

## ORIGINAL ARTICLE

# Diversity exists in development parameters and enhancement of antioxidant mechanisms of some einkorn and bread wheats under combined water deficits and salt stress

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**Summary.** *Introduction.* Climatic changes worsen the production of wheat, an important stable crop while improving its some nutritional quality characteristics. *Methods.* Therefore, the purpose here was to evaluate some yield limiting factors as well as some quality characteristics in 8 bread wheat (*Triticum aestivum* L.) cultivars (Kıraç - 66, Kenanbey, Flamura - 85, Momtchill, Bayraktar - 2000, Tosunbey, Pandas, and Pehlivan) and 8 einkorn (*Triticum monococcum* spp. *monococcum*) populations (Populations - 4, 5, 6, 9, 10, 11, 14, and 15) under three different osmotic pressures (0.0 MPa., -0.5 MPa., or -1.0 MPa.) and three different salt concentrations [0.0 (distilled water), 50 mM or 100 mM NaCl]. Moreover, total phenolic content (TPC) and total flavonoid content (TFC), and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities were determined. *Results.* Among bread wheat cultivars, Pandas had the longest shoot length, root length, the heaviest fresh shoot weight, dry shoot weight, and fresh root weight in the control group. In the einkorn populations, Pop - 6 had the longest shoot length, root length, the heaviest fresh shoot weight, dry shoot weight, and fresh root weight again in the control group. The heaviest dry root weight was obtained in Momtchill and Pop - 9 under drought stresses. The TPC was the highest in Kıraç - 66 and in Pop - 10 under salt stress and the TFC was in Pehlivan under salt stress. The methanol extracts of Kıraç - 66, Pop - 9, and Pop - 15 obtained under salt stress had stronger free radical scavenging activity than by ascorbic acid. *Conclusion:* Kıraç - 66 had the highest free radical scavenging antioxidant activity under salt stress and higher secondary metabolite products (as flavonoids), which indicated the highest tolerance system under both drought and salt stress. Kıraç - 66, which was improved for poorer, drier, and cooler lands had better root and metabolite production under combined drought and salt stress as expected.

**Key words:** antioxidant activity, Kıraç - 66, phenolics, seed germination, abiotic stress

## Introduction

Drought and salinity restrict plant growth through changes in water and nutrient relations, decreases water use efficiency, and photosynthesis ratio in plants (1-4) and, therefore, decreases yield in many plant species (5). Thus, drought and salinity tolerance are significant in plants (8, 9). The yield and production of bread wheat, depend on rainfall for production swing frequently because of highly restrictive non - environmental and environmental stresses including drought

and salinity. The harvest is what is left from those most destructive stresses. Stresses, which widely fluctuate across the world, ruin wheat crop depending upon the degree and duration, growth stage of plant, and time of the stress. Plants, on the other hand, avoid from drought (6) and salinity (7) through various modified morphological, anatomical, physiological, and biochemical processes. Developed efficient testing and rapid screening methods are also means of aids for plants to overcome these stresses. Widely adapted stress enduring wheat genotypes yield higher and esca-

late wheat production across many countries.

Drought and salt stressed plants could produce varied levels of free radicals such as flavonoid, phenolic acid, and antocyanin (10, 11) which provide an escape from inflammation, ischemia, arthritis, asthma, cancer, neuro - degeneration, Parkinson's diseases, mongolism, ageing, and dementia (12-16). Plant - derived natural free radical scavenger antioxidant foods are potentially safer, effective, and cheaper than industrially produced ones (17), and, moreover, encourage regeneration.

Wheat (*Triticum* spp.), which has evolved from wild ancestors of cultivated einkorn (*Triticum monococcum* spp. *monococcum*) and emmer (*Triticum dicoccum* Schrank.), (18-21), highly contributed to human diet and health (22-27). Einkorn (*Triticum monococcum* spp. *monococcum*) was first domesticated and cultivated around Karacadag Mountains, Diyarbakir, Turkey (28). Its wider adaptation, better disease resistance, and some enhanced quality characteristics (29) have been, to some extent, introduced into modern wheat cultivars. Unfortunately, its cultivation has been marginalized to Kastamonu, Bolu, Bilecik, and Sinop provinces in Turkey (20) and some parts in the Caucasus, Balkans, Spain, and Italy (30, 31) today.

Based on the points we explained below, we, here, studied the relationship among drought stress, salt stress, growth parameters, total phenolic content, total flavonoid content, and free radical scavenging activities of 8 bread wheat (*Triticum aestivum* L.) and 8 hulled einkorn (*Triticum monococcum* ssp. *monococcum*) populations.

## Material and Methods

### Seed material

The seed material was 8 bread wheat (*Triticum aestivum* L.) cultivars ('Kıraç - 66', 'Kenanbey', 'Flamura - 85', 'Momtchill', 'Bayraktar - 2000', 'Tosunbey', 'Pandas', and 'Pehlivan') and 8 einkorn (*Triticum monococcum* ssp. *monococcum*) populations (Pop - 4, 5, 6, 9, 10, 11, 14, and 15) from Seben - Bolu and İhsangazi - Kastamonu. Bread wheat cultivars were kindly provided by Central Research Institute for Agricultural Research (CRIFC), Ankara, Thrace Agricultural Research Institute, Edirne, and Cukurova Agricultural Research Institute, Adana. Einkorn populations were kindly provided by Bolu Quality and Feed Industry Corporation - Bolu (Table 1).

**Table 1.** Species, names, and locations of used wheat samples.

No	Cultivars or Populations of Wheat	Common Name	Species and Subspecies	Location
1	Kıraç- 66	Bread wheat	<i>T. aestivum</i> L.	CRIFC <sup>1</sup> , Ankara
2	Kenanbey	Bread wheat	<i>T. aestivum</i> L.	CRIFC <sup>1</sup> , Ankara
3	Flamura- 85	Bread wheat	<i>T. aestivum</i> L.	TARF <sup>3</sup> , Edirne
4	Momtchill	Bread wheat	<i>T. aestivum</i> L.	TARF <sup>3</sup> , Edirne
5	Bayraktar - 2000	Bread wheat	<i>T. aestivum</i> L.	CRIFC <sup>1</sup> , Ankara
6	Tosunbey	Bread wheat	<i>T. aestivum</i> L.	CRIFC <sup>1</sup> , Ankara
7	Pandas	Bread wheat	<i>T. aestivum</i> L.	CARI <sup>4</sup> , Adana
8	Pehlivan	Bread wheat	<i>T. aestivum</i> L.	TARF <sup>3</sup> , Edirne
9	Pop- 4	Einkorn	<i>T. monococcum</i> ssp. <i>monococcum</i>	Field # 2; Kavaklı Yazı Village, Seben, Bolu
10	Pop- 5	Einkorn	<i>T. monococcum</i> ssp. <i>monococcum</i>	Field # 3; Kavaklı Yazı Village, Seben, Bolu
11	Pop- 6	Einkorn	<i>T. monococcum</i> ssp. <i>monococcum</i>	Fie id# 4; Kavaklı Yazı Village, Seben, Bolu
12	Pop- 9	Einkorn	<i>T. monococcum</i> ssp. <i>monococcum</i>	Field # 1; Çatalyazı Village, İhsangazi, Kastamonu
13	Pop- 10	Einkorn	<i>T. monococcum</i> ssp. <i>monococcum</i>	Fie id# 2; Çatalyazı Village, İhsangazi, Kastamonu
14	Pop- 11	Einkorn	<i>T. monococcum</i> ssp. <i>monococcum</i>	Fie id# 3; Çatalyazı Village, İhsangazi, Kastamonu
15	Pop- 14	Einkorn	<i>T. monococcum</i> ssp. <i>monococcum</i>	Field # 4; Çatalyazı Village, İhsangazi, Kastamonu
16	Pop- 15	Einkorn	<i>T. monococcum</i> ssp. <i>monococcum</i>	Field # 5; Çatalyazı Village, İhsangazi, Kastamonu

CRIFC<sup>1</sup>: Central Research Institute for Agricultural Research; TARF<sup>3</sup>: Thrace Agricultural Research Institute; CARI<sup>4</sup>: Cukurova Agricultural Research Institute

### *Growth traits at germination*

Totally 16 different wheat genotypes were counted (100 pieces of seeds) and put into beaker (250 ml) separately. Seeds were surface-sterilized in 150 ml of 5% sodium hypochlorite (NaClO) for 15 min and thoroughly rinsed 4 - 5 times in distilled water. Ten sterilized seeds are placed between the sterile filter papers placed in the petri plates of 10 ml distilled water for control, 10 ml -0.0 MPa., -0.5 MPa. or -1.0 MPa. Polyethylene Glycol (PEG) 600 for drought stress, and 10 ml of 50 mM ve 100 mM sodium chloride (NaCl) for salt stress were put on petri plates. The pH of each concentration was adjusted to pH 5.8 and seeds were germinated at  $22 \pm 2^\circ\text{C}$  in a dark growth room and, then, transferred into a enlightened one. Five replicate petri dishes were prepared for each group (5 petri  $\times$  10 seeds). Paraffin wrapped around the petris againsts evaporation. All petri dishes were kept at  $22 \pm 2^\circ\text{C}$  in the dark room for 4 days and in a growth room with a 16h photoperiod following 6 days. Filter papers and test solutions in petri dishes were renewed day by day. Growth parameters of "shoot length (cm)", "root length (cm)", "fresh shoot weight (mg)", "fresh root weight (mg)", dry shoot weight (mg)", and "dry root weight (mg)" for 15 randomly selected seedlings after 10 days from sowing were measured under control, drought, and salt stress. Dry weight of germinated seeds were measured after stood them at  $105^\circ\text{C}$  (etuv) for 2 hours.

### *Plant extraction*

The plantlets of bread and einkorn wheat entries were collected from 10 day old germinated seeds. They were liquid nitrogen dried and powdered in a porcelain mortar. One g of powdered plant material was transferred to a glass test tube containing 10 ml of 80% methanol (MeOH) for 18 h at  $35^\circ\text{C}$  in an agitated hot water bath for extraction. Then, the test tubes were centrifuged at 5000 rpm for 10 min. The supernatant was filtrated by 0.45  $\mu\text{m}$  pore size Whatman syringe filter and transferred to a new glass test tube. The 80% methanol extract solutions of wheat seedlings obtained from bread and einkorn wheat were kept at  $-20^\circ\text{C}$  for all further analyses.

### *Determination of antioxidant activities*

#### *Total phenolics*

The total phenolic content (TPC) of methanol extracts obtained from bread and einkorn wheat

plantlets was determined in triplicate using the Folin - Ciocalteu reagent (32, 33), expressed as mg gallic acid equivalent (GAE) and mg tannic acid equivalent (TAE) in g dried weight (dw) of plant material from the calibration curves.

#### *Total flavonoids determination*

The total flavonoid content (TFC) of methanol extracts from bread and einkorn wheat seedlings was determined in triplicate by aluminum chloride ( $\text{AlCl}_3$ ) colorimetric assay (34) and expressed mg catechol equivalents (CE) and mg quercetin equivalents (QE)/g dw of plant material according to the calibration curves.

#### *Determination of free radical scavenging activity by DPPH assay*

The free radical scavenging antioxidant activity of methanol extracts from seedlings of bread and einkorn wheat was spectrophotometrically determined by 2,2 - diphenyl - 1 - picrylhydrazyl (DPPH) (Sigma-Aldrich Chemie, Steinheim, Germany) assay. The free radical scavenging activity of extracts was measured by slightly modified method of Brand - Williams (35) as described in Pehlivan Karakas (33). Radical scavenging activity (% inhibition) of methanol extracts from seedlings of bread and einkorn wheat was calculated according to Gulcin et al.'s (36) formula: % Inhibition =  $[(\text{AB} - \text{AA}) / \text{AB}] \times 100$ , where: AB = absorption of blank sample (control); AA = absorption of tested extract solution at 517 nm. The results were also expressed as  $\text{IC}_{50}$  (mg/L), which meant the amount of samples necessary to decrease the absorbance of DPPH in 50%.

#### *Statistical analysis*

The statistical analysis of the experimental data was performed using SPSS Version 22.0 (SPSS Inc., Chicago, IL, USA). First, one way ANOVA for the significance of cultivars and treatments, and, then, Duncan's multiple range for differences among means ( $p < 0.05$ ) were run. The results were calculated as a mean  $\pm$  SD (standard deviation). Small varying letters (a, b, c, d, and e) indicated the differences between means in the same column.

## Results and discussion

### *Effects of drought and salinity stresses on growth parameters*

Biotic and abiotic stress factors in natural environmental conditions frequently change and inhibit the growth and developmental stages in many plant species (37). In the present study, we measured mean shoot length (Figure 1A, 1B), root length (Figure 1C, 1D), fresh shoot weight (Figure 2A, 2B), fresh root weight (Figure 3A, 3B), dry shoot weight (Figure 2C, 2D), and dry root weight (Figure 3C, 3D) in 10 day-old seedlings of 8 bread and 8 einkorn wheat entries under control (distilled water), drought (-0.5 MPa.), or salinity (50 mM NaCl). Drought and salinity stresses caused a significant reduction in all bread and einkorn wheat growth parameters (Figures 1 and 2). The mean shoot length in bread wheat cultivars decreased more under drought stress than salinity except Pehlivan (Figure 1A). On the contrary, mean root length under salinity shortened more than all bread wheat cultivars under drought (Figure 1C). The longest shoot length was in Pehlivan ( $12.77 \pm 0.62$  cm) under control, the shortest shoot length was in Bayraktar - 2000 ( $2.30 \pm 0.30$  cm) under drought (Figure 1A). However, the longest root length was in Pandas ( $9.15 \pm 0.38$  cm) under control, the shortest root length was in Kırac - 66 ( $3.62 \pm 0.50$  cm) under salinity (Figure 1C). While drought decreased the mean shoot lengths of bread wheat cultivars, salinity decreased the mean root lengths. The heaviest fresh shoot weight ( $97.84 \pm 6.20$  mg), dry shoot weight ( $10.88 \pm 0.51$  mg) and fresh root weight ( $103.67 \pm 6.00$  mg) were Pandas under control, the heaviest dry root weight was in the Momtchill under drought (Figure 3C). Kırac - 66 ( $22.99 \pm 2.10$  mg), Flamura - 85 ( $27.74 \pm 0.80$  mg), and Tosunbey ( $27.42 \pm 3.10$  mg) had the lowest fresh shoot weight under drought (Figure 2A). Kırac - 66 had also the lowest dry shoot weight ( $3.49 \pm 0.30$  mg) (Figure 2C) and fresh root weight ( $38.13 \pm 4.60$  mg) under drought (Figure 3A). Flamura - 85 had the lowest dry root weight ( $4.70 \pm 0.57$  mg) under salinity (Figure 3C). The mean fresh and dry shoot weight obtained from bread wheat cultivars were more affected by drought than salinity except for Momtchill (Figure 2A, 2C). The dry root weights of bread wheats were heavier under drought than salinity (Figure 3C). Development of tolerance of

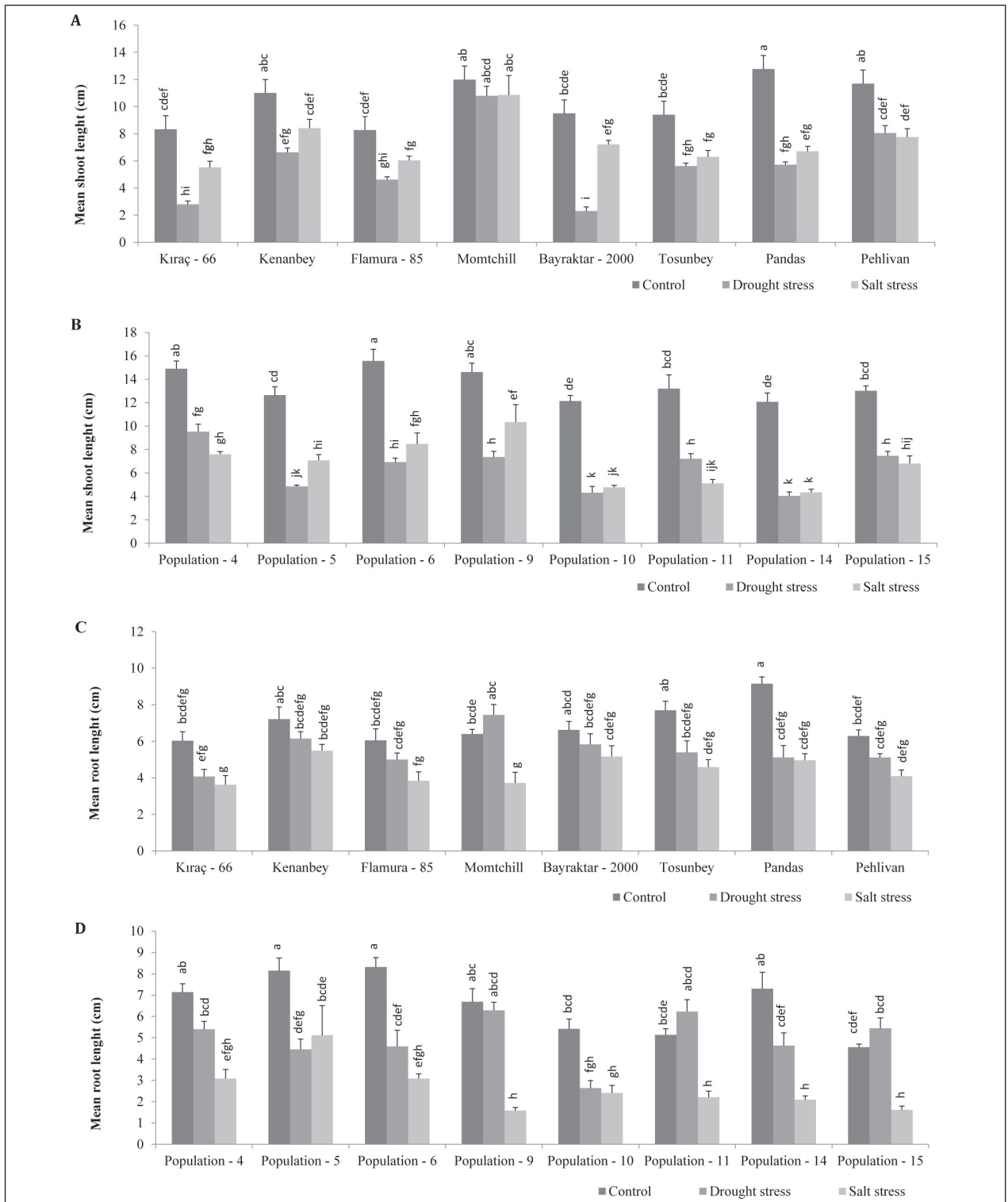
drought and salinity in plants are expected to increase crop productivity through plant breeding (9).

The shortage in the mean shoot length of einkorn populations was higher under drought than salinity except Pop - 4, Pop - 11, and Pop - 15 (Figure 1B). On the contrary, the shortage of mean root length was higher under salinity than drought for all tested einkorn populations except for Pop - 5 (Figure 1D). When the longest shoot length ( $15.56 \pm 1.0$  cm) (Figure 1B), root length ( $8.32 \pm 0.44$  cm) (Figure 1D) and the heaviest fresh shoot weight ( $98.91 \pm 6.49$  mg) (Figure 2B), dry shoot weight ( $10.03 \pm 0.89$  mg) (Figure 2D), and fresh root weight ( $78.1 \pm 4.45$  mg) were in Pop - 6 under control (Figure 3B), the heaviest dry root weight was in Pop - 9 ( $8.27 \pm 0.47$  mg) under drought (Figure 3D). However, the shortest shoot was observed in Pop - 10 under drought, ( $4.3 \pm 0.55$  cm), Pop - 14 under drought ( $4.04 \pm 0.34$  cm) and salt stress ( $4.34 \pm 0.28$  cm) (Figure 1B), the shortest root length was in Pop - 9 ( $1.58 \pm 0.14$  cm), Pop - 11 ( $2.2 \pm 0.29$  cm), Pop - 14 ( $2.09 \pm 0.18$  cm), and Pop - 15 ( $1.61 \pm 0.18$  cm) under salinity (Figure 1D). The lightest fresh shoot ( $3.48 \pm 0.56$  mg) (Figure 2B), dry shoot ( $0.67 \pm 0.12$  mg) (Figure 2D), and fresh root ( $20.13 \pm 2.3$  mg) (Figure 3B) were in Pop - 10 under drought, the lightest dry root was in the Pop - 15 under salinity (Figure 3D).

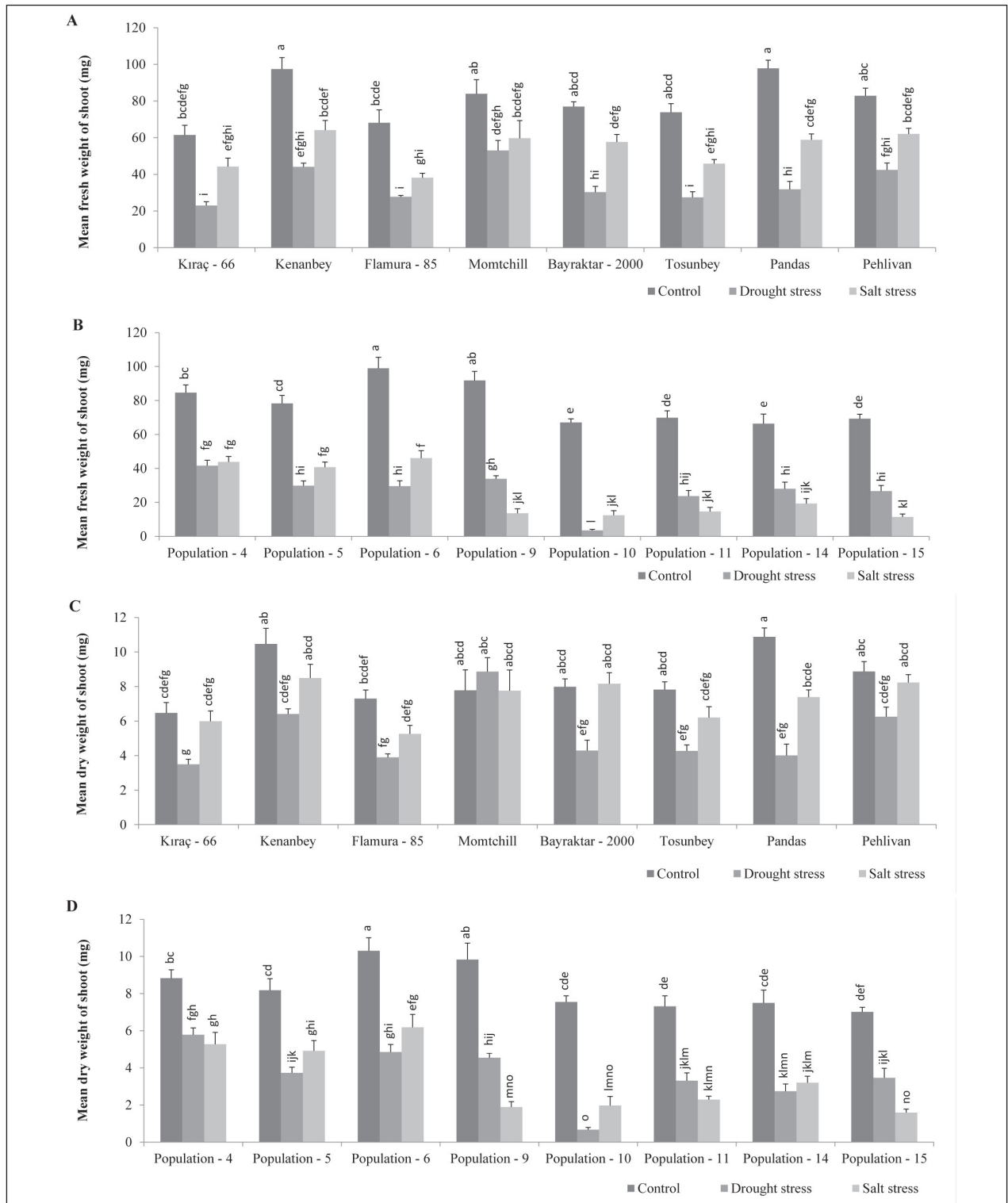
Drought and salt reduce plant growth while they generally enhance the concentration of secondary products in plants. Thus, the overall amount of natural products on fresh or dry weight base could simply be increased (38) most likely due to the reduction in biomass. Of growth parameters, shoot and root lengths, fresh and dry weights decreased under drought and salt stress as expected. Drought, which is a non - phase specific stress in wheat, affects all growth stages (1, 39, 40) Salt stress produces similar symptoms in the plants under water deficit, drought conditions (41).

### *Total phenolic and flavonoid contents in 8 bread wheat cultivars and 8 einkorn populations*

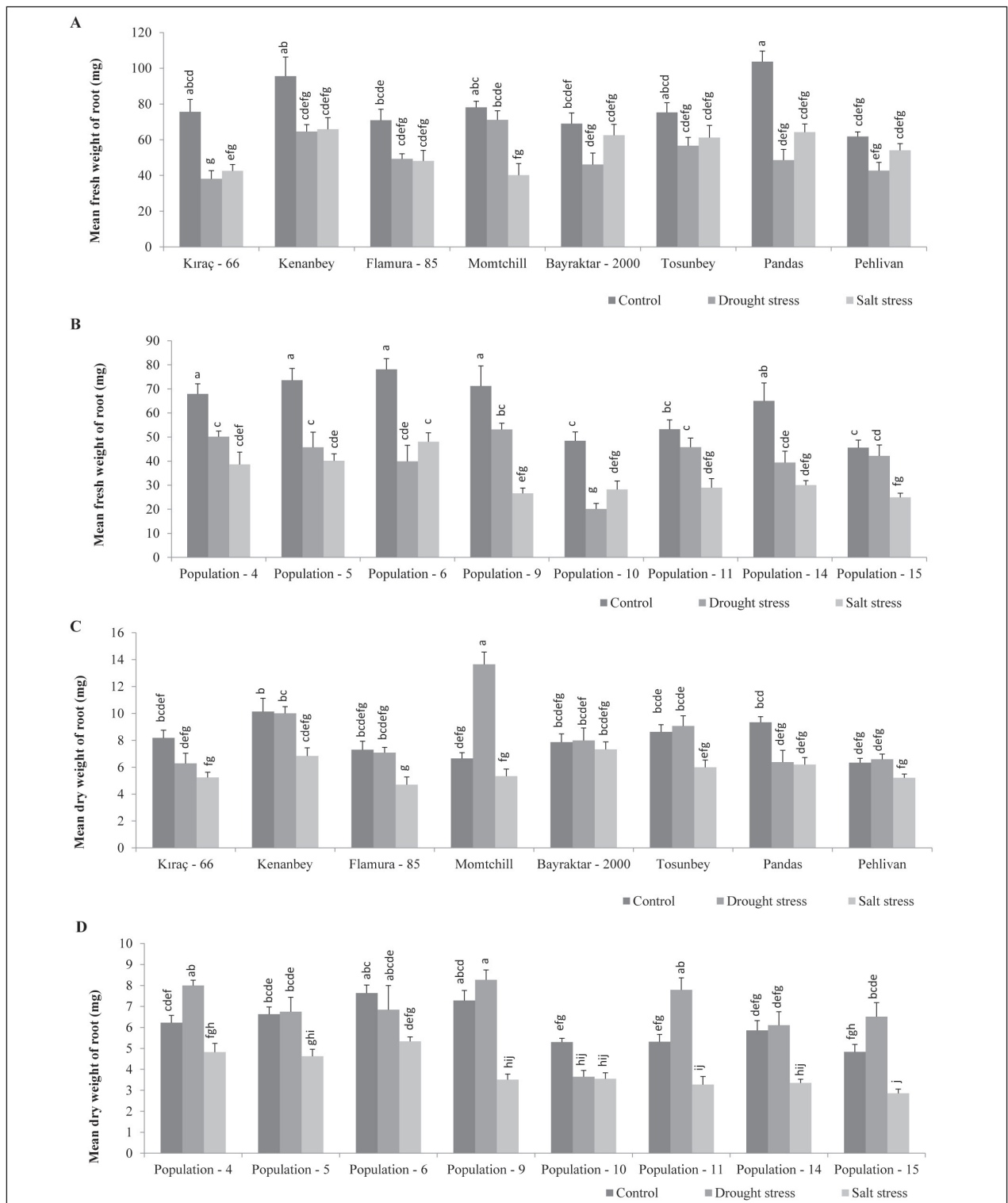
The total phenolic content (TPC) was expressed as GAE and TAE in mg/g dw, and total flavonoid content (TFC) was expressed as CE and QE in mg/g dw of plant material in 16 wheat entries (Table 2). TPC ranged from 13.69 to 53.92 mg/g GAE and 10.72 to 57.66 mg/g TAE in bread wheat cultivars (Table



**Figure 1.** Mean shoot and root length of germinated wheats under control, drought, and salt conditions. A: Mean shoot length of 8 bread wheat cultivars. B: Mean shoot length of 8 einkorn populations. C: Mean root length of 8 bread wheat cultivars. D: Mean root length of 8 einkorn populations. Outcomes were presented as means ± SD. Different superscript letters in a column indicate significant differences ( $P < 0.05$ ).



**Figure 2.** Mean fresh and dry weight of shoots under control, drought and salt conditions. A: Mean fresh weight of shoots of 8 bread wheat cultivars. B: Mean fresh weight of shoots of 8 einkorn populations. C: Mean dry weight of shoots of 8 bread wheat cultivars. D: Mean dry weight of shoots of 8 einkorn populations. Outcomes were presented as means  $\pm$  SD. Different superscript letters in a column indicate significant differences ( $P < 0.05$ ).



**Figure 3.** Mean fresh and dry weight of roots under control, drought and salt conditions. A: Mean fresh weight of roots of 8 bread wheat cultivars. B: Mean fresh weight of roots of 8 einkorn populations. A: Mean dry weight of roots of 8 bread wheat cultivars. B: Mean dry weight of roots of 8 einkorn populations. Outcomes were presented as means ± SD. Different superscript letters in a column indicate significant differences ( $P < 0.05$ ).

2). Kıraç - 66, a salt and drought resistance cultivar, exhibited the highest TPC with GAE (53.92 mg/g) and TAE (57.66 mg/g) under salt. Kenanbey (13.69 mg/g GAE; 10.72 mg/g TAE) had the lowest TPC under drought (Table 2). The total flavonoid content of 8 bread wheat cultivars significantly ranged from 1.66 to 87.66 mg/g CE and from 16.22 to 87.89 mg/g QE (Table 2). Pehlivan (87.66 mg/g CE; 87.89 mg/g QE), and Kıraç - 66 (83.00 mg/g CE; 84.00 mg/g QE) had the highest TFC (Table 2) under salt stress. Bayraktar - 2000 had the lowest TFC value (1.66 ± CE; 16.22 mg/g QE) under drought condition. The treatments including 50 mM NaCl increased TPC and TFC in

Kıraç - 66, Flamura - 85, Momtchill, and Pehlivan (Table 2). On the other hand, drought decreased TPC and TFC in Kenanbey, Bayraktar - 2000, and Pehlivan, (Table 2). The TPC of Bayraktar - 2000 and TFC of Momtchill were not affected by both stresses (Table 2). These outcomes showed that salt and drought were significantly induced phenolic, flavonoid, and antioxidant enzyme biosynthesis in wheat. The similar studies, which each plant material had needed special abiotic stresses for activation of antioxidant defence system such as polyphenol synthesis were reported in *Bellis perennis* (42, 43). The phenolic and flavonoid content composition of plants usually depends on ge-

**Table 2.** The total phenolic (TPC) and flavonoid contents (TFC), and DPPH free radical activity of 8 bread wheat cultivars (*Triticum aestivum* L.) under three different treatments.

Bread wheats	Treatments	TPC mg/g <sup>w</sup> dw	TPC mg/g <sup>x</sup> dw	TPC mg/g <sup>y</sup> dw	TPC mg/g <sup>z</sup> dw	IC <sub>50</sub> mg/L
Kıraç- 66	Control	<sup>b</sup> 33.09 ± 0.93 <sup>de</sup>	<sup>b</sup> 33.36 ± 1.08 <sup>de</sup>	<sup>b</sup> 29.00 ± 6.92 <sup>efg</sup>	<sup>b</sup> 38.99 ± 5.77 <sup>efg</sup>	15.40 ± 0.01 <sup>c</sup>
	Drought stress	<sup>b</sup> 40.71 ± 4.64 <sup>c</sup>	<sup>b</sup> 42.25 ± 5.42 <sup>c</sup>	<sup>b</sup> 29.66 ± 1.15 <sup>efg</sup>	<sup>b</sup> 39.55 ± 0.96 <sup>efg</sup>	12.04 ± 0.12 <sup>b</sup>
	Salt stress	<sup>a</sup> 53.92 ± 4.72 <sup>a</sup>	<sup>a</sup> 57.66 ± 5.51 <sup>a</sup>	<sup>a</sup> 83.00 ± 4.0 <sup>a</sup>	<sup>a</sup> 84.00 ± 3.33 <sup>a</sup>	8.36 ± 0.05 <sup>a</sup>
Kenanbey	Control	<sup>a</sup> 23.33 ± 1.14 <sup>s</sup>	<sup>a</sup> 21.97 ± 1.33 <sup>s</sup>	<sup>a</sup> 23.00 ± 4.00 <sup>gh</sup>	<sup>a</sup> 34.00 ± 3.33 <sup>gh</sup>	80.85 ± 0.92 <sup>o</sup>
	Drought stress	<sup>b</sup> 13.69 ± 0.89 <sup>j</sup>	<sup>b</sup> 10.72 ± 1.04 <sup>j</sup>	<sup>b</sup> 7.66 ± 6.42 <sup>jk</sup>	<sup>b</sup> 21.22 ± 5.35 <sup>jk</sup>	67.56 ± 0.88 <sup>n</sup>
	Salt stress	<sup>a</sup> 23.21 ± 0.94 <sup>g</sup>	<sup>a</sup> 21.83 ± 1.09 <sup>g</sup>	<sup>a</sup> 26.33 ± 4.16 <sup>fg</sup>	<sup>a</sup> 36.78 ± 3.47 <sup>fg</sup>	26.45 ± 0.31 <sup>c</sup>
Flamura- 85	Control	<sup>b</sup> 27.85 ± 0.35 <sup>f</sup>	<sup>b</sup> 27.25 ± 0.42 <sup>f</sup>	<sup>b</sup> 17.66 ± 5.03 <sup>hi</sup>	<sup>b</sup> 29.55 ± 4.19 <sup>hi</sup>	17.86 ± 0.26 <sup>d</sup>
	Drought stress	<sup>a</sup> 23.69 ± 0.74 <sup>s</sup>	<sup>a</sup> 22.39 ± 0.86 <sup>s</sup>	<sup>a</sup> 33.0 ± 2.0 <sup>ef</sup>	<sup>a</sup> 42.33 ± 1.66 <sup>ef</sup>	26.31 ± 0.30 <sup>c</sup>
	Salt stress	<sup>a</sup> 33.33 ± 0.20 <sup>de</sup>	<sup>a</sup> 33.64 ± 0.24 <sup>de</sup>	<sup>b</sup> 11.66 ± 6.11 <sup>ij</sup>	<sup>b</sup> 24.55 ± 5.09 <sup>ij</sup>	16.14 ± 0.14 <sup>c</sup>
Momtchill	Control	<sup>c</sup> 35.83 ± 2.58 <sup>d</sup>	<sup>c</sup> 36.55 ± 3.01 <sup>d</sup>	<sup>a</sup> 69.66 ± 8.32 <sup>b</sup>	<sup>a</sup> 72.89 ± 6.94 <sup>b</sup>	48.52 ± 0.75 <sup>kl</sup>
	Drought stress	<sup>b</sup> 40.24 ± 1.25 <sup>c</sup>	<sup>b</sup> 41.69 ± 1.45 <sup>c</sup>	<sup>a</sup> 60.33 ± 3.05 <sup>c</sup>	<sup>a</sup> 65.11 ± 2.54 <sup>c</sup>	48.81 ± 0.73 <sup>kl</sup>
	Salt stress	<sup>a</sup> 44.16 ± 1.48 <sup>b</sup>	<sup>a</sup> 46.27 ± 1.73 <sup>b</sup>	<sup>a</sup> 62.33 ± 7.57 <sup>bc</sup>	<sup>a</sup> 66.77 ± 6.31 <sup>bc</sup>	46.00 ± 0.71 <sup>ji</sup>
Bayraktar - 2000	Control	<sup>a</sup> 16.54 ± 1.76 <sup>ij</sup>	<sup>a</sup> 14.05 ± 2.05 <sup>ij</sup>	<sup>b</sup> 9.66 ± 3.05 <sup>j</sup>	<sup>b</sup> 22.89 ± 2.54 <sup>j</sup>	46.08 ± 0.18 <sup>ji</sup>
	Drought stress	<sup>a</sup> 15.83 ± 1.14 <sup>ij</sup>	<sup>a</sup> 13.22 ± 1.33 <sup>ij</sup>	<sup>c</sup> 1.66 ± 1.15 <sup>k</sup>	<sup>c</sup> 16.22 ± 0.95 <sup>k</sup>	49.22 ± 0.20 <sup>l</sup>
	Salt stress	<sup>a</sup> 16.07 ± 1.0 <sup>ij</sup>	<sup>a</sup> 13.50 ± 1.25 <sup>ij</sup>	<sup>a</sup> 33.66 ± 3.05 <sup>ef</sup>	<sup>a</sup> 42.89 ± 2.54 <sup>ef</sup>	44.66 ± 0.1 <sup>i</sup>
Tosunbey	Control	<sup>c</sup> 18.69 ± 0.74 <sup>hi</sup>	<sup>c</sup> 16.55 ± 0.86 <sup>hi</sup>	<sup>b</sup> 34.55 ± 5.59 <sup>ef</sup>	<sup>b</sup> 43.63 ± 4.65 <sup>ef</sup>	64.25 ± 0.52 <sup>m</sup>
	Drought stress	<sup>a</sup> 30.47 ± 0.82 <sup>ef</sup>	<sup>a</sup> 30.30 ± 0.96 <sup>ef</sup>	<sup>b</sup> 32.33 ± 4.16 <sup>ef</sup>	<sup>b</sup> 41.78 ± 3.47 <sup>ef</sup>	41.93 ± 0.36 <sup>h</sup>
	Salt stress	<sup>b</sup> 21.07 ± 0.94 <sup>gh</sup>	<sup>b</sup> 19.33 ± 1.09 <sup>gh</sup>	<sup>a</sup> 43.66 ± 3.05 <sup>d</sup>	<sup>a</sup> 51.22 ± 2.54 <sup>d</sup>	41.16 ± 0.27 <sup>gh</sup>
Pandas	Control	<sup>a</sup> 28.97 ± 1.01	<sup>a</sup> 28.54 ± 1.19 <sup>f</sup>	<sup>b</sup> 46.77 ± 3.67 <sup>d</sup>	<sup>b</sup> 53.81 ± 3.05 <sup>d</sup>	40.05 ± 0.30 <sup>g</sup>
	Drought stress	<sup>b</sup> 19.28 ± 2.23 <sup>hi</sup>	<sup>b</sup> 17.25 ± 2.60 <sup>hi</sup>	<sup>b</sup> 48.77 ± 3.67 <sup>d</sup>	<sup>b</sup> 55.48 ± 3.06 <sup>d</sup>	48.92 ± 0.08 <sup>l</sup>
	Salt stress	<sup>a</sup> 28.25 ± 3.35 <sup>f</sup>	<sup>a</sup> 27.71 ± 3.91 <sup>f</sup>	<sup>a</sup> 63.00 ± 1.33 <sup>bc</sup>	<sup>a</sup> 67.33 ± 1.11 <sup>bc</sup>	25.03 ± 0.11 <sup>c</sup>
Pehlivan	Control	<sup>b</sup> 19.16 ± 2.42 <sup>hi</sup>	<sup>b</sup> 17.11 ± 2.83 <sup>hi</sup>	<sup>b</sup> 56.33 ± 3.05 <sup>c</sup>	<sup>b</sup> 61.77 ± 2.54 <sup>c</sup>	47.05 ± 0.54 <sup>jk</sup>
	Drought stress	<sup>b</sup> 18.45 ± 1.15 <sup>hi</sup>	<sup>b</sup> 16.27 ± 1.33 <sup>hi</sup>	<sup>c</sup> 35.66 ± 5.03 <sup>c</sup>	<sup>c</sup> 44.55 ± 4.19 <sup>c</sup>	33.68 ± 0.17 <sup>f</sup>
	Salt stress	<sup>a</sup> 24.52 ± 1.96 <sup>g</sup>	<sup>a</sup> 23.36 ± 2.29 <sup>g</sup>	<sup>a</sup> 87.66 ± 3.05 <sup>a</sup>	<sup>a</sup> 87.89 ± 2.54 <sup>a</sup>	44.53 ± 4.61 <sup>i</sup>
	Ascorbic acid	-	-	-	-	13.30 ± 0.20 <sup>bd</sup>

Values are mean (n=3) ± SD. Mean values followed by different superscript letters in a column were significantly different (P < 0.05). Superscripts on the right showed the differences among the all groups of bread wheats. Superscripts on the left showed the statistically significant differences within the same cultivar of bread wheat (control, drought, and salt condition). <sup>w</sup> Gallic acid equivalents (GAE). <sup>x</sup> Tannic acid equivalents (TAE). <sup>y</sup> Catechol equivalents (CE). <sup>z</sup> Quercetin equivalents (QE).



netic structure, origin, and abiotic and biotic environmental factors (17).

The methanol extracts from 8 einkorn populations significantly differed for TPC and TFC (Table 3). They ranged from 9.04 to 41.78 mg/g GAE; from 5.30 to 43.50 mg/g TAE; from 1.66 to 77.00 mg/g CE; and from 16.22 to 79.00 mg/g QE, in einkorn populations, respectively (Table 3). Pop - 10 contained the highest TPC (41.78 mg/g GAE; 43.50 mg/g TAE) under salt stress while the highest TFC existed in Pop - 11 (77.00 mg/g CE; 79.00 mg/g QE). The lowest TPC was in Pop - 6 (9.04 mg/g GAE; 5.30 mg/g TAE) under drought. The lowest TFC was also

in Pop - 6 (1.66 mg/g CE; 16.22 mg/g QE) and Pop - 10 (1.66 mg/g CE; 16.77 mg/g QE) under control. Our results showed that 50 mM NaCl application on wheat seedlings generally increased the TPC values except Pop - 4, Pop - 5, and Pop - 9 (Table 3) and TFC values except Pop - 4, Pop - 5, and Pop - 14 (Table 3). A great number of research exhibited that some medicinal plants exposed to drought stress accumulate higher concentrations of secondary metabolites than those cultivated under well - watered applications (38). On the contrary, drought stress (-0.5 MPa.) decreased overall TPC values in all einkorn populations except Pop - 14 (Table 3). Otherwise, we compared TFC val-

**Table 3.** The total phenolic (TPC) and flavonoid contents (TFC), and DPPH free radical activity of 8 hulled einkorn populations (*Triticum monococcum* ssp. *monococcum*) under three different treatments.

Einkorns	Treatments	TPC mg/g <sup>w</sup> dw	TPC mg/g <sup>x</sup> dw	TPC mg/g <sup>y</sup> dw	TPC mg/g <sup>z</sup> dw	IC <sub>50</sub> mg/L
Pop- 4	Control	<sup>a</sup> 32.50 ± 1.07 <sup>d</sup>	<sup>a</sup> 32.67 ± 1.25 <sup>d</sup>	<sup>a</sup> 21.66 ± 5.03 <sup>c</sup>	<sup>a</sup> 32.88 ± 4.19 <sup>c</sup>	31.32 ± 0.25 <sup>cf</sup>
	Drought stress	<sup>c</sup> 17.74 ± 1.25 <sup>h</sup>	<sup>c</sup> 15.44 ± 1.45 <sup>h</sup>	<sup>b</sup> 11.66 ± 6.1 <sup>f</sup>	<sup>b</sup> 24.55 ± 5.09 <sup>f</sup>	61.07 ± 0.58 <sup>a</sup>
	Salt stress	<sup>b</sup> 21.90 ± 1.25 <sup>f</sup>	<sup>b</sup> 20.30 ± 1.45 <sup>f</sup>	<sup>b</sup> 3.0 ± 2.0 <sup>gh</sup>	<sup>b</sup> 17.33 ± 1.66 <sup>gh</sup>	87.80 ± 0.265
Pop- 5	Control	<sup>a</sup> 23.21 ± 1.88 <sup>f</sup>	<sup>a</sup> 21.83 ± 2.20 <sup>f</sup>	<sup>a</sup> 37.66 ± 6.11 <sup>bcd</sup>	<sup>a</sup> 46.22 ± 5.09 <sup>bcd</sup>	42.79 ± 0.05 <sup>i</sup>
	Drought stress	<sup>b</sup> 14.40 ± 2.68 <sup>j</sup>	<sup>b</sup> 11.55 ± 3.13 <sup>j</sup>	<sup>a</sup> 42.11 ± 1.54 <sup>b</sup>	<sup>a</sup> 49.92 ± 1.28 <sup>b</sup>	65.33 ± 0.44 <sup>p</sup>
	Salt stress	<sup>a</sup> 23.17 ± 0.99 <sup>f</sup>	<sup>a</sup> 21.78 ± 1.15 <sup>f</sup>	<sup>a</sup> 37.22 ± 2.34 <sup>bcd</sup>	<sup>a</sup> 45.85 ± 1.95 <sup>bcd</sup>	55.17 ± 0.22 <sup>m</sup>
Pop- 6	Control	<sup>b</sup> 15.47 ± 0.54 <sup>i</sup>	<sup>b</sup> 12.80 ± 0.63 <sup>i</sup>	<sup>b</sup> 1.66 ± 1.15 <sup>h</sup>	<sup>b</sup> 16.22 ± 0.95 <sup>h</sup>	63.45 ± 1.05 <sup>o</sup>
	Drought stress	<sup>c</sup> 9.04 ± 1.15 <sup>l</sup>	<sup>c</sup> 5.30 ± 1.33 <sup>l</sup>	<sup>b</sup> 3.00 ± 2.00 <sup>gh</sup>	<sup>b</sup> 17.33 ± 1.66 <sup>gh</sup>	45.12 ± 0.15 <sup>j</sup>
	Salt stress	<sup>a</sup> 23.33 ± 1.14 <sup>f</sup>	<sup>a</sup> 21.97 ± 1.33 <sup>f</sup>	<sup>a</sup> 40.33 ± 2.30 <sup>bcd</sup>	<sup>a</sup> 48.44 ± 1.92 <sup>bcd</sup>	32.36 ± 0.3 <sup>l</sup>
Pop- 9	Control	<sup>a</sup> 28.45 ± 1.09 <sup>e</sup>	<sup>a</sup> 27.94 ± 1.27 <sup>e</sup>	<sup>b</sup> 11.00 ± 4.00 <sup>fg</sup>	<sup>b</sup> 24.00 ± 3.33 <sup>fg</sup>	23.82 ± 0.46 <sup>c</sup>
	Drought stress	<sup>b</sup> 19.64 ± 1.28 <sup>g</sup>	<sup>b</sup> 17.66 ± 1.49 <sup>g</sup>	<sup>c</sup> 3.66 ± 1.15 <sup>gh</sup>	<sup>c</sup> 17.88 ± 0.96 <sup>gh</sup>	29.74 ± 3.97 <sup>de</sup>
	Salt stress	<sup>c</sup> 5.95 ± 0.90 <sup>m</sup>	<sup>c</sup> 1.69 ± 1.05 <sup>m</sup>	<sup>a</sup> 33.00 ± 4.0 <sup>cd</sup>	<sup>a</sup> 42.33 ± 3.33 <sup>cd</sup>	10.65 ± 0.44 <sup>a</sup>
Pop- 10	Control	<sup>b</sup> 37.26 ± 0.54 <sup>b</sup>	<sup>b</sup> 38.22 ± 0.63 <sup>b</sup>	<sup>b</sup> 1.66 ± 1.15 <sup>h</sup>	<sup>b</sup> 16.77 ± 0.95 <sup>h</sup>	16.03 ± 0.11 <sup>b</sup>
	Drought stress	<sup>c</sup> 34.52 ± 0.90 <sup>c</sup>	<sup>c</sup> 35.03 ± 1.04 <sup>c</sup>	<sup>a</sup> 29.66 ± 4.16 <sup>d</sup>	<sup>a</sup> 39.55 ± 3.46 <sup>d</sup>	28.86 ± 0.33 <sup>d</sup>
	Salt stress	<sup>a</sup> 41.78 ± 0.94 <sup>a</sup>	<sup>a</sup> 43.50 ± 1.10 <sup>a</sup>	<sup>a</sup> 32.33 ± 4.16 <sup>cd</sup>	<sup>a</sup> 41.78 ± 3.47 <sup>cd</sup>	15.10 ± 0.10 <sup>b</sup>
Pop- 11	Control	<sup>b</sup> 16.43 ± 0.36 <sup>hi</sup>	<sup>b</sup> 13.91 ± 0.41 <sup>hi</sup>	<sup>c</sup> 6.33 ± 6.11 <sup>gh</sup>	<sup>c</sup> 20.11 ± 5.09 <sup>gh</sup>	47.40 ± 0.16 <sup>k</sup>
	Drought stress	<sup>c</sup> 11.43 ± 0.94 <sup>k</sup>	<sup>c</sup> 8.08 ± 1.10 <sup>k</sup>	<sup>b</sup> 36.33 ± 5.03 <sup>bcd</sup>	<sup>b</sup> 45.11 ± 4.19 <sup>bcd</sup>	48.98 ± 0.40 <sup>k</sup>
	Salt stress	<sup>a</sup> 22.26 ± 0.54 <sup>f</sup>	<sup>a</sup> 20.72 ± 0.63 <sup>f</sup>	<sup>a</sup> 77.00 ± 10.00 <sup>a</sup>	<sup>a</sup> 79.00 ± 8.33 <sup>a</sup>	40.86 ± 0.29 <sup>h</sup>
Pop- 14	Control	<sup>c</sup> 16.90 ± 0.74 <sup>hi</sup>	<sup>c</sup> 14.47 ± 0.86 <sup>hi</sup>	<sup>a</sup> 34.33 ± 2.30 <sup>bcd</sup>	<sup>a</sup> 43.44 ± 1.92 <sup>bcd</sup>	70.58 ± 0.46 <sup>r</sup>
	Drought stress	<sup>b</sup> 21.78 ± 0.71 <sup>f</sup>	<sup>b</sup> 20.16 ± 0.83 <sup>f</sup>	<sup>a</sup> 35.66 ± 6.11 <sup>bcd</sup>	<sup>a</sup> 44.55 ± 5.09 <sup>bcd</sup>	71.30 ± 1.61 <sup>r</sup>
	Salt stress	<sup>a</sup> 27.97 ± 0.74 <sup>c</sup>	<sup>a</sup> 27.39 ± 0.86 <sup>c</sup>	<sup>a</sup> 34.33 ± 2.30 <sup>bcd</sup>	<sup>a</sup> 43.44 ± 4.19 <sup>bcd</sup>	50.75 ± 0.131
Pop- 15	Control	<sup>b</sup> 27.02 ± 0.90 <sup>e</sup>	<sup>b</sup> 26.28 ± 1.04 <sup>e</sup>	<sup>b</sup> 6.33 ± 4.16 <sup>gh</sup>	<sup>b</sup> 20.11 ± 3.4 <sup>gh</sup>	37.37 ± 0.16 <sup>g</sup>
	Drought stress	<sup>c</sup> 22.86 ± 1.07 <sup>f</sup>	<sup>c</sup> 21.42 ± 1.25 <sup>f</sup>	<sup>a</sup> 33.66 ± 4.61 <sup>bcd</sup>	<sup>a</sup> 42.89 ± 3.84 <sup>bcd</sup>	68.70 ± 0.86 <sup>q</sup>
	Salt stress	<sup>a</sup> 37.26 ± 0.89 <sup>b</sup>	<sup>a</sup> 38.22 ± 1.04 <sup>b</sup>	<sup>a</sup> 37.00 ± 2.0 <sup>bcd</sup>	<sup>a</sup> 45.66 ± 1.66 <sup>bcd</sup>	11.36 ± 0.48 <sup>a</sup>
	Ascorbic acid	-	-	-	-	13.3 ± 0.20 <sup>ab</sup>

Results are presented as mean (n=3) ± SD. Mean values followed by different superscript letters in a column were significantly different (P < 0.05). Superscripts on the right showed differences among the all groups of einkorn populations. Superscript letters on the left showed the statistically significant differences within the same einkorn population (control, drought and salt). <sup>w</sup> Gallic acid equivalents (GAE). <sup>x</sup> Tannic acid equivalents (TAE). <sup>y</sup> Catechol equivalents (CE). <sup>z</sup> Quercetin equivalents (QE).

ues between control and drought treatments, TFC was higher in Pop – 5, Pop – 6, Pop – 10, Pop – 11, and Pop – 15 under drought than control.

#### *DPPH free radical scavenging activity of the methanol extracts of wheats*

The methanol extracts from 8 bread and 8 einkorn wheat entries exhibited a significantly reduced power variation (Table 2, 3). The IC<sub>50</sub> values of DPPH free radical scavenging potential ranged from 8.36 to 87.80 mg/L. In bread wheats, the methanol extract obtained from Kıraç – 66, with an IC<sub>50</sub> value of 8.36 ± 0.05 mg/L, was the highest DPPH free radical scavenging activity under salt stress (Table 2). The lowest DPPH free radical scavenging activity (80.85 mg/L) was detected in Kenanbey under control (Table 2). Here, Kıraç – 66 also had the highest TPC, TFC, and DPPH free radical scavenging activity. The higher antioxidant potential of plant extracts on DPPH might have resulted from their phenolic and flavonoid owed hydrogen-donating capability (44). Kıraç – 66 had the highest activity among other bread and einkorn wheat entries. This might be because of higher tolerance against salt and drought stress of Kıraç – 66 in genetic background. Kıraç – 66, gathered drought and salt tolerance from its ancestors (45).

The methanol extract obtained from Pop – 9 and Pop – 15 had the highest DPPH free radical scavenging activity with the lowest IC<sub>50</sub> values of 10.65 mg/L and 11.36 mg/L among einkorn populations under salt stress. They showed the better antioxidant activity than positive control of ascorbic acid (Table 3). According to DPPH outcomes, Pop – 4 had the lowest DPPH free radical scavenging activity with the highest IC<sub>50</sub> value (87.80 mg/L) under salt stress (Table 3). There was no significant relationship among TPC, TFC, and antioxidant activity of the methanol extracts of einkorn populations. Babbar et al. (46) similarly demonstrated that phenolic molecules not only were completely responsible for the antioxidant activity of plants but also for other secondary metabolites such as tocopherols, terpenes, alkaloids, ascorbates, carotenoids, and pigments as well as the synergistic, which could presumably promote the total antioxidant activity (47). Other non – detected secondary metabolites in einkorn populations may be the reason for antioxidant activity.

## Conclusion

The results of the present study supported the hypothesis that growth parameters (shoot length, root length, fresh shoot weight, fresh root weight, dry shoot weight, and dry root weight), total phenolic content, total flavonoid content, and antioxidant activity in 10 day – old-seedlings of 8 bread wheat cultivars and 8 hulled einkorn wheat populations were significantly affected by salinity (50 mM NaCl) and drought (-0.5 MPa. osmotic pressure). Momtchill and Pop – 9 were more tolerant to drought and salt during early growth stages. Cultivar “Kıraç – 66” and “Pehlivan” and populations “Pop – 10” and “Pop – 11” proved to produce more secondary metabolite under salt stress. Thus, they may protect their tissue composition from salt toxicity and tolerate against the salinity. Kıraç – 66, Pop – 9 and Pop – 15 contained the strongest free radical scavenging antioxidant activity under salt stress. Salt stress may induce the activation of antioxidant defense system in these wheats. Stress factors, while on one hand decrease yield, on the other hand provide opportunities for the production of healthier secondary metabolites. Therefore, further studies on these subjects needed to be carried out.

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