



Variation in Chemical Constituents of Siyez Wheat (*Triticum monococcum* L.) in Response to Some Abiotic Stress Factors

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ARTICLE INFO	ABSTRACT
<p>Research Article</p> <p>Received : 15/10/2018 Accepted : 21/12/2018</p> <p>Keywords: Abiotic stresses Siyez Tolerance Wheat Antioxidant</p>	<p>Main aim of this study was to determine the effects of different salt contents (75 mM, 150 mM and 225 mM NaCl), heavy metal (0.2 mg/L FeCl₃, NiCl₂, ZnCl₂), lime (2 mg/L CaCO₃), drought (50%) and pollution (0.2 mg/L dust of factories) on photosynthetic pigments, malondialdehyde (MDA), hydrogen peroxide (H₂O₂) levels, the ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (GPOX) and superoxide dismutase (SOD) in Siyez wheat (<i>Triticum monococcum</i> L.). All experiments were carried out under laboratory conditions with 16 hour-day and 8 hour-night photoperiod in an incubator at 23 ± 1°C. Results showed that mean chlorophyll-a concentration was highest in the siyez seedlings treated with the pollution, while both mean chlorophyll-b and total chlorophyll concentrations were highest with 75 mM salt application. Mean total carotenoid was, however, highest with the drought treatment and mean relative water content was highest with NiCl₂ application. Mean MDA and H₂O₂ contents were found to be highest in the siyez seedlings treated with 225 mM salt, whereas they were lowest with NiCl₂ treatment. Mean proline content was highest with the NiCl₂ treatment compared to the lowest concentration in the control siyez seedlings (82 µmol/g). Mean APX, CAT and GPOX activities were noted to be highest in the siyez seedlings treated with NiCl₂. In general, the siyez seedlings showed high tolerance to the pollution, NiCl₂ and drought with having highest photosynthetic pigments, proline, protein content and enzymes activities. Among all treatments, 225 mM NaCl and CaCO₃ negatively influenced chemical compounds of the siyez seedling. When all data are taken into consideration, it can be said that higher photosynthetic pigments, proline contents, antioxidant enzymes activities and lower MDA and H₂O₂ levels play an important role in the resistance of siyez seedlings against abiotic stress conditions.</p>

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Siyez Buğday Çeşidinin Kimyasal Bileşenlerinin Bazı Abiyotik Stres Koşullarına Karşı Değişimi

MAKALE BİLGİSİ	ÖZ
<p>Araştırma Makalesi</p> <p>Geliş : 15/10/2018 Kabul : 21/12/2018</p> <p>Anahtar Kelimeler: Abiyotik stres Siyez Tolerans Buğday Antioksidan</p>	<p>Bu çalışmanın esas amacı farklı konsantrasyonlarda tuz (75 mM, 150 mM ve 225 mM NaCl, ağır metal (0.2 mg/L FeCl₃, NiCl₂, ZnCl₂), kireç (2 mg/L CaCO₃), kurak (%50) ve kirlilik (0.2 mg/L fabrika baca tozu) uygulamalarının Siyez buğdayının (<i>Triticum monococcum</i> L.) fotosentetik pigment, malondialdehit (MDA), hidrojen peroksit (H₂O₂), prolin, toplam çözünür protein, askorbat peroksidaz (APX), katalaz (CAT), guaiakol peroksidaz (GPOX) ve süperoksit dismutaz (SOD) aktiviteleri üzerine etkilerini araştırmaktır. Bulgulara göre klorofil a miktarı kirlilik uygulamasında, klorofil b ve toplam klorofil miktarı ise 75 mM tuz uygulamasında en yüksektir. Bununla birlikte toplam karotenoid kuraklık uygulamasında ve bağıl su içeriği de NiCl₂ uygulamasında en yüksek değere ulaşmıştır. MDA ve H₂O₂ içeriği 225 mM tuz uygulamasında en yüksek, NiCl₂ uygulamasında en düşüktür. Prolin içeriği kontrole göre (82 µmol/g) NiCl₂ uygulamasında en yüksektir. APX, CAT ve SOD aktiviteleri NiCl₂ uygulamasında yüksek olarak bulunmuştur. Sonuç olarak siyez fideleri yüksek pigment, prolin, protein ve enzim aktiviteleri ile kirlilik, NiCl₂ ve kurak uygulamalarına yüksek tolerans göstermiştir. Uygulamalardan 225 mM tuz ve CaCO₃ siyez fidelerindeki kimyasal bileşenleri negative olarak etkilemiştir. Tüm veriler göz önünde bulundurulduğunda yüksek fotosentetik pigment, prolin ve antioksidan enzim aktiviteleri ve düşük MDA ve H₂O₂ miktarlarının siyez in abiyotik stres koşullarına toleransında önemli rol oynadığı söylenebilir.</p>

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Introduction

Wheat (*Triticum L. spp.*) is one of the most important cereal crops around the world, which can be cultivated in a wide variety such as temperate, high-rainy areas and warm, dry and cold environment. It has over 713 million tons in 2013 as annual production (Faostat, 2014). However, generally warm and drought climatic conditions create the ideal environment for salinity and barrenness formation in any region where the wheat grows (Başer et al., 2005). On the other hand, the accumulation of zinc, iron, lead, cadmium, nickel and other heavy metals in dense industrial zones which are close to cultivation lands affect wheat crop production by leading to heavy metal toxicity (Mutlu et al., 2013; Mutlu et al., 2014; Mutlu et al., 2016; Mutlu and Kurnaz, 2017; Barut et al., 2017; Kurnaz and Turfan, 2017; Sariyıldız et al., 2017; Mutlu and Kurnaz, 2018). Many authors stated that salinity, heavy metals and element toxicity, excessive calcareous soil and also drought especially before grain filling may reduce leaf and stem properties such as leaf area, length of leaf, length and strength of internode (Ostrowska et al., 2014; Turkyılmaz et al., 2018). It has been reported that deviations from optimal growth and development of crops repress photosynthetic activity which is main factor on grain quality and yield (Saeidi and Abdoli, 2016; Konuşkan et al., 2017). On the other side, those conditions can stimulate oxidative stress that leads to disruption of chloroplast structure, destruction of photosynthetic pigments, degradation protein and amino acid, inhibition of enzymes, increasing free radicals and malondildehyde (Neto et al., 2006; Turkyılmaz et al., 2014). Due to the increased nutrient requirements and the limited availability of agricultural lands, in parallel with population increase, selecting wild and improved genotypes with high tolerance to stress factors in regions where salinity, lime/drought and heavy metal toxicity are dominant will contribute to more efficient utilization of existent land resources. In this context, Siyez (einkorn) grown well around Kastamonu region is an important ancestral gene source. It has been reported that einkorn is an ancient wheat which originates in the mountainous areas of Turkey and its wild progenitor (*T. baeoticum Boiss.*) (Lüje et al., 2003). In addition, compared to common wheat, einkorn is generally more resistant to diseases and has the ability to withstand drought, but the yields of einkorn are less compared to the common wheat variety (Shewry and Hey, 2015; Nakov et

al., 2016). San et al., (2015) analysed the polymorphism in seed endosperm proteins for Turkish cultivated einkorn wheat (*Triticum monococcum ssp. monococcum*). They showed that it had the the high number of proteins and genetic variation, and increased interest in organic products. In order to better understand the mechanism of resistance to stress factors, the determination of morphological parameters as well as physiological measurements in different wheat varieties can provide us more accurate steps to select the appropriate species and varieties. We, therefore set up a study to investigate the effects of salt, heavy metals, drought and lime treatments on the green parts photosynthetic pigments, proline, total soluble protein, MDA, H₂O₂ amount and APX, CAT, GPOX and SOD activities were siyez wheat (*Triticum monococcum L.*). We used FeCl₃, NiCl₂ and ZnCl₂ in this present study in order to understand the effects of the heavy metal on siyez wheat since those three heavy metals are known to reduce plant growth and also they are the elements in the components of photosynthesis, carbohydrate and respiratory reactions.

Materials and Methods

Laboratory Incubations

All experiments were carried out under laboratory conditions with 16 hour-day and 8 hour-night photoperiod in an incubator at 23 ± 1°C. The seeds were planted in the plastic seeding pots (Figure 1) containing 1:1:1 garden soil, peat and sand (three replicate each) and placed in the incubators until analyses (Figure 2). In order to apply salt, heavy metal, CaCO₃ and pollution to the seedlings, each treatment group was dissolved in ArnonHoagland (Hoagland and Arnon, 1950) nutrient solution. The nutrient solution consisted of 2.5 mM NO₃⁻, 0.5 mM NH₄⁺, 2 mM K⁺, 1 mM Ca²⁺, 0.5 mM Mg²⁺, 0.05 mM Fe-EDTA, 5 & mu; M Mn²⁺, 0.5 & mu; M Zn²⁺, 0.5 & mu; M Cu²⁺, 1 mM Cl, 0.55 mM SO₄⁻², 0.5 mM PO₄⁻³, 1.5 & mu; M BO₃, 0.1 & mu; M MoO₄. The applications were made twice a week as stress application and only once a week as nutrient solution. In each case 25 ml was added. The drought application was carried out using 12.5 ml according to the soil susceptibility, while the nutrient solution and 25 ml were applied on the control group. All applications were carried out for 5 weeks.

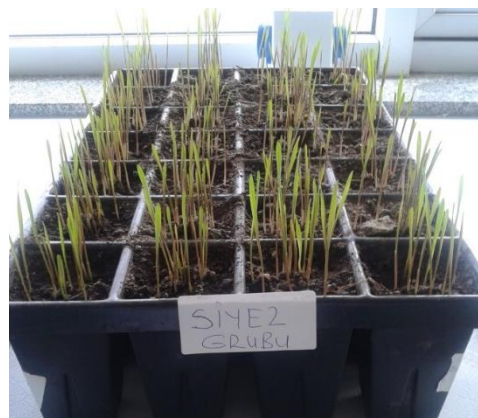


Figure 1 The siyez seeds were planted in the plastic pots (left). The development of the siyez seedlings under laboratory conditions (right)

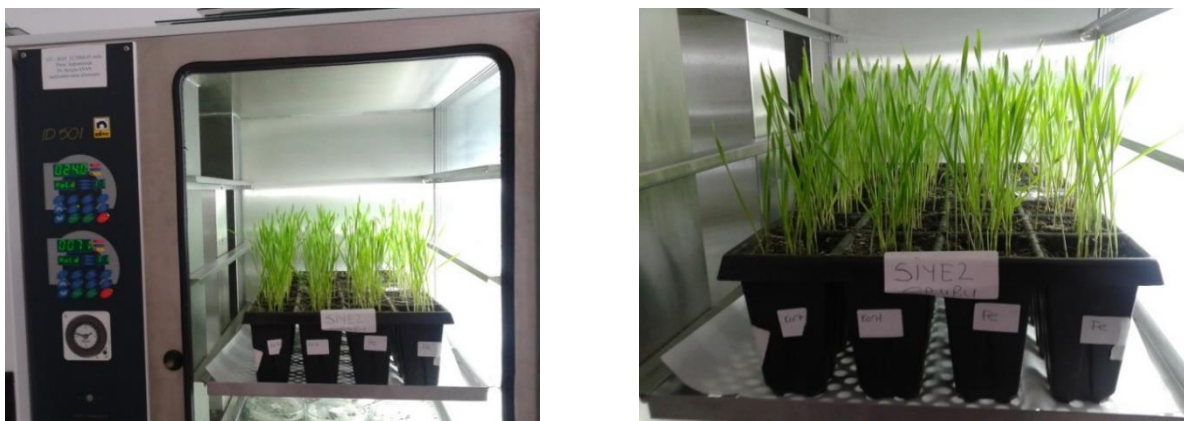


Figure 2 The growing siyez seeding treated with different amount salt, heavy metals, drought, CaCO₃ and pollutant were kept in the incubators for 5 weeks

Chemical Analyses

The leaf samples were collected at the end of the fifth week and analysed for photosynthetic pigments (chlorophyll- a, chlorophyll-b and carotenoids), proline, total soluble protein, glucose, sucrose, total soluble sugar, peroxidation level (MDA-malondialdehyde), hydrogen peroxide (H₂O₂) and antioxidants such as ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD) activities. Analyses were carried out in triplicate. Chlorophyll content of the leaf sample was measured by the method of Arnon (1949). For this, 500 mg of leaf samples were extracted with 80% acetone and centrifuged at 3000 rpm for 15 minutes. The extract was utilized for chlorophyll estimation. Carotenoid amount was estimated by Jaspars Formula according to the method by Witham et al. (1971). Proline content of leaf tissues was estimated spectrophotometrically following the ninhydrin method described by Bates et al. (1973). 500 mg of leaf sample were homogenized in 3% of sulphosalicylic acid. Samples were mixed, centrifuged at 10.000 ×g for 15 min, and added on the supernatants 2 mm glacial acetic acid and ninhydrin reagent 83% (w/v) ninhydrin in 60% (v/v) 6 M phosphoric acid) in order. All samples were kept at 90°C for 1 h. After icecooling, 4 ml cooling toluene poured on the samples, and then the absorbance of the upper (toluene) phase was determined at 520 nm against a zerotime blank. Proline concentrations were calculated using proline standards (0-100 µg mL⁻¹) in identical manner. The level of lipid peroxidation products was determined using the thiobarbituric (TBA) method which decompose and product of peroxidized polyunsaturated fatty acid component of membrane lipids. 500 mg sample were homogenized in 5 ml 0.1% (w/v) trichloroacetic acid (TCA) using a chilled mortar and pestle. The homogenate was centrifuged at 15,000 g for 15 min. To 1 ml aliquot of supernatant, 4 ml 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) TCA was added. The mixture was heated at 95°C for 30 min. The mixture was then transferred to an ice bath and centrifuged at 10,000 g for 10 min. Then the absorbance of the supernatant was recorded at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. MDA content was expressed as µmol g⁻¹ of MDA formed using an extinction coefficient of 155 mM⁻¹ cm⁻¹ as µmol MDA according to Lutts et al. (1996). Hydrogen peroxide in the plant samples was determined by the method of Velikova et al. (2000). 500 mg of fresh leaf samples were homogenized with 5 mL of 0.1% (w/v) trichloroacetic acid

and then centrifuged at 12,000 g for 15 minutes. Later, 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide were added to 0.5 mL of the supernatant. Finally, the absorbance was recorded at 390 nm. The amount of H₂O₂ expressed as µmol g⁻¹ FW. For the determination of the enzyme activity, the extracts were prepared from the first three leaves of the plants which were treated as the control and the stress factor. Accordingly, nearly 0.5-gram fresh leaf samples were homogenized with 50 mM (pH 7.6) phosphate (P) buffer solution (10 mL) ground in liquid nitrogen and containing 0.1 mM Na-EDTA. 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 (GPOX) and superoxide dismutase (SOD) activities were measured according to the methods of Bergmeyer and Grabl (1983), Nakano and Asada (1981), Chance and Maehley (1995) and Çakmak (1994) respectively under nitro blue tetrazolium chloride (NBT) light by O₂⁻ reduction. 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.

Relative water content in the leaves (RWC) was determined by the method of Ekanayake et al. (1993). The fresh leaf samples were cut about 5 cm² with the scissors and weighted (FW). Then samples were placed in tube contain 50 ml distilled water and kept at +4°C for 24 h. Turgid weight (TW) were determined at the end of this period and then samples were dried at 65°C for 24 h in an oven. Dry weight of the leaf discs was recorded (DW), and RWC of the controls and the stressed seedling was calculated using the equation (1).

$$\text{RWC (\%)} = [(\text{FW}-\text{DW}) / (\text{TW}-\text{DW})] \times 100 \quad (1)$$

Statistical Analysis of Data

Analysis of variance (ANOVA) was applied for analysing the differences in the chemical composition of Siyez wheat between the different treatments and the controls using the SPSS program (Version 20 for Windows). Following the results of ANOVAs, Tukey's honestly significance difference (HSD) test ($\alpha = 0.05$) was used for significance.

Results

Mean chlorophyll-a, chlorophyll-b, total chlorophyll, total carotenoid, relative water content and the ratio of chlorophyll a/b in the siyez seedlings treated with the different salts, heavy metals, drought and pollutant were shown in Table 1. All photosynthetic pigments and relative water content varied significantly with all treatments ($P<0.01$). Mean chlorophyll-a content was lowest in the siyez seedlings treated with the NiCl₂, 75 mM salt and the control samples (0.592, 0.603 and 0.604 mg/g respectively), whereas it was highest in the siyez seedlings treated with the pollution (0.691 mg/g). The control siyez seedling showed the lowest mean chlorophyll-b, total chlorophyll, total carotenoid and relative water content compared to the all treatments (Table 1). Both mean chlorophyll-b and total chlorophyll concentrations were, however, highest with the 75 mM salt application, mean total carotenoids was highest with the drought treatment and mean relative water content was highest with the NiCl₂ application (Table 1). The ratio of chlorophyll a/b was also highest in the siyez seedling treated with the NiCl₂, while the highest ratio of chlorophyll a/b was noted with the control siyez seedling. Mean MDA, H₂O₂, proline and total soluble protein contents in the siyez seedlings treated with the different salts, heavy metals, drought and pollutant were shown in Table 2. All MDA, H₂O₂, proline and total

soluble protein contents varied significantly with all treatments ($P<0.01$). Mean MDA and H₂O₂ contents were highest in the siyez seedlings treated with the 225 mM salt, whereas they were lowest with the NiCl₂ treatment (Table 2). However, proline content was highest with the NiCl₂ treatment (103 μmol/g) compared to the lowest content in the control siyez seedlings (82 μmol/g). Protein content was lowest (9.04 mg/g) with the 225 mM salt application, but it was highest 75 mM salt application (14.7 mg/g) (Table 2). Mean APX, CAT, GPOX and SOD activities in the siyez seedlings treated with the different salts, heavy metals, drought and pollutant were shown in Table 3. All APX, CAT, GPOX and SOD activities varied significantly with all treatments ($P<0.01$). Mean APX, CAT and GPOX activities were highest (0.150, 0.042 and 0.052 EU respectively) in the siyez seedlings treated with the NiCl₂, whereas they were lowest with the 225 mM salt treatment (0.055, 0.023 and 0.029 EU respectively). SOD activity was also lowest (89.5 EU) with the 225 mM salt application, but it was highest 75 mM salt application (122.4 EU). Significant differences have been found between the element amounts in the factory dust. Especially the elements such as zinc, iron, chlorine, bismuth, aluminum, lead, arsenic and boron are toxic (Table 4).

Table 1 Mean chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoids, relative water content (RWC) and the ratio of chlorophyll a/b in the siyez seedlings treated with the different salt contents (75, 150 and 225 mM NaCl), heavy metals (0.2 mg/L FeCl₃, NiCl₂ and ZnCl₂), lime (2 mg/L CaCO₃), drought (50%) and pollution (0.2 mg/L dust of factories)

	Chlorophyll a mg/g	Chlorophyll b mg/g	Total Chlorophyll mg/g	Ratio of Chl. a/b	Total Carotenoids mg/g	RWC (%)
Control	0.604±0.003 ^{c*}	0.264±0.004 ^a	0.868±0.004 ^a	2.29:1	7.31±0.052 ^a	82.4±0.23 ^b
75 mM	0.603±0.002 ^c	0.656±0.004 ⁱ	1.259±0.006 ^f	0.92:1	8.16±0.021 ^b	90.6±0.31 ^c
150 mM	0.610±0.003 ^e	0.576±0.010 ^h	1.186±0.011 ³	1.06:1	8.12±0.006 ^b	81.9±0.18 ^a
225 mM	0.614±0.001 ^f	0.503±0.002 ^e	1.117±0.002 ^c	1.22:1	8.15±0.004 ^b	87.1±0.44 ^c
FeCl ₃	0.618±0.001 ^f	0.541±0.003 ^f	1.158±0.003 ^c	1.14:1	8.25±0.047 ^b	92.3±0.34 ^d
NiCl ₂	0.592±0.001 ^a	0.277±0.010 ^b	0.869±0.010 ^a	2.14:1	8.11±0.014 ^b	105.7±0.16 ^e
ZnCl ₂	0.607±0.004 ^d	0.569±0.015 ^g	1.175±0.015 ^d	1.07:1	8.24±0.048 ^b	102.5±0.18 ^e
CaCO ₃	0.596±0.007 ^b	0.327±0.001 ^c	0.922±0.007 ^b	1.84:1	7.92±0.027 ^a	92.7±0.67 ^d
Drought	0.617±0.004 ^f	0.570±0.007 ^g	1.188±0.004 ^e	1.09:1	8.49±0.010 ^c	93.4±0.9 ^d
Pollution	0.691±0.001 ^g	0.483±0.001 ^d	1.101±0.001 ^b	1.28:1	8.34±0.038 ^b	93.3±0.51
F	9.053	391.132	378.014	284.211	101.186	269.95
Sig.	0.002	0.001	0.002	0.001	0.002	0.001

*: a,b,c...i = means within the same column with different superscripts are significantly ($P<0.05$) different.

Table 2 Mean MDA, H₂O₂, proline and total soluble protein contents in the siyez seedlings treated with the different salt contents (75, 150 and 225 mM NaCl), heavy metals (0.2 mg/L FeCl₃, NiCl₂ and ZnCl₂), lime (2 mg/L CaCO₃), drought (50%) and pollution (0.2 mg/L dust of factories)

	MDA μmol/g	H ₂ O ₂ μmol/g	Proline μmol/g	Protein mg/g
Control	18.5±0.24 ^{c*}	31.6±0.22 ^d	82.1±0.24 ^b	10.6±0.01 ^b
75 mM	15.5±0.23 ^b	23.8±0.11 ^c	93.2±0.16 ^e	14.7±0.22 ^d
150 mM	22.4±0.2 ^d	34.9±0.14 ^e	77.3±0.18 ^a	10.4±0.01 ^b
225 mM	26.6±0.2 ^e	45.5±0.17 ^f	94.5±0.21 ^e	9.04±0.001 ^a
FeCl ₃	15.5±0.06 ^b	16.8±0.12 ^b	97.4±0.22 ^f	9.90±0.01 ^a
NiCl ₂	12.6±0.20 ^a	13.7±0.22 ^a	103.6±0.21 ^g	11.8±0.01 ^c
ZnCl ₂	24.0±0.06 ^d	17.5±0.21 ^b	88.5±0.14 ^c	10.8±0.01 ^b
CaCO ₃	14.4±0.03 ^b	36.4±0.11 ^e	92.8±0.24 ^d	10.1±0.01 ^a
Drought	14.6±0.20 ^b	16.4±0.19 ^b	92.6±0.16 ^d	10.5±0.01 ^b
Pollution	25.8±0.16 ^e	22.7±0.14 ^c	93.5±0.17 ^e	10.7±0.01 ^b
F	893.481	4107.948	1469.364	462.087
Sig.	0.003	0.003	0.001	0.002

*: a,b,c...i = means within the same column with different superscripts are significantly ($P<0.05$) different

Table 3 Mean APX, CAT, GPOX and SOD activities in the siyez seedlings treated with the different salt contents (75, 150 and 225 mM NaCl), heavy metals (0.2 mg/L FeCl₃, NiCl₂ and ZnCl₂), lime (2 mg/L CaCO₃), drought (50%) and pollution (0.2 mg/L dust of factories)

	APX	CAT	GPOX	SOD
	EU/mg Protein	EU/mg Protein	EU/mg Protein	EU/mg Protein
Control	0.112±0.0001 ^{fs}	0.028±0.0002 ^d	0.046±0.0005 ^e	104.1±0.01 ^b
75 mM	0.090±0.0008 ^d	0.034±0.0002 ^e	0.036±0.0002 ^c	122.6±0.45 ^g
150 mM	0.076±0.0002 ^b	0.024±0.0005 ^b	0.032±0.0001 ^b	109.5±0.01 ^c
225 mM	0.055±0.0002 ^a	0.023±0.0001 ^a	0.029±0.0002 ^a	89.5±0.34 ^a
FeCl ₃	0.137±0.0015 ^g	0.034±0.0003 ^e	0.049±0.0003 ^f	110.6±0.01 ^d
NiCl ₂	0.150±0.0022 ^h	0.042±0.0002 ^h	0.052±0.0003 ^g	117.7±0.43 ^f
ZnCl ₂	0.089±0.0012 ^d	0.036±0.0002 ^g	0.042±0.0003 ^d	118.3±0.01 ^f
CaCO ₃	0.106±0.0018 ^e	0.026±0.0003 ^c	0.047±0.0003 ^f	122.3±0.04 ^g
Drought	0.138±0.0019 ^g	0.043±0.0003 ⁱ	0.049±0.0002 ^f	117.0±0.01 ^f
Pollution	0.085±0.0013 ^c	0.035±0.0002 ^f	0.042±0.0001 ^d	114.5±0.34 ^e
F	523.717	1064.885	953.660	4835.953
Sig.	0.003	0.002	0.002	0.001

*: a,b,c...i = means within the same column with different superscripts are significantly (P<0.05) different

Discussion

Chlorophyll pigments play an important role in photosynthetic metabolism and it they have been considered as one of the parameters of stress tolerance in crop plants (Panda et al. 2013; Şevik et al., 2015). In this present study, a significant variation in the pigment contents, especially chlorophyll-b and total chlorophyll was observed. Mean chlorophyll-a content was highest in the siyez seedlings treated with the pollution (0.691 mg/g), while mean chlorophyll-b and total chlorophyll concentrations were highest with the 75 mM salt application (Table 2). Other studies have also revealed that salinity, heavy metals, lime, drought, pollution and other stress conditions can cause significant reduction in the photosynthetic pigment level in some susceptible species. Langmeier et al., (1993), Torun et al. (2017) expressed that the amount of chlorophyll pigment in sensitivity plants was lowered by heavy metals such as Ni, Cd, Zn, Fe and Hg. Çakmak et al., (2000) and Gruber and Kosegarten (2002) found that calcareous soil decreased pigment content and non-chlorotic area in durum wheat genotypes Chernane et al. (2015) showed that chlorophyll a content was lower under salt condition, while total chlorophyll level was higher in the control seedling compared to the stressed seedlings. Some authors (Parida et al., 2007) showed that photosynthetic pigments were altered under drought conditions for wheat genotypes and cotton plants. Many authors reported that the amount of pigments decreased when a plant species was exposed to salt, excess heavy metals and deficient of nutrition elements, drought and lime stresses (Bavaresco et al., 1994; Parida et al., 2007). They have stated that abiotic stress conditions can cause leaf senescence by loss of chlorophyll, destruction of chloroplast membrane and accumulation of excess free radicals (Molas, 2002; Gregersen et al., 2008; Konoşkan et al., 2017)). Changes in relative water content has been used as an indicator of phytotoxicity under drought, salty, excess heavy metal stress, deficient of nutrition and calcareous conditions for herbal plants. In this present study, percent relative water content was lowest in the siyez seedlings treated with 150 mM NaCl, but highest in the siyez seedlings treated with NiCl₂ and ZnCl₂ (Table 1). It has been shown by a number of authors (Kadioğlu and Terzi,

2007; Keyvan, 2010) that salinity, drought, heavy metals, ion toxicity, pollution damaged water relations and osmotic balance stress lead to decline in plant growth and development. On the other hand, plants may prevent the harmful effect of imbalance osmotic adjustment by accumulation osmolytes such as proline, soluble protein, and reduced sugars. Zhu (2002) and Farouk (2011) showed that salt condition induced reduction in relative water content as well as water and osmotic potential but tolerant wheat genotypes increased osmolytes synthesis and regulated water relation. Hui et al. (2012) and Keyvan (2010) found that leaf relative water content decreased with drought stress but it was higher in some genotypes due to accumulating osmoprotective compounds. Carvajal et al. (1996) and Gajewska et al. (2006) for wheat genotypes, Cseh et al. (2000) for cucumber and Llamas et al. (2000) for rice showed that the amount of relative water content was reduced insufficient of nutrient and heavy metals such as Pb and Ni. They stated that the stress induced stomata closure by direct interaction of toxic metals with guard cells and preventing of water movement water into the vascular system. During plant growth and development, membrane properties and compositions can change by cell dividing, new tissues and organs forming (Berger et al., 2001). But under stress conditions, chemical bound in membrane lipids can be loosen by enzymatically or non-enzymatically and stimulate toxic molecules such as malondialdehyde (MDA), ketones and also accumulate free radicals like singlet oxygen, hydrogen peroxide and super oxide anions (Terzi and Kadioğlu, 2006). However, it has been reported that the synthesis of enzymatic and non-enzymatic antioxidant compounds increased in tolerant crops (Ashram et al., 2007). In stressed leaf sample, our results showed that the amount of MDA was highest the siyez seedlings treated with the 225 mM salt, pollution and ZnCl₂, whereas it was lowest under the drought, CaCO₃ and NiCl₂ conditions (Table 2). H₂O₂ concentration in the siyez seedlings was lowest under the heavy metals and drought stress, but it significantly increased in the siyez seedlings under 225 mM NaCl and pollution condition compared to the control siyez seedlings (Table 2).

Table 4 Mean elemental profile of dust factories

	ppm
Na	55.92±0.240
Mg	47.89±0.003
Al	357.35±0.001
Si	2033.22±0.001
P	81.48±0.001
S	3029.48±0.010
Cl	35324.00±0.003
K	644.28±0.003
Ca	4906.27±0.007
Ti	279.01±0.002
V	71.20±0.001
Cr	20.42±0.001
Mn	2615.91±0.001
Fe	73377.60±0.020
Ni	29.55±0.001
Cu	61.83±0.001
Zn	85577.80±0.010
Ga	95.86±0.001
As	319.42±0.003
Br	1548.83±0.001
Rb	295.19±0.001
Sr	319.98±0.001
Y	52.93±0.001
Cd	22.11±0.001
Sn	49.91±0.001
I	60.50±0.001
Ba	329.88±0.001
Ta	29.97±0.001
Tl	58.93±0.001
Pb	10691.40±0.003
Bi	2151.08±0.001

Antioxidant enzyme activities were higher at FeCl₃, NiCl₂ and drought generally (Table 3). However, CAT was higher under heavy metals, drought and pollution conditions, while APX increased under FeCl₃, NiCl₂ and drought conditions. Mean GPOX activity was stimulated by FeCl₃, drought and NiCl₂ conditions, while SOD activity decreased with the application of 225 mM NaCl (Table 3). The increase of MDA and H₂O₂ concentrations and the reduction of APX, CAT, GPOX and SOD activities under higher salt (225 and 150 mM NaCl), pollution and ZnCl₂ conditions indicated that there was a negative interaction between MDA and H₂O₂ levels and antioxidant enzymes (Verma and Dubey, 2003). Sairam et al. (2005) and Abd-Elgawad et al. (2016) observed that under salt stress, MDA and H₂O₂ level increased in the susceptible wheat genotypes but antioxidant activity was higher in the resistant types. Neto et al. (2006) also found that MDA and H₂O₂ increased with salts treatments in sensitive maize leaf and root cells, whereas SOD, CAT, APX contents were significantly greater in tolerant types than sensitive ones. It was reported for cereals that excess heavy metals and pollutions induced lipid peroxidation and H₂O₂ accumulation, but moderate concentrations of heavy metals and pollutions increased SOD (Verma and Dubey, 2003; Sharmila and Saradhi, 2002). Similarly, under the drought conditions, APX, SOD and GPOX activity increased in tolerant genotypes, but MDA and ROS levels decreased (Mohammadi et al., 2011; Sun et al., 2016).

Jakovljević et al. (2017) investigated the salt stress on antioxidant enzyme for the early growth in sweet basil seedlings. Their results showed that guaiacol peroxidase (GPOX) activity increased, but CAT activity was seen to be the most salinity-sensitive enzyme examined. Under calcareous stress, Shukry et al. (2007) found that there was a decrease in photosynthetic pigments, but phenol, lipid peroxidation, CAT, SOD and POX activities increased. Gruben and Kosegarten (2002), Bavaresco and Poni (2002) have stated that calcareous soils induce iron deficiency and inhibition of enzymes activities responsible chlorophyll synthesis in plants which are characterized as chlorosis by Mg lacking. In many plants, osmolytes such as free proline, glycinebetain, and soluble protein, accumulates in response to abiotic and biotic stress conditions (Ashram et al., 2007; Xu et al. 2012). The results from this present study showed that mean proline content in the siyez seedlings was higher than the control seedling. It only decreased with the application of 225 mM NaCl. Total soluble protein content was however reduced under all salt applications, CaCO₃ and drought conditions (Table 2). Those findings for proline and protein were in agreement with other studies. An increase in proline amount due to the drought stress was reported by Keyyvan (2010), Giancarla et al. (2011). Terzi and Yıldız (2013) and Turkyılmaz et al. (2014) found that proline level increased in tolerant genotypes under salty conditions. Effects of heavy metals on proline content were studied by many researchers. For example, Kao et al. (2007) and Gajewska et al. (2006) showed that Ni treatments increased proline level in the stressed seedlings due to protein hydrolysis, a reduce in proline dehydrogenase activity and a decrease in proline utilization. Zengin and Kirbag (2007) found for sunflower seedlings, a decrease in protein content with increasing copper (Cu) concentration, but an increase in proline accumulation. They explained that the effect of Cu on proline and protein contents were dose-dependent. Under alkali and calcareous conditions, Gruber and Kosegarten (2009) and Yang et al. (2007) observed that proline enhanced tolerance capacity of plants. It has been shown that the level of soluble protein was higher in resistant species under salt stress (Davies, 1987; Crawford, 1995) and drought conditions (Habibi, 2014; Parida et al., 2009) and also heavy metals or lacking of minerals (Chen et al., 2001; Singh and Tewari, 2003). The accumulations of compatible solutes, such as proline and soluble proteins are considered as one of the main factors responsible for their tolerance to abiotic stress. They prevent cellular structures and components by scavenging ROS level, and also proteins can catabolize to proline and its content can decrease. The results have shown that abiotic factors, in this present study salts, heavy metals, drought, pollution and calcareous factors can significantly influence the photosynthetic pigments as chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid concentrations, RWC, proline and soluble protein concentrations, lipid peroxidation, hydrogen peroxide, and antioxidant activities such as APX, CAT, GPOX and SOD activities in Siyez seedlings. Some of those chemical compounds in Siyez cultivar could be ascribed to determine the effects of salt, heavy meals, drought and calcareous stresses on the resistance mechanisms of wheat genotypes. For example, the results from the pigment analyses have also shown that

siyez seedlings are highly resistant to FeCl₃ and drought. But based on the others results it is more tolerant to NiCl₂, 75 Mm NaCl as well as FeCl₃. According to all results, it is concluded that the resistance of a plant species against abiotic stress is not uniform and it varies with the stress types and its concentrations.

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