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Determination of Resistance Cumhuriyet-75 and Selimiye-95 Wheat (*Triticum Aestivum* L.) Varieties Against to Some Abiotic Stress Factories

Nezahat Turfan[1*], Ekrem Mutlu[2]

[1]*Department of Biology, Faculty of Arts and Sciences, Kastamonu University, 37200 Kastamonu, Turkey*
[2]*Aquaculture Department, Faculty of Fisheries, Kastamonu University, 37200 Kastamonu, Turkey*

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*Corresponding Author:
E-mail: nturfan@kastamonu.edu.tr

A B S T R A C T

In this study, resistance mechanism of two wheat genotypes against salt, heavy metal, lime and drought (50%) treatments were investigated in summer Cumhuriyet-75 and winter Selimiye-95. According to results chlorophyll a, b, total chlorophyll and carotenoid level increased in $FeCl_3$, drought and 225 mM NaCl in Cumhuriyet-75 but they were higher at NaCl, $FeCl_3$ and $ZnCl_2$ treatments in Selimiye-95 comparison to control. While H_2O_2 content rose all stres treatments in both varieties but Malondialdehyde (MDA) decreased in Selimiye with all applications. The amount of proline is lower in Cumhuiyet-75 but higher in Selimiye-95. Total soluble protein was found higher at salt concentartion and drought in both varieties. Ascorbate peroxidase (APX), Süperoxide dismutase (SOD) activity increased in salt and $FeCl_3$ in Selimiye-95 but SOD ativity were higher at salt treatments in Cumhuriyet-75. And also in both varieties APX and Guaiacol peroxidase (GuPX) increased at $FeCl_3$ but Catalase (CAT) were higher in only $FeCl_3$ in Cumhuriyet-75. As a result Selimiye-95 showed tolerance to salt and $FeCl_3$ with high photosynthetic pigment, proline and soluble protein content with lower MDA but it is sensitive to $NiCl_2$ and drought. Whereas Cumhuriyet-75 cultivar is resistan to drought, FeCl3 and 225 mM NaCl depended on pigment, protein content and APX, CAT, GuPX and SOD activities. When all the data are taken into consideration, it was concluded that the responses of the varieties to the treatments changed according to the type and concentration of stress, and Selimiye-95 variety was tolerant compared to Cumhuriyet-75 variety.

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Cumhuriyet-75 ve Selimiye-95 Buğday Çeşitlerinin Bazı Abiyotik Stres Faktörlerine Toleranslarının Belirlenmes

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*Sorumlu Yazar:
E-mail: nturfan@kastamonu.edu.tr

Ö Z E T

Bu çalışmada Cumhuriyet-75 ve Selimiye-95 buğday çeşitlerinin tuz, ağır metal, kireç ve kurak (%50) uygulamalarına toleransları araştırılmıştır. Bulgulara göre Cumhuriyet-75'te klorofil a, klorofil b, toplam klorofil ve karotenoit miktarı $FeCl_3$, kurak ve 225 mM NaCl uygulamalarında kontrole göre artış gösterirken Selimiye-95'te NaCl konsantrasyonları, $FeCl_3$ ve $ZnCl_2$ uygulamalarında en yüksek değere ulaşmıştır. Hidrojen peroksit miktarı (H_2O_2) miktarı her iki buğday çeşidinde yüksektir ancak malondialdehit (MDA) miktarı Selimiye-95'te tüm uygulama gruplarında azalmıştır. Prolin içeriği Cumhuriyet'te tüm uygulama gruplarında azalırken Selimiye-95'te artmıştır. Toplam çözünür protein her iki buğday çeşidinde NaCl konsantrasyonları ve kuraklık uygulamasında yüksek bulunmuştur. Askorbat peroksidaz (APX) ve Süperoksit dismutaz (SOD) aktivitesi Selimiye-95'te NaCl ve $FeCl_3$ uygulamasında, Cumhuriyet-75'te ise SOD aktivitesi NaCl uygulamalarında yüksek bulunmuştur. Ayrıca APX ve Guaiakol peroksidaz (GuPX) aktivitesi her iki çeşitte $FeCl_3$ uygulamasında artış gösterirken, CAT aktivitesi sadece Cumhuriyet-75'te $FeCl_3$ uygulamasında yüksektir. Sonuç olarak Selimiye-95 buğday çeşidi yüksek pigment içeriği, prolin, çözünür protein ve düşük MDA değeri ile NaCl konsantrasyonları ve $FeCl_3$ uygulamalarına tolerans gösterirken, $NiCl_2$ ve kuraklık uygulamalarına ise duyarlı bulunmuştur. Cumhuriyet-75 çeşidi pigment, protein miktarı ve APX, Katalaz (CAT), GuPX ve SOD aktivite değerlerine bağlı olarak kuraklık, $FeCl_3$ ve 225 mM NaCl uygulamalarına dayanıklıdır. Tüm veriler değerlendirildiğine çeşitlerin stres uygulamalarına tepkisi stresin çeşidi ve konsantrasyonuna göre değiştiği ve ayrıca Selimiye-95 çeşidinin Cumhuriyet-75'e göre dayanıklı olduğu sonucuna varılmıştır.

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Introduction

Wheat ranks first in terms of area of planting and production in field crops due to its being a good source of nutrients, its broad adaptation limits and ease of transport, storage and processing. Wheat is grown in almost every part of our country however most of the wheat grown in our country (80%) is grown under rain-dependent conditions. Moreover, the general warmt and drought climatic conditions of our country create the ideal environment for salinity and barrenness formation (Öztürk and Aydın, 2004; Başer et al., 2005). The accumulation of zinc, iron, lead, cadmium, nickel and other heavy metals in dense industrial zones close to cultivated lands affect crop production by leading to heavy metal toxicity. As is seen, wheat production areas are not uniform, some factors required for growth and development are wanting while some factors are far from being optimal. The cited conditions affect wheat production and cause loss of quality and efficiency (Gupta et al., 2011; Öz et al., 2016). As such, it is very important to rehabilitate and utilize such agricultural areas in an economic and economical way. Due to the increased nutrient requirements and the limited availability of agricultural lands in parallel with population increase, selecting genotypes with high tolerance to stress factors in the regions where salinity, lime/drought and heavy metal toxicity are dominant will contribute to more efficient utilization of existing land resources. Determination of morphological parameters as well as physiological measurements in the varieties in order to determine the mechanism of resistance to stress factors will enable more accurate steps to be taken for determining the appropriate species and varieties. It is reported that changes in photosynthetic activity are the main factors that decrease yield and quality in wheat within this context (Makino, 2001; Molas, 2006). Öncel and Keleş (2002), Blake et al. (2007), Razi et al. (2016) have reported that abiotic stress conditions, salinity, heavy metal toxicity and drought change the leaf morphology of wheat and causes oxidative stress in the leaf tissue thereby damaging the chloroplast membrane respectively. While decrease in area and length of leaf cause reduction of the photosynthesis capacity, oxidative stress leads to degradation of chloroplast structure thereby breaking up the pigments, reducing enzyme activities responsible for chlorophyll synthesis and consequently decreasing the amount of photosynthetic pigments (Hernandez et a 2001; Çimen et al., 2013). The amounts of proline (Chen et al., 2001), total soluble protein (Nagoor, 1999; Sharma and Dietz, 2006), soluble carbohydrates and starch (Pattanagul and Thitisaksakul, 2008) also vary in wheat under stress conditions. Furthermore, stress conditions increase the accumulation of ROS such as hydrogen peroxide, hydroxy radicals, singlet oxygen and superoxide anions (Kholová et al., 2010) Excessive ROS accumulation causes excessive accumulation of malondialdehyde (MDA) which induces peroxidation in membrane lipids causing protein, DNA, RNA denaturation (Neto et al., 2006). Researchers have reported that the activity of enzymes such as ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD) and peptidase (POD) have a role in reducing oxidative stress and lipid peroxidation damage in tolerant species (Terzi and Yıldız, 2013). That said, yield and quality in wheat vary according to the ecological structure of the region and the applied cultivation processes (Zheng et al., 2009; Waters et al., 2009). Effects of salt, heavy metals, drought and lime treatments in green parts photosynthetic pigments, proline, total soluble protein, MDA, H_2O_2 amount and APX, CAT, GuPX and SOD activities of summer Cumhuriyet-75 and winter Selimiye-95 variety wheat bread genotypes have been researched in this study.

Material and Method

Plant Material

Cumhuriyet-75 (bread type) variety registered by the Ege (Agean) Institute of Agricultural Research in 1976 and Selimiye-95 (bread) variety produced by crossbreeding registered Trakya (Thrace) Institute of Agricultural Research in 2009 were used in the study. Cumhuriyet-75 is a summer type spiny wheat which that the biggest grain among the white wheat varieties. Its planting is recommended in coastal zones and rural and basal areas. Selimiye-95 is a winter variety without spines. It is recommended for regions where planting is made in winter.

Planting Operations of Varieties

The study was launched in 3rd week of October. 25 seeds were planted in a pot with three replications for each treatment group in each pot. The pots have a volume of 5 L in and consist of a mixture in ration of garden turf: peat: sand (1: 1: 1). Salt treatment (75 mM, 150 mM and 225 mM NaCl), heavy metal treatment (0.2 mg/L in the form of $FeCl_3$, $NiCl_2$ and $ZnCl_2$) and lime treatment (2 mg/L in the form of $CaCO_3$) were solubilized in tap water with ds 0.04 and with freshly prepared solutions each time. For 50% drought treatments trap water was used 275 ml kg^{-1}. The treatments were made while the seedlings were at 4-5 leaf stage by treatment of twice a week for week five weeks. After five weeks, the fully expanded leaf was randomly collected. In leaf sample photosynthetic pigments as chlorophyll a, chlorophyll b, total chlorophyll and carotenid, lipid peroxidation level (malondialdehyde-MDA), hydrogen peroxide (H_2O_2), glucose and starch amount as well as APX, CAT, GuPX and SOD activities were determined.

Chemical Analyzes

Chlorophyll content of the leaves was measured by the method of Arnon (1949). Carotenoid amount was estimated by Jaspars Formulated according to the method Witham et al. (1971). Proline content was determined according to the modified method of Bates et al. (1973). Total soluble protein contents were determined according to the method of Bradford (1976) using the Bio-Rad assay kit with bovine serum albumin as a calibration standard. The level of lipid peroxidation products was determined and expressed as MDA content according to Luts et al. (1996). Hydrogen peroxide in the plant samples was determined by the method of Velikova et al. (2000).

The extracts were prepared from first three leaves of the plants which were treated by control and stress. Accordingly, nearly 0.5 g fresh leaf samples were homogenized with 50 mM (pH 7.6) phosphate buffer solution (10 mL) ground in liquid nitrogen and containing 0.1 mM Na-EDTA (Ethylenediaminetetraacetic acid). The homogenized samples were centrifuged for 15 min at 15000 g and +4 °C, and then the enzyme activities in the resulting supernatant were determined according to the methods of Çakmak (1994). Catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GuPX) and superoxide dismutase (SOD) activities were measured according to the methods of Bergmeyer (1974), Nakano and Asada (1981), Chance and Maehley (1995) and respectively under nitro blue tetrazolium chloride (NBT) light by 0_2^- reduction

Statistical Analysis of Data

The statistical analysis of the data obtained as a result of the study was conducted according to the ANOVA and Tukey tests at 95% confidence interval by virtue of the SPSS 20 program.

Results

Photosynthetic pigments showed differences basing on wheat varieties and stress treatments. While chlorophyll a, chloropphyll b, total chlorophyll and carotenoid levels increased in drought, $FeCl_3$, 225 mM and 75 mM NaCl compared to control group but it was lower in other treatments in Cumhuriyet-75. The lowest values of those pigments were obtained with $NiCl_2$, $CaCO_3$ and $ZnCl_2$ applications in orderly (Table 1). It was found that the amount of chlorophyll a was lowest value in 75 mM NaCl while chlorophyll b content lowered in drought, $NiCl_2$ and $CaCO_3$ treatments in Selimiye-95 respectively. Whereas total chlorophyll content increased with salts, $FeCl_3$ and $ZnCl_2$ treatments but increased in drought and $NiCl_2$. And also total carotenoid amount was lower at $CaCO_3$ only in Selimiye-95 (Table 1).

The proline content of Cumhuriyet-75 decreased in all stress treatments but it was found higher in Selimiye-95 with respect to control plants (Table 2). In Cumhuriyet-75 the amount of proline was lowest in 50 %drought (53.64%), in $ZnCl_2$ (45.26%), in $NiCl_2$ (44.92 %). But the highest proline values were determined in $CaCO_3$ (74.62 %), $FeCl_3$ (64.13 %), $NiCl_2$ (50.22 %) in Selimiye-95 (Table 2). In Cumhuriyet the total soluble protein content was found lower in $NiCl_2$, $ZnCl_2$ and $CaCO_3$ but it was lowest in only heavy metals treated plants in Selimiye-95 variety compared to control seedling (Table 2). Increasing in the content of soluble protein was determined in 150 mM NaCl as 94.35 %, in 225 mM NaCl as 90.5 %, 75 mM NaCl as 74.46 % and in $FeCl_3$ as14.2% for Cumhuriyet-75 (Table 2). In Selimiye-96 content of protein increased with salt concentration. The lowest amount of protein was recorded in $NiCl_2$ (31.28%), $FeCl_3$ (17.49%) and $ZnCl_2$ (9.97%) treatments (Table 2).

Table 1 Effects of salt, heavy metals, lime and drought (50%) treatments in clorophyll a, chlorophyll b, total chlorophyll, ratio of chloroğhyll a/ chlorophyll b and carotenoids content in the Cumhuriyet-75 and Selimiye-95 variety.

T	Cumhuriyet				
	Chl a	Chl b	Tot. Chl	Chl a/b	Tot.Car.
Control	$0.152±0.001^d$	$0.104±0.0004^f$	$0.256±0.0001^e$	$1.459±0.010^{cd}$	$8.01±0.02^d$
75 mM	$0.153±0.001^e$	$0.096±0.0003^d$	$0.249±0.0004^d$	$1.582±0.010^d$	$8.01±0.02^d$
150 mM	$0.151±0.001^d$	$0.099±0.0003^e$	$0.250±0.0003^d$	$1.528±0.010^{cd}$	$7.96±0.01^d$
225 mM	$0.153±0.001^e$	$0.108±0.0004^g$	$0.260±0.0005^f$	$1.413±0.001^c$	$7.86±0.01^d$
$FeCl_3$	$0.154±0.001^e$	$0.154±0.0001^h$	$0.307±0.0003^h$	$1.000±0.001^b$	$8.26±0.03^e$
NiCl2	$0.094±0.001^a$	$0.032±0.0005^a$	$0.126±0.0004^a$	$2.980±0.060^g$	$5.87±0.01^a$
$ZnCl_2$	$0.117±0.001^c$	$0.047±0.0004^c$	$0.164±0.0004^c$	$2.511±0.040^e$	$6.86±0.02^c$
$CaCO_3$	$0.107±0.001^b$	$0.041±0.0004^b$	$0.147±0.0003^b$	$2.645±0.040^f$	$6.28±0.01^b$
Drought	$0.156±0.001^f$	$0.107±0.0006^g$	$0.262±0.0004^g$	$1.457±0.010^{cd}$	$8.50±0.02^e$
F	5787.79	18102.58	51951.91	833.42	467.96
Sig.	0.000	0.000	0.000	0.000	0.000
T	Selimiye				
	Chl a	Chl b	Tot. Chl	Chl a/b	Tot.Car.
Control	$0.162±0.0005^b$	$0.192±0.0002^c$	$0.353±0.0001^c$	$0.844±0.001^e$	$9.11±0.005^a$
75 mM	$0.159±0.0002^a$	$0.294±0.0001^h$	$0.452±0.0001^h$	$0.540±0.001^a$	$9.46±0.004^d$
150 mM	$0.165±0.0002^c$	$0.232±0.0001^e$	$0.397±0.0001^e$	$0.710±0.001^d$	$9.20±0.004^b$
225 mM	$0.164±0.0002^c$	$0.253±0.0001^f$	$0.417±0.0001^f$	$0.650±0.001^c$	$9.26±0.136^c$
$FeCl_3$	$0.161±0.0005^b$	$0.274±0.0002^g$	$0.435±0.0003^h$	$0.589±0.003^b$	$9.30±0.003^c$
NiCl2	$0.166±0.0002^c$	$0.175±0.0002^b$	$0.341±0.0002^b$	$0.953±0.002^g$	$9.42±0.029^d$
$ZnCl_2$	$0.166±0.0005^c$	$0.229±0.0005^e$	$0.395±0.0005^e$	$0.724±0.001^d$	$9.23±0.004^b$
$CaCO_3$	$0.169±0.0001^d$	$0.189±0.0004^c$	$0.358±0.0003^c$	$0.893±0.002^f$	$9.05±0.029^a$
Drought	$0.161±0.0002^b$	$0.155±0.0002^a$	$0.315±0.0002^a$	$1.039±0.003^h$	$9.54±0.004^e$
F	90.67	355.14	427.58	203.02	13.16
Sig.	0.000	0.000	0.000	0.000	0.000

T: Treatments, Chl a: Clorophyll a (mg/g), Chl b: Clorophyll b (mg/g), Tot.Chl: Total chlorophyll (mg/g), Chl a/b: Ratio of chloroğhyll a/ chlorophyll b, Tot. Car.: Total carotenoids content (mg/g), *The difference between the averages indicated by the same letter in the same column are not important (P<0.05).

Table 2 Effects of salt, heavy metals, lime and drought (50%) treatments on proline, total soluble protein, malondialdehyde (MDA)and hydrogen peroxide (H_2O_2) in the Cumhuriyet-75 and Selimiye-95 variety.

T	Cumhuriyet			
	Prolin (µmol/g)	Protein (mg/g)	MDA (µmol/g)	H_2O_2 (µmol/g)
Control	52.96±0.17^{g*}	25.5±0.23^{c}	3.19±0.08^{a}	15.77±0.11^{a}
75 mM	45.21±0.02^{e}	44.4±0.21^{e}	5.07±0.07^{d}	24.53±0.15^{c}
150 mM	34.97±0.02^{d}	49.5±0.12^{g}	4.94±0.13^{b}	59.60±0.09^{f}
225 mM	33.27±0.02^{c}	48.5±0.21^{f}	5.26±0.08^{f}	32.17±0.09^{e}
$FeCl_3$	33.36±0.02^{c}	35.62±0.19^{b}	5.05±0.08^{d}	17.15±0.06^{b}
$NiCl_2$	29.17±0.03^{b}	12.84±0.18^{b}	5.00.4±0.09^{c}	14.83±0.09^{a}
$ZnCl_2$	28.99±0.04^{b}	10.49±0.24^{a}	5.27±0.08^{f}	28.81±0.08^{d}
$CaCO_3$	46.43±0.02^{f}	24.8±0.09^{c}	5.18±0.09^{e}	20.47±0.07^{c}
Drought	24.55±0.03^{a}	37.2±0.10^{d}	5.09±0.09^{d}	27.31±0.10^{d}
F	195075.82	7632.92	551012	18465.97
Sig.	0.003	0.002	0.001	0.002
T	Selimiye			
	Prolin (µmol/g)	Protein (mg/g)	MDA (µmol/g)	H_2O_2 (µmol/g)
Control	24.44±0.12^{a}	16.87±0.04^{d}	6.17±0.07^{g}	18.13±0.06^{a}
75 mM	24.74±0.06^{a}	75.12±0.05^{i}	2.75±0.05^{b}	43.35±0.08^{d}
150 mM	27.66±0.09^{b}	63.63±0.05^{h}	3.39±0.12^{c}	56.30±0.08^{g}
225 mM	35.42±0.06^{e}	58.23±0.03^{g}	4.29±0.08^{e}	59.83±0.08^{h}
$FeCl_3$	40.12±0.06^{g}	13.92±0.04^{b}	4.36±0.09^{e}	40.32±0.09
$NiCl_2$	36.70±0.09^{f}	11.58±0.07^{a}	5.74±0.12^{f}	53.22±0.07^{f}
$ZnCl_2$	34.08±0.06^{d}	15.19±0.04^{c}	3.68±0.09^{d}	38.37±0.10^{c}
$CaCO_3$	42.68±0.10^{h}	25.60±0.04^{e}	1.40±0.10^{a}	30.65±0.08^{b}
Drought	33.31±0.06^{c}	38.85±0.03^{f}	3.46±0.13^{c}	47.26±0.06^{e}
F	639082.59	350418.87	17623.18	220.93
Sig.	0.003	0.002	0.001	0.002

T: Treatments, *The difference between the averages indicated by the same letter in the same column are not important ($P<0.05$).

Table 3 Effects of salt, heavy metals, lime and drought (50%) treatments in the APX, GuPX, CAT and SOD activities in the Cumhuriyet-75 and Selimiye-95 variety (EU/mg Protein)

T	Cumhuriyet			
	APX	CAT	GuPX	SOD
Control	0.016±0.001^{d}	0.094±0.001^{e}	0.0310±0.0001^{d}	130.55±0.18^{h}
75 mM	0.011±0.001^{b}	0.091±0.001^{e}	0.0195±0.0002^{a}	145.48±0.21^{e}
150 mM	0.017±0.001^{d}	0.048±0.001^{a}	0.0180±0.0003^{a}	137.68±0.09^{d}
225 mM	0.009±0.001^{a}	0.057±0.002^{b}	0.025±0.0003^{b}	134.37±0.13^{e}
$FeCl_3$	0.011*±0.001^{b}	0.121±0.001^{e}	0.038±0.0006^{d}	129.53±0.10^{g}
$NiCl_2$	0.014±0.001^{c}	0.135±0.003^{f}	0.054±0.0005^{e}	120.53±0.17^{b}
$ZnCl_2$	0.021±0.001^{e}	0.085±0.002^{d}	0.027±0.0002^{c}	123.46±0.15^{d}
$CaCO_3$	0.022±0.001^{e}	0.090±0.001d^{e}	0.030±0.0006^{d}	122.34±0.11^{c}
Drought	0.036±0.002^{f}	0.073±0.001^{c}	0.027±0.0005^{c}	118.29±0.102^{a}
F	423.15	1083.91	768.34	692.23
Sig.	0.000	0.000	0.000	0.000
T	Selimiye			
	APX	CAT	GuPX	SOD
Control	0.073±0.001^{c}	0.102±0.001^{i}	0.012±0.0001^{d}	139.69±0.01^{d}
75 mM	0.153±0.002^{f}	0.093±0.001^{d}	0.015±0.0003^{f}	172.82±0.26^{h}
150 mM	0.141±0.003^{e}	0.084±0.001^{f}	0.013±0.0002^{e}	158.57±0.22^{g}
225 mM	0.131±0.002^{e}	0.078±0.001^{h}	0.011±0.0002^{de}	152.56±0.21^{f}
$FeCl_3$	0.134±0.015^{b}	0.071±0.001^{e}	0.014±0.0005^{c}	129.66±0.44^{c}
$NiCl_2$	0.026±0.001^{a}	0.059±0.001^{g}	0.007±0.0001^{a}	126.13±0.05^{a}
$ZnCl_2$	0.070±0.001^{c}	0.042±0.001^{c}	0.008±0.0001^{b}	127.32±0.01^{b}
$CaCO_3$	0.078±0.001^{cd}	0.043±0.001^{b}	0.011±0.0001^{c}	148.77±0.27^{e}
Drought	0.096±0.002^{d}	0.038±0.001^{a}	0.014±0.0003^{f}	147.87±0.27^{e}
F	93.85	6663.57	402.79	4508.96
Sig.	0.000	0.000	0.000	0.000

T: Treatments, *The difference between the averages indicated by the same letter in the same column are not important ($P<0.05$).

The amount of malondialdehyde (MDA) was observed higher in all types of stress treatments in Cumhuriyet-75 variety compared to control group, but it lowered in leaf samples of Selimiye-95 variety (Table 3). In Cumhuriet-75 in $ZnCl_2$ (5.27 µmol), 225 mM NaCl (5.26 µmo, $CaCO_3$ (5.18 µmol) treatments MDA level was the highest level respectively. Chlorophyll b, total chlorophyll and carotenoid contents increased in salt concentrations, $FeCl_3$, $ZnCl_2$ and $CaCO_3$ treatments ($P<0.05$; Table 1). There was a decrease in H_2O_2 concentration 6% only in $NiCl_2$ treatment (14.83 µmol) in the Cumhuriyet-75 variety compared to control group (15.77 µmol) (Table 2). But in Selimiye-95 variety H_2O_2 concentration showed significant increase in all treatment groups compared to control, especially in 225 mM (59.83 µmol), 150 mM (56.30 µmol) NaCl and $NiCl_2$ (53.22 µmol) treatments (Table 2).

There were significant differences between enzyme activities of wheat varieties ($P<0.05$). The level of APX (ascorbate peroxidase), CAT (catalase), GuPX (guaiacol peroxidae) and SOD (superoxide dismutase) activities were higher in Selimiye-95 than Cumhuriyet-75. However in Cumhuriyet APX activity was found higher in drought (2.31 time), $CaCO_3$ (38.93%), $ZnCl_2$ (32.1%) and 150 mM (5.17%) with respect to control. CAT and GuPX activity were higher in $NiCl_2$ (0.195 EU and 0.054 EU) and $FeCl_3$ (0.121 EU and 0.038 EU) but lower in all other treatments. The lowest activity was determined in 150 mM NaCl for both enzymes as 0.48 EU and 0.18 EU in order. SOD activity was higher value of salt treatments, but it was reduced with cocentrations (Table 3). In Selimiye APX activity increased with salts (0.153 EU, 0.141 EU, 0.131 EU) and $FeCl_3$ (0.134 EU) application but CAT activity decreased in all treatments, especially in heavy metals and 225 mM NaCl applications. GuPX activity reduced heavy metals (0.007 EU, 0.008, 0.011 EU) and in 225 MM NaCl (0.011 EU) while SOD were determined lower in only heavy metals (129.66 EU, 126.12 EU, 127.32 EU) (Table 3).

Discussion

Photosynthetic pigments have a central role in capturing light, driving electron transport and generation of chemical energy and reducing power in the form of ATP and NADPH, respectively. Their content in photosynthetic active tissue varies depends on genotype, maturity of leaf and whole plant and also environmental conditions such as climatic factors, mineral status of soil, drought and salty (Molas, 2002; Raines, 2011). The amount of chlorophyll a, b, total chlorophyll and carotenoid were higher in Selimiye generally but there was no significant difference between species in terms of chlorophyll a/b ratio (Table 1). In Cumhuriyet photosynthetic pigments were most influenced by $NiCl_2$, $ZnCl_2$, $CaCO_3$ treatments negatively. The amount of chlorophyll b and total chlorophyll were the highest at FeCl3 in both varities but they were lowest in $NiCl_2$ for Cumhuryet-75 and in drought for Selimiye-95 (Table 1). Photosynthetic pigment results showed that Cumhuriyet-75 is more sensitive to NiCl2, $ZnCl_2$, $CaCO_3$ but it is tolerant to $FeCl_3$, drought and 225 mM NaCl treatments. On the other hand Selimiye-95 sowed sensitivity to drought and $NiCl_2$ but was found resistant to salts and $FeCl_3$ treatments (Table 1). Photosynthetic pigment results are similar to those found in this field. For example Öncel and Keleş (2002), Parida et al. (2002), Santos (2002) reported that chlorophyll content was higher in leaves of tolerant cultivars while Kholová et al. (2010), Terzi and Yıldız (2013) stated that pigment levels were lower in salts condition in susceptible. However they noted that their content may be lower at the beginning of growth phase but they can higher by the age of maturity in plant. Kumar et al (2012), Dubey and Pandey (2011) in heavy metal the amount of chlorophyll pigment reduced in susceptible crops. It has been expressed that iron (Fe) toxicity (Schmidt and Fühner 1998; Molas 2002), zinc (Zn) toxicity in agricultural soils (Nagaiyot et al., 2010) repressed chlorophyll synthesis, stimulated leaf chlorosis due to iron deficiency, and inhibit the absorption of phosphorus and iron. On the other hand Gruber and Kosegarten (2002) found that chlorine areas in leaves increase due to iron lacking and inhibition of chlorophyll synthesis in grapes in calcareous soil. In sensitive varieties decreasing in chlorophyll under salt, drought, heavy metal and limy stresses is mainly the result of damage to chloroplast membrane, increasing of chlorophyll and protein degradation, decreasing biosynthesis and inhibition enzymes which responsible of biosynthesis of chlorophyll pigment by stimulated oxidative reactions (Sairam et al. 205; Foyer and Shigeoka 2011). Total carotenoids areless affected by drought than chlorophyll (Efeoglu and Terzioglu, 2009). Our carotenoid findings are consistent with this information especially in Selimiye-95.

One of the most abundant compounds in plant tissues is soluble nitrogen compounds. Researchers stated that prolines and total soluble proteins are necessary at all stages of growth (Parida et al., 2002; Sharma and Dietz, 2006). The content of proline was very lower compared to the control group in the stress treatment groups in the Cumhuriyet-75 but it was higher in Selimiye-95 in all applications (Table 2). In Cumhuriyet the amount of proline was the lowest value especially in drought, heavy metals ($ZnCl_2$, $NiCl_2$, and $FeCl_3$) treatments while it was maximum level at $CaCO_3$ and $FeCl_3$ (Table 2). Within the scope of total soluble protein quantity changes heavy metals and drought have decreased the amount of it in both wheat varieties and also it increased with salt concentrations (Table 2). The results of proline and protein are in agreement with other investigations. Demiral and Türkan (2005), Turkyılmaz et al. (2014), determined that proline level increased in tolerant genotypes order under salty conditions; Keyvan (2010), Razi et al. (2016), found that by drought cause an increase proline content in resistant species. Chen et al. (2001), Sharma and Dietz (2006) observed that proline content reduced by excess elements and heavy metals, and also Gregersen et al. (2008), Çakmak et al. (2001) noted that exposed to limy stress decreased level of it in susceptible plants. It has been proven that protein content was higher in resistant compared to susceptible species and varieties in salt stress by Ashraf and Harris (2004) and Crawford (1995), and drought conditions by Başer et al. (2005), Parida et al. (2007). Chen et al. (2001), Singh and Tewari (2003), found that excess levels of heavy metals (Zn, Cu, Cd, Co, Pb, Ni and Ag) inhibit the binding of metals to sulfhydryl groups in amino acids and proteins. As a result of this may repress its synthesis and impaire the functions (Davies, 1987; Sharma and Dietz, 2006). It has been reported that tolerance genotypes accumulate more osmolytes such as proline and soluble proteins during stress conditions and play an important role in prevention of the cellular structures and components (Szabados and Savoure 2009). And also proteins can caatbolise to proline (Kavir Kishor et al., 2005) while proline can metabolize to glucose and cause synthesis of chlorophyll by glutamate pathway (Sharma et al., 2011). It was found out that n cumhuriyet SOD activity is higher as protein content increases but CAT and GuPX activity are lower with higher MDA and H_2O_2 levels. However CAT andd GuPX activities are the highest value at lowest H_2O_2 level. APX is maximum activity due to higher protein in drought (Table 2, Table 3). In Selimiye CAT activity is lower due to higher H_2O_2 concentration but APX and SOD activity are higher by higher proline, soluble protein as well lower MDA. SOD activity is lower at heavy metals concentrations (Table 2, Table 3).

Results of MDA, H_2O_2 and enzyme activities are similar to other works of researchers which determined that ROS, MDA level were higher by abiotic stress conditions such as salinity, drought, heavy metals. For example Sairam et al. (2005) investigated that effect of long-term sodium chloride salinity in tolerant and susceptible wheat genotypes. They found that salt treatment decreased membrane stability index (MSI), activities of SOD, APX and GR but increased the contents of H_2O_2. Turkyılmaz et al. (2014) determined that the activities of APX, CAT, POX and SOD increased in tolerant barley seedling significantly under NaCl stress while Kholová et al. (2010) measured that NaCl decreased antioxidant enzymes in maize genotypes. Sofo et al. (2015), Razi et al. (2016), have reported that enzyme activities are decreased in susceptible varietiesin drought conditions; Singh and Tewari (2003) and Pandey and Sing (2011) have reported that concentrations of heavy metals (Fe, Cd, Cu, Zn, Ni, Pb) reduce SOD, APX, CAT activities in safflower, rice and spinach respectively.

As a result, it was shown in this study that the differences in growth, photosynthetic pigments as chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents, proline and soluble protein content, lipid peroxidation, hydrogen peroxide, and antioxidant activities such as APX, CAT, GuPX and SOD Activities in the two wheat cultivars could be ascribed for determination of salt, heavy meals, drought and calcareous stress effects on resistance mechanisms of wheat genotypes. Depends on chemical compound results Selimiye-95 variety is more resistant to NaCl, FeCl3 and limedrought but it showed moderate tolerance to $CaCO_3$. Cumhuriyet-75 cultivar was found as sensivite types comparison to Selimiye but it is tolerant to drought, $FeCl_3$ and 225 mM NaCl.

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