method of Arnon (1949). The chlorophyll stability index (CSI) was determined according to Sairam et al. (1997) and calculated as follows:

$$CSI = (\text{Total Chl under stress}/\text{Total Chl under control}) \times 100$$

Enzyme extraction

For protein and antioxidant enzyme assays, frozen leaves were ground to a fine powder with liquid nitrogen and were extracted with ice-cold 0.1 M Tris-HCl buffer (pH 7.5) containing 5% (w/v) sucrose and 0.1% 2-mercaptoethanol (3:1 buffer volume/FW). The homogenate was centrifuged at 10000 g for 20 min, at 4°C, and the supernatant was used for enzyme activity and protein determinations. Preparations for enzyme extraction and enzyme assay were carried out at 4°C.

Protein determination

The concentration of protein was determined by the method of Bradford (1976) using BSA as a standard.

Enzymes assay

CAT activity was determined by monitoring the disappearance of H_2O_2 at 240 nm (ε = 40 mM−1 cm−1) according to the method of Aebi (1983). The reaction mixture contained 50 mM K-phosphate buffer (pH 7.0), 33 mM H_2O_2 and enzyme extract.

For peroxidase activity, the leaves were homogenized on ice in 10 ml cold sodium phosphate buffer (pH 7.0). Activity was determined spectrophotometrically according to Rodriguez and Sanchez (1982). POD activity was analyzed in 50 mM phosphate buffer (pH 6.5) containing 40 mM guaiacol and 26 mM H_2O_2. The increase of absorbance at 420 nm was recorded within 180 s after adding 26 mM H_2O_2.

Data analysis

Data analysis was carried out through Minitab and MSTAT-C softwares and Excel software was used for drawing diagrams. Means were compared by Duncan's multiple range test at a probability level of 5%.

RESULTS AND DISCUSSION

Data analysis showed that the investigated lines were

Table 1. Variance analysis related to the rate of damage to cell membrane and chlorophyll resistance index as a result of drought stress after pollination.

SOV	df	MS			
		Chlorophyll stability index	Chlorophyll b stability index	Chlorophyll a stability index	Cellular damage to the Communion
Rep	2	827.11**	1182.34**	1225.1*	44.726
Genotypes	6	609.17**	732.13**	353.7	321.48**
Error	12	85.36	85.19	342.3	40.57

* and **, significant at 5 and 1% levels of probability, respectively.

Table 2. Variance analysis of drought stress effect on chlorophyll content, antioxidant enzymes and the investigated lines function.

SOV	df	MS					
		Yield	Catalase activity	Peroxidase activity	Total chlorophyll	Chlorophyll b	Chlorophyll a
Rep	2	0.031	0.1713**	3.1407	0.0137**	0.0015**	0.07562**
Condition	1	4.598**	0.2115**	31.201**	0.2711**	0.0532**	0.12453*
Genotype	6	0.153**	0.2212	106.106**	0.0023	0.00072*	0.00801
G×C	6	0.111**	0.0722	5.933**	0.0029*	0.00101**	0.00421
Error	22	0.034	0.0221	1.432	0.0014	0.00024	0.04547

* and **, significant at 5 and 1% levels of probability, respectively.

significantly different in cell membrane stability and resistance (Table 1), so that Rasad and Homalines won the most stable membranes and line SARA-PBWYT-85-86-22-5 had the most unstable membrane. So drought stress caused the most deleterious effect on SARA-PBWYT-85-86-22-5 line cell membrane (Table 1). Drought stress effect on the amount of *a*, *b* and total chlorophyll was significant (Table 1). In all lines, the amount of chlorophyll *a*, *b* and *a*+*b* (total chlorophyll) reduced in the stressed treatment.

Results showed that in the different lines, stability index of chlorophyll *b* and total chlorophyll were significantly different statistically, but in the case of chlorophyll *a* stability index, there was no significant difference between the lines (Table 1). The highest stability index of chlorophyll *b* was related to Homa and Unknown 11 and the lowest belonged to SHARK-4-0YC-0YC-0YC-5YC-0YC, respectively (Figure 1b). Also from the investigated, lines of stability index of total chlorophyll the lines Ohadi, Unknown 11, Homa and Sabalan acquired the highest content.

The effect of drought stress on antioxidant enzymes activity was significant, statistically (Table 2). Different lines showed different reactions in terms of peroxidase enzyme activity, in drought stress condition (Table 2). In drought tolerant (Ohadi and Unknown 11) and semi-tolerant (Rasad) lines, the activity of this enzyme increased by 183 and 107% compared to the control, respectively. While increasing the activity of this enzyme in the two sensitive lines (SARA-PBWYT-85-86-22-5 and SHARK-4-0YC-0YC-0YC-5YC-0YC) was very small. Although, there was no significant increase in peroxidase enzyme activity in Homa line at drought conditions, it had the highest activity of the enzyme in stressed conditions (Figure 2a).

Unlike the peroxidase, the catalase activity in different lines under drought stress remained unchanged or decreased, as against control conditions (Figure 2b). But among the different lines (drought tolerant and sensitive) no particular pattern of activity of this enzyme was observed. In drought condition, different lines had different yields (Figure 2c). The highest decrease in the yield at stress conditions was seen in the sensitive lines (SARA-PBWYT-85-86-22-5 and SHARK-4-0YC-0YC-0YC-5YC-0YC). A very strong negative corre-lation was observed between peroxidase enzyme activity and damage to cell membranes in stress conditions while correlation between catalase enzyme and this trait was not significant. Peroxidase enzyme activity showed a positive correlation with grain yield and stability of chlorophyll *b* but the traits showed no correlation with the catalase enzyme activity. Further-more, the catalase enzyme activity in drought conditions showed a positive correlation with the traits of total chlorophyll and chlorophyll *b* contents. The correlations between peroxidase enzymes with these two traits were not significant. Water stress reduced membrane stability index in all lines investigated and the intensity of decrease was greater in lines sensitive to stress. One of the reactions thatspeed up in the presence of reactive oxygen species is peroxidation of membrane lipids which leads to the production of aldehydes like mlondialdehyde and other products like ethylene (Liu and Hoang, 2000; Jiang and Hoang, 2001).

High concentrations of malondialdehyde increases lipid peroxidation and oxidation of cell membrane fatty acids

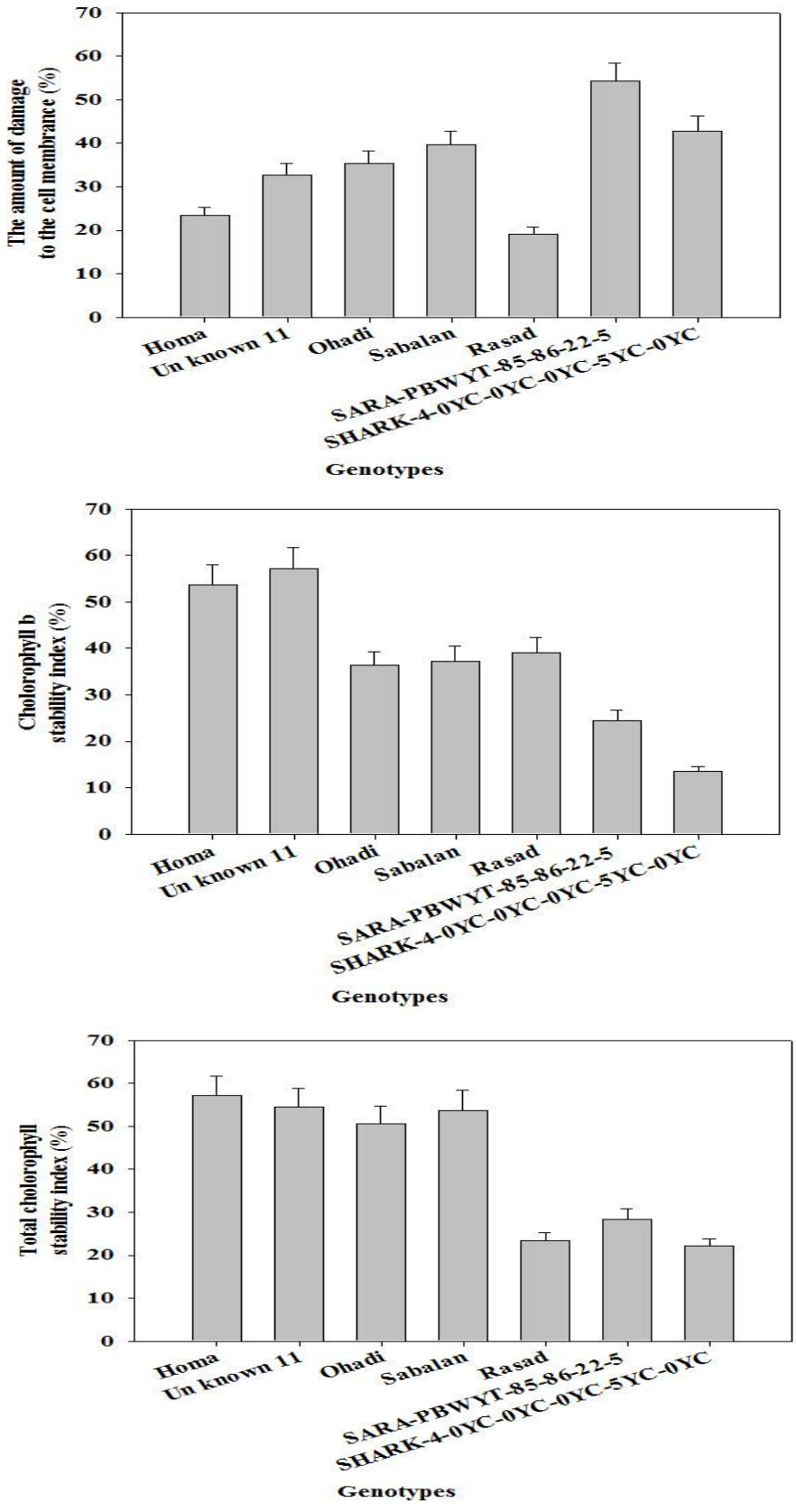

Figure 1. Damage rate average to cell membrane in the result of drought function. (A) Chlorophyll *b* resistance index. (B) all chlorophylls resistance index. (C) After pollination in the investigated lines.

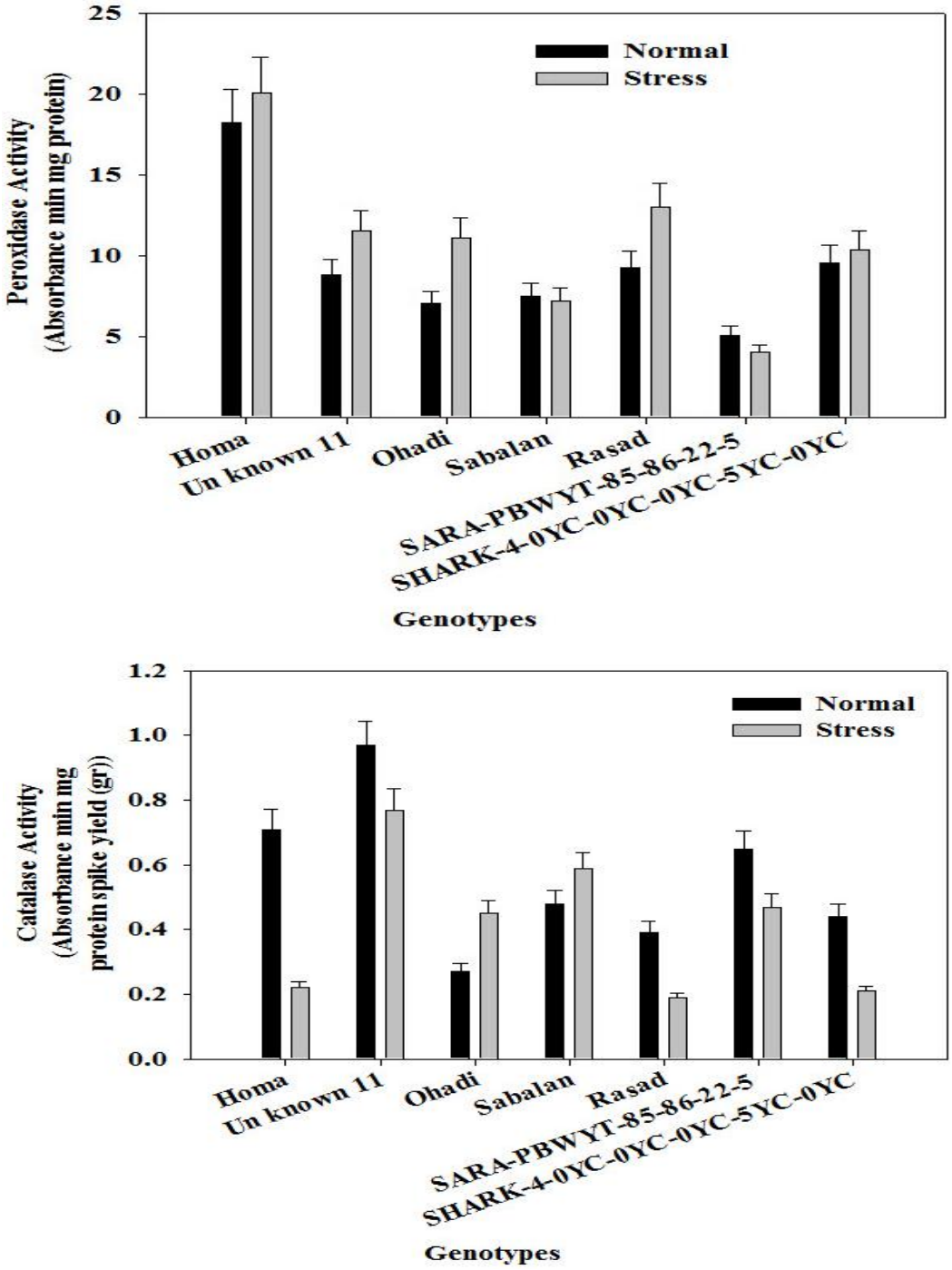

Figure 2. Peroxidase enzyme activity average. (a) Catalase. (b) also function (c) in drought stress condition and control in the tested lines.

reactions, which finally decreases cell membrane stability index (Hong et al., 2006; Dacosta and Hoang, 2007). Increasing the production of reactive oxygen species cause peroxidation of photosynthetic pigments and at the end, lead to their break down due to drought stress. The result of these reactions will decreased chlorophyll content (a, b and total) and the reduction rate is greater in more sensitive lines (Jiang and Zhang, 2004; Nikolaeva et al., 2010). Moreover, the observed correlation in the stress condition between total chlorophyll content with the yield emphasizes the importance of keeping an optimum content of the pigments to produce the yield. Peroxidase activity in drought conditions increased in semi-tolerant and tolerant lines, and the highest activity of this enzyme

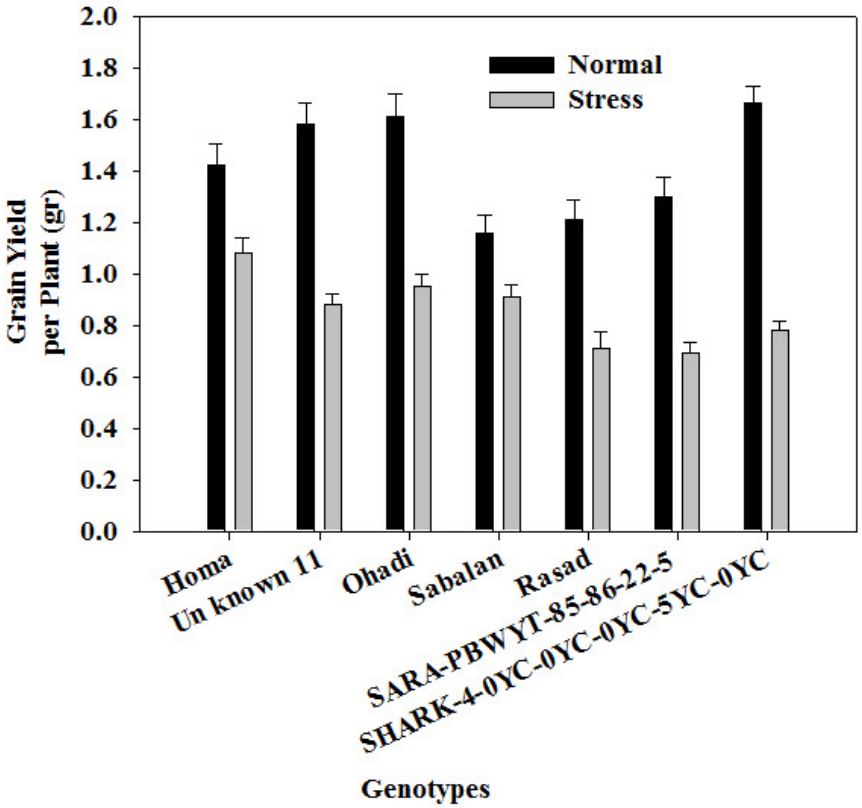

Figure 2c. Contd.

Table 3. Correlation between different traits in drought stress condition.

Traits	Damage to the cell membrane	Stability of chlorophyll b	Total chlorophyll	Chlorophyll b	Yield
Peroxidase	-0.71**	0.49*	0.23	0.15	0.47*
Catalase	28.9	0.11	0.44*	0.39*	0.31

* and **, significant at 5 and 1% levels of probability, respectively.

was observed in the tolerant line Unknown 11. Peroxidase enzyme can efficiently remove H_2O_2 both in the cytosol and chloroplast. So, increasing the activity of this enzyme in drought stress perhaps shows the accumulation of H_2O_2 in the condition (Csiszar et al., 2005). Furthermore, a significant negative correlation was perceived at drought condition between peroxidase enzyme and damage on cell membrane so that Homa and Rasad lines had high rate of peroxidase activity in this condition. The rate of damage to its cell membrane was low and SARA-PBWYT-85-86-22-5 which had the lowest peroxidation in stress condition had the most sensitive membrane to drought condition. Accordingly, it can be concluded that drought stress prevents harmful effects of reactive oxygen species on the cell membrane by increasing peroxidase activity as possible (Csiszar et al., 2005). Moreover, the positive correlation between peroxidase activity and chlorophyll stability index in stress condition is another testimony on the capability of this enzyme to eliminate the toxic effects of reactive oxygen species.

The resistant lines, because of the high activity of peroxidase enzyme in drought condition as well as higher chlorophyll and membrane stability index, have higher yields which justifies the perceived positive correlation between enzyme activity and yield in drought stress condition (Table 3). In this study, the Catalase enzyme activity in the investigated lines at stress conditions either remained constant or declined compared to the control and in general, no significant trend was perceived between the investigated lines. Catalase is responsible for decomposition and detoxification of H_2O_2 in the Peroxisomes. The activity of this enzyme is sensitive to both drought and heat stresses (Jiang and Hoang, 2001). Decreasing the activity of this enzyme may relate to either photo inactivation of the enzyme (Polle, 1997) which is a sign for the advent of light stress in the plant that usually cause photo-inhibition of photosystem II, and

this condition itself leads to H_2O_2 concentration and damage to cell membrane (Jang, 2004), or prevention of new enzyme synthesis that occurs in darkness, is another factor which decreases the activity of this enzyme (Dat et al., 1998). The decrease in the activity of this enzyme is usually considered as reduction in the capability of leaves to breakdown H_2O_2. In this study, there was a positive correlation in drought condition among catalase enzyme with chlorophyll *b* and total chlorophyll contents, which shows the role of this enzyme in eliminating the adverse effects of H_2O_2. So, that of Unknown 11, Sabalan and Ohadi lines high in enzyme activity at drought condition had high amount of chlorophyll, too. Eventually, stomatal closure under drought stress will cause the cessation of CO_2 fixation which leads to $NADP^+$ limitation, resulting in electron transfers to oxygen and accumulation of hydrogen superoxide and hydrogen peroxide and this causes disruptions in the activity of some scavenger enzymes of reactive oxygen species like catalase at drought stress, which will increase lipid peroxidation resulting in damages to chlorophyll and cell membrane.

Plant increase the activity of some involved enzymes in removing reactive oxygen species, like peroxidase, through stimulation of gene expression to alleviate the adverse effects of oxidative stress caused by drought stress.This condition is more visible in the resistant lines. The stress-resistant lines having these traits reduce damaging effects of oxidative stress through degradation and inactivation of reactive oxygen species and because of more stability of cell membrane and chlorophyll under these conditions, access to high yields in this lines is probable due to sustaining photosynthetic capacity.

REFERENCES

Aebi H (1983) Catalase. In: Bergmeyer (ed.), Methods of Enzymatic Analysis, Vol. 3, pp: 273–277. VerlagChemie, Weinheim, Germany.

Arnon DI (1949) Copper enzymes in isolated chloroplasts, polyphenoloxidases in Beta vulgaris. Plant Physiol., 24: 1–15.

Baruah A, Simkova K, Apel K, Laloi C (2009) Arabidopsis mutants reveal multiple singlet oxygen signaling pathways involved in stress response and development. Plant Mol. Biol., 70: 547-563.

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

Csiszar J, Feher-Juhasz E, Kotai E, Ivankovits- Kiss O, Horvath GV, Mai A, Galle A, Tari I, Pauk J, Dudits D, Erdei L (2005) Effect of osmotic stress on antioxidant enzyme activities in transgenic wheat call bearing MsALR gene. Acta Biologica Szegediensis 49: 49–50.

Dacosta M, Huang B (2007) Changes in antioxidant enzyme activities and lipid peroxidation for bent grass species in responses to drought stress. J. Am. Soc. Hortic. Sci., 132: 319–326.

Dat JF, Lopez-Delgado H, Foyer CH, Scott IM (1998). Parallel changes in H2O2 and catalase during thermo tolerance induced by salicylic acid or heat acclimation in mustard seedlings. Plant Physiol., 116: 1351-1357.

FeyziaslVV, Kasraei r, Moghadam M, Valizadeh G (2004) Studying the detection of the shortage and limitations of food elements absorption by using different methods with consumption of phosphor and zinc in rain-watered Sardari wheat. Agronomical Sci. Nat. resour. Gorgan Un., (3)11. Page 23-33.

FeyziaslVV, Valizadeh G (2003).The effect of azote consumption and time on rain-watered wheat function. Water soil J., (1)17.page 29-38.

FeyziaslVV, ValizadehG (2001) Measuring the nitrogen and phosphor need of Sabaln type in complementary irrigation and rain-watered conditions. J. Iran agronomical sci., pp. 23-28.

Friso G, Giacomeli L, Ytterberg AJ, Peltier JB, Rudella A, Sun Q (2004) In-depth analysis of the thylakoid membrane proteome of Arabidopsis thaliana chloroplasts; new proteins, new functions, and a plastid proteome database. Plant Cell16: 478-499.

Gechevet TS, Breusegem FV., Stone JM., Denev I, Laloi C (2006) Reactive oxygen species as signals that modulate plant stress responses and programmed cell death.Bio. Essays 28: 1091–1101.

Hong S, Suo LZ MingAn S (2006) Osmotic regulation of 10 wheat(Triticumasstivum L.) genotypes at soil water deficits. Sci. direct 47: 132 – 139.

Jiang MY,Zhang JH (2004)Abscisic acid and antioxidant defense in plant cells. Acta Bot. Sin., 46: 1–9

Jiang Y, Huang N (2001) Drought and Heat stress injury to two cool-season turf grasses in relation to antioxidant metabolism and lipid peroxidation. Crop Sci., 41: 436-422.

Jang S (2004) Variation in antioxidant metabolism of yong and mature leaves of Arabidopsis thaliana subjected to drought. Plant Sci., 166: 459 – 466.

Khanna-Chopra R, Selote D S (2007) Acclimation to drought stress generates oxidative stress tolerance in drought-resistant than susceptible wheat cultivar under field conditions. Environ. Exp. Bot., 60: 276-283.

Liu X, Huang B (2000) Heat stress injury in relation to membrane lipidperoxidation in creeping bent grass. Crop Sci., 40, 503-510.

Nikolaeva MK, Maevskaya SN, Shugaev AG, Bukhov NG (2010) Effect of drought on chlorophyll content and antioxidant enzyme activites in leaves of three wheat cultivars varying in productivity.Russian Journal of Plant Physiol., 57: (1) 87 -95.

Polle A (1997) Defense against photo oxidative damage in plants. p. 783 – 813. In: J. Scandalios, ed. Oxidative Stress and the molecular biology of oxidative defense. Cold Spring Harbor Laboratory press, Cold Spring Harbor, 1VY.

Ramachandra RA, Choityana KV, Ivekanadan A (2004) Drought-induced response of photosynthesis and antioxidant metabolism in higher plants. J. Plant Physiol., 161: 1189- 1202.

Sairam RK, Deshmukh PS, Shukla DS (1997) Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. J. Agronomy Crop Sci., 178: 171–178.

Srivalli BG, Sharma, Khanna-Chopra R (2003) Anti oxidative defense system in an upland rice cultivar subjected to increasing intensity of water stress following by recovery. Physiol. Planta 119: 503-512.

Tas S, Tas B (2007) Some physiological responses of drought stress in wheat genotypes with different ploidity in Turkiye. World J. Agric. Sci., 3: 178–183.

Valentovic P, Luxova M, Kolarovic L, Gasparikova O (2006) Effect of osmotic stress on compatible solutes content, membrane stability and water relations in two maize cultivars. Plant Soil Environ., 52(4): 186-191.

Feyziasl VV, Valizadeh G (2001) Measuring the nitrogen and phosphor need of Sabaln type in complementary irrigation and rain-watered conditions. J. Iran agron. sci., pp. 23-28