

# Evaluation of pigment, antioxidant capacity and bioactive compounds in microgreens of wheat landraces and cereals

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## ABSTRACT

Landraces that adapt to all kinds of stress factors over thousands of years are considered a very important genetic resource. In this study, antioxidant activities, bioactive compounds, and pigment contents of microgreen in Kose and Kirik wheat landraces, which are used extensively in bread making especially in rural areas of Eastern Anatolia, and some cereals, were examined. In the study, chlorophyll (CHLdx), flavonol (FLV), anthocyanin (ANT), and N balance index (NBI) were measured in fresh material with a Dualex device. Also, total antioxidant capacity (TAC), total phenolic content (TPC), total flavonoid content (TFC), ascorbic acid (AAC) content and pigment values such as total chlorophyll (TCHL), chlorophyll a (CHLa), chlorophyll b (CHLb) and carotenoid content (CAR) were determined. It was observed that TAC capacity of wheat landraces was more than twice that of other cereals (except 'Alparslan') and their NBI, AAC, ANT and FLV contents were low. Kirik landrace had high TCHL, CHLa, CHLb and CAR content, while Kose landrace had low all pigment values. Besides, NBI content of barley, AAC and FLV content of oat cultivars and ANT content of wheat 'Alparslan' were high as a group. The CHLdx values of barley and oats were twice that of wheat. A very important and positive correlation was determined between TCHL with CHLa, CHLb, and CAR contents. Also, correlations between CHLa with CHLb and CAR, and between CHLb with CAR was important and positive. In the study, especially TAC capacities of local wheat varieties were significantly higher.

**Key words:** Antioxidant capacity, cereals, microgreens, secondary metabolites, wheat landraces.

## INTRODUCTION

Microgreen is a food source that has recently been used in raw nutrition. Unlike sprouts, it is germinated in bright conditions having more variety of aroma and leaf color. Microgreens are harvested after the emergence of the cotyledon and their baby leaves are older than the first shoots are younger from the sprouts (Xiao et al., 2012).

Microgreens are generally consumed in salads, sauces, sandwiches, soups, and mixed with drinks. It offers a rich food variety to vegans and vegetarians (Renna, 2015). Not every herb can be considered as microgreen as some cause toxicity for those have allergy (Aksoy, 2017). The richness of microgreen with minerals might be a source to overcome mineral deficiency, which has become a global problem in human nutrition (Islam et al., 2020). Some studies had shown that microgreens contain more carotenoids, vitamins (C, K, and E), mineral substances (Ca, Zn, Fe, Mg, Mo, Mn, and Se), ascorbic acid and less nitrate than grains and plant (Xiao et al., 2012).

Furhermore, it has been determined that microgreens are good antioxidant with health-beneficial compounds (Xiao et al., 2012). Even though the daily consumption of microgreens varies according to the species, they are harvested within 7-21 d, when true leaves are formed and the plant is 5-10 cm height (Reed et al., 2018).

Cereals are produced in most of the climate zones worldwide, they have rich content in terms of the antioxidant and bioactive compounds. It contains more beneficial antioxidant, flavonoids, phenolics, vitamin C, and anthocyanins, especially than wheat grains (Islam et al., 2020).

Landraces are very valuable breeding materials that have been used in agricultural production since the beginning of the agriculture. They are characterized with resistant to biotic and abiotic stress, and adapted to most ecological conditions (Ozberk et al., 2016). Anatolia is the gene center of the landraces with a wide genetic center.

In this study, microgreens of wheat landraces and other cereal crops were examined in terms of pigment, antioxidant and bioactive compounds. Kirik and Kose wheat landraces that are intensively grown in the countryside of Eastern Anatolia (Ozberk et al., 2016). In addition to these, cool climate cereals such as wheat, barley and oat, which are most grown in Turkey and preferred in similar studies, were used in the study. In the study, chlorophyll, flavonol, anthocyanin and N balance indexes of this material were measured with the Dualex device. In addition, the total antioxidant capacity, total phenolic, total flavonoid, ascorbic acid contents and total chlorophyll, chlorophyll a, chlorophyll b and carotenoid contents of this material were determined.

## MATERIALS AND METHODS

This research was carried out in in 2021, Van Yuzuncu Yil University, Faculty of Agriculture, Field Crops Department Research Laboratory, in Van, Turkey. The location where the research was conducted is in Eastern Anatolia Region. In this region, Kose and Kirik wheat landraces are heavily preferred for tandoori bread. Alparslan is a cultivar registered by the Eastern Anatolian Research Institute located in this region. This cultivar is a preferred variety for cultivation in the harsh climatic conditions of the region. For these reasons, these three wheat genotypes were used in the study. Barley and oats used in the research are the cool climate cereals most grown in Turkey after wheat and preferred in such studies.

### Plant materials

The supplying place and variety information of the material used in the research are given in Table 1. The genotypes in the study were Kose (wheat landrace, *Triticum aestivum* L.) obtained from the farmers of Bitlis city region, and Kirik (wheat landrace) and Alparslan (wheat cultivar, *T. aestivum* L. emend. Fiori et Paol.) from the Eastern Anatolia Agricultural Research Institute. ‘Beysehir’ barley (*Hordeum vulgare* L.) and ‘Seydisehir’ oat (*Avena sativa* L.) were obtained from Bahri Dagdas Agricultural Research Institute, and ‘Sari’ and ‘Fetih’ oats were obtained from the Aegean Agricultural Research Institute.

The research is mostly focused on landraces. Thus, a group of landraces were formed and tables were created to see their comparisons with other cereal crops. In addition, analysis tables have been created so that the status of the examined features based on variety can also be seen.

### Experimental design, sampling, and measurements

Trial design was implemented according to Niroula et al. (2019a), Vastakaite et al. (2015) and Samuoliene et al. (2017) procedures. Seeds were planted by light pressing to cover 2/3 of the 500 cm<sup>3</sup> plastic box, in a mixture sterile peat, cockpit (coconut shell) and perlite obtained from a certified commercial company The seeds were covered with the same mixture in 2 cm thickness and lightly pressed. The prepared material was placed in a fully controlled climate cabinet at 21 ± 2 °C/17 ± 2 °C in accordance with the references 50%-60% humidity and 16/8 h light/dark period. Plants

**Table 1. The supply and group distributions of the herbal material used in the research.**

Groups	Genotypes (varieties)	Species	Supplying institution/Place
Wheat landraces	Kose	<i>Triticum aestivum</i> L.	Farmers of Bitlis City in the Eastern Anatolia
	Kirik	<i>T. aestivum</i>	Eastern Anatolia Agricultural Research Institute
Wheat	Alparslan	<i>T. aestivum</i> L. emend. Fiori et Paol.	Eastern Anatolia Agricultural Research Institute
Barley	Beysehir	<i>Hordeum vulgare</i> L.	Bahri Dagdas Agricultural Research Institute
Oats	Seydisehir	<i>Avena sativa</i> L.	Bahri Dagdas Agricultural Research Institute
	Sari	<i>A. sativa</i>	Aegean Agricultural Research Institute
	Fetih	<i>A. sativa</i>	Aegean Agricultural Research Institute

were daily sprayed with an adequate amount of pure water. Harvesting for microgreen was made on the 7<sup>th</sup> day for wheat, 9<sup>th</sup> day for barley, the 8<sup>th</sup> for 'Seydischir' and 'Sari' and 13<sup>th</sup> days for oats microgreens. For the sampling process, the grown microgreens were cut with sterile scissors at soil surface after taking necessary measurements on leaves with a chlorophyll sensor device (Dualex, Force-A, Paris, France).

Chlorophyll (CHLdx), total chlorophyll (TCHL), chlorophyll a (CHLa), chlorophyll b (CHLb), carotenoid (CAR), N balance index, anthocyanin and flavonol values were measured in fresh weight (FW). Chlorophyll, N balance index, anthocyanin and flavonol values were measured on the fresh leaves of microgreens with the Dualex device. Total chlorophyll, CHLa, CHLb and CAR contents were also determined in fresh weight (FW) according to the method determined by Lichtenthaler and Wellburn (1983). Total phenolic, flavonoid, ascorbic acid and antioxidant content of microgreens were measured in DM in accordance with the methods used in similar studies (Djordjevic et al., 2011; Niroula et al., 2019b).

### **Measurements by Dualex device**

Dualex device (Force-A, <https://www.force-a.com/products/dualex>) is a floodable sensor that measures flavonol, anthocyanin and chlorophyll index. The parameters are measured on the leaf in a short time and non-destructively. Geolocalization is done with the GPS built into the sensor. The device data logger provides a large data memory for data acquisition and storage. Total chlorophyll ( $\mu\text{g cm}^{-2}$ ), flavonol (dualex index), anthocyanin (dualex index) and N balance index (dualex index) were measured in the fresh material (Cerovic et al., 2012; Vastakaite et al., 2015; Hosseini et al., 2019; Matysiak and Kowalski, 2019).

### **Determination of total antioxidant capacity**

Leaves samples (0.2 g) from microgreens were passed through an homogenizer by adding 5 mL methanol, then centrifuged at 12000 rpm for 15 min, and supernatant part removed. Later, ferric reducing antioxidant power (FRAP) reagent was prepared after 300 mM acetate buffer (pH 3.6), 10 mM L<sup>-1</sup> 2,4,6-tripyridyl-s-triazine (TPTZ) prepared by dissolving in 40 mM HCl and 20 mM L<sup>-1</sup> FeCl<sub>3</sub>·6H<sub>2</sub>O solutions by mixing at a ratio 10:1:1, respectively. The mixture prepared for analysis with 2850  $\mu\text{L}$  FRAP reagent was diluted 20 times with methanol, then 150  $\mu\text{L}$  sample was mixed and kept at room temperature for 1 h. The resulting ferrus tripyridyltriazine complex was measured at 593 nm in the spectrophotometer and the results were reported as mg Trolox equivalents (TE) g<sup>-1</sup> DM (Lutz et al., 2011). Trolox concentration range has been studied as 0-500 mg L<sup>-1</sup>.

### **Determination of total phenolic content**

The method developed by modifying the Folin-Ciocalteu spectrophotometric method specified by Obanda et al. (1997) was used to determine the total phenolic compound content. The Folin-Ciocalteu solution was diluted 1:10. Saturated sodium carbonate (20%) solution; 20 g sodium carbonate was dissolved in distilled water, completed to 100 mL, and then filtered after being left overnight. Gallic acid stock solution (500  $\mu\text{g mL}^{-1}$ ) was prepared by dissolving 50 mg gallic acid in 100 mL pure water. Gallic acid working solution; every 500  $\mu\text{g mL}^{-1}$  stock solution was prepared in 5 mL measuring flasks, as nine separate solutions with a concentration varying between 0 and 55  $\mu\text{g mL}^{-1}$ . A sample (0.2 g) was taken from the microgreens and 5 mL methanol was added, and then it was passed through a homogenizer. After centrifuging at 12000 rpm for 15 min, the supernatant remaining on top was taken; 150  $\mu\text{L}$  extract was taken and 2400  $\mu\text{L}$  distilled water was added first, then, 150  $\mu\text{L}$  Folin-Ciocalteu solution was added. After shaking this solution with vortex for 35-40 s, it was waited for 5 min. Then, 300  $\mu\text{L}$  20% sodium carbonate was added and left at room temperature for 60 min, the absorbance value of the blue color formed was read in the spectrometer at 725 nm (Zin et al., 2006). The calibration curve was obtained by plotting the absorbance values read against these different concentrations of gallic acid and calculated as mg gallic acid equivalent (GAE) 100 g<sup>-1</sup> DM.

### **Determination of total flavonoid content**

Total flavonoid content was determined according to the method developed by Quettier-Deleu et al. (2000); 2 mL 2% AlCl<sub>3</sub> were added onto 2 mL extract and were kept at room temperature and in the dark for 1 h. Total flavonoid content of the extracts was measured with a spectrophotometer at 415 nm wavelength by performing two parallel runs in each sample and calculated in mg quercetin equivalent (QE) 100 g<sup>-1</sup> DM by using the calibration curve prepared using standard quercetin.

### Determination of ascorbic acid content

Fresh microgreen samples (2 g) were homogenized (HG-15A homogenizer, Witeg Labortechnik GmbH, Wertheim, Germany) with 20 mL oxalic acid (0.4%) and kept at 5 °C in a circular shaking oven (ACMI 006) for 24 h. It was then centrifuged at 5000 rpm for 10 min. The supernatant was used for ascorbic acid determination spectrophotometrically at 520 nm by adding 400 µL 0.4% oxalic acid and 4.5 mL (30 ppm) 2,6-dichlorophenolindophenol solution onto 100 µL supernatant (AOAC, 1990). The amount of ascorbic acid in the samples was calculated in mg 100 g<sup>-1</sup> DM with the help of the calibration curve drawn with pure ascorbic acid.

### Pigment analysis

Determination of photosynthetic pigments was performed according to Lichtenthaler and Wellburn (1983), 0.2 g (200 mg) fresh microgreen sample was extracted with 10 mL 80% acetone and centrifuged at 4600 rpm for 15 min. The absorbance values of the aliquots were taken after centrifugation, then spectrophotometer (T60 UV-Vis, PG Instruments, Wibtsoft, UK) readings were recorded at 662, 652, 645 and 470 nm. Total chlorophyll (TCHL), CHLa, CHLb and CAR concentrations were calculated with Lichtenthaler and Wellburn (1983) formulas as below:

$$\text{TCHL } (\mu\text{g g}^{-1} \text{FW}) = (27.8 \times A_{652})$$

$$\text{CHLa } (\mu\text{g g}^{-1} \text{FW}) = (11.75 \times A_{662}) - (2.350 \times A_{645})$$

$$\text{CHLb } (\mu\text{g g}^{-1} \text{FW}) = (18.61 \times A_{645}) - (3.960 \times A_{662})$$

$$\text{CAR } (\mu\text{g g}^{-1} \text{FW}) = [(1000 \times A_{470}) - (2.270 \times \text{CHLa})] - (81.4 \times \text{CHLb}/227)$$

where FW is fresh weight, A662 is absorbance reading at 662 nm, A652 is absorbance reading at 652 nm, A645 is absorbance reading at 645 nm, A470 is absorbance reading at 470 nm.

### Statistical analysis

The data of the research were subjected to ANOVA according to the completely randomized experimental design. Statistical calculations were made using the program CoStat version 6.303 (CoHort Software, Birmingham, UK) according to three replicates. The differences between means were determined according to the least significant difference (LSD) comparison method.

A group table was created regarding the averages of data obtained in triplicate from the microgreens of the examined cereal species (wheat, barley and oats) on the basis of varieties (genotypes) and groups (species) (Table 1). Since three different cereals species were studied, it was preferred to subject the results to a group analysis on the basis of species together with the varieties in order to better see the results. Besides, since the study focuses more on the comparison of landraces (Kose and Kirik) with other species, the tables are necessarily divided into two parts as varieties and groups. The data of the analyses obtained in triplicate are given as decimal with their standard deviations. Thus, the status of landraces according to both varieties and wheat, barley and oat groups was clearly shown and interpreted. According to results, differences between averages obtained in all parameters examined were significant.

## RESULTS AND DISCUSSION

### Total antioxidant capacity and bioactive compounds in genotypes and groups

Total antioxidant capacity (TAC), phenolic (TPC), flavonoid (FLAV), N balance index (NBI), ascorbic acid (AAC), anthocyanin (ANT) and flavonol (FLV) contents of the two wheat landraces and some cereals are given as form of genotypes and groups in Table 2. Values are shown with their standard deviations (SD). Accordingly, differences between all the traits examined in terms of genotypes and groups were significant. The TAC contents of the examined genotypes vary between 2542.500 and 5386.250 mg TE g<sup>-1</sup> DM. The highest TAC was obtained from the Kose wheat landrace and the lowest value was obtained from 'Beysehır' barley. It was determined that Kirik landrace contains TAC close to the highest level (4689.375 mg TE g<sup>-1</sup> DM). According to the average of the studied material based on the groups, the highest TAC (5037.813 mg TE g<sup>-1</sup> DM) was obtained from the wheat landraces and the lowest value (3337.292 mg TE g<sup>-1</sup> DM) from oat cultivars (Table 2). When TAC and bioactive compounds were evaluated based on genotypes and groups, it was determined that wheat landraces (Kose and Kirik) and the standard 'Alparslan' wheat had a TAC capacity two times higher than the other cereals. The TAC varies according to the analysis method used (Lutz et al., 2011; Urias-Lugo et al., 2015; Chomchan et al., 2016; Islam et al., 2019).

**Table 2. Total antioxidant capacity (TAC) and bioactive compounds of wheat landraces and cereals.**

Genotypes	TAC	TPC	TFC	NBI
	mg TE g <sup>-1</sup> DM	mg GAE 100 g <sup>-1</sup> DM	mg QE 100 g <sup>-1</sup> DM	dualex index
Kose (landrace)	5386.250 ± 40.625a	549.000 ± 5.000d	398.758 ± 4.442c	71.383 ± 1.776b
Kirik (landrace)	4689.375 ± 37.500b	581.222 ± 13.889c	408.708 ± 11.439bc	47.467 ± 2.122d
Alparslan (cultivar)	4667.500 ± 21.875b	529.556 ± 0.000e	476.986 ± 6.989a	67.300 ± 2.851b
Beysehir barley	2542.500 ± 71.875f	465.111 ± 5.555g	302.066 ± 1.549e	100.767 ± 10.276a
Seydisehir oat	3358.125 ± 31.250d	601.778 ± 4.444b	258.893 ± 3.321f	42.500 ± 2.516d
Sari oat	2830.000 ± 34.375e	496.778 ± 5.000f	363.321 ± 4.354d	57.567 ± 1.855c
Fetih oat	3823.750 ± 40.625c	634.556 ± 25.000a	418.672 ± 6.642b	46.067 ± 4.990d
CV, %	0.399	1.523	1.576	7.826
LSD	18.162	9.779	6.892	5.639
Groups				
Wheat (landraces)	5037.813 ± 383.292A	565.111 ± 19.965A	403.733 ± 9.483B	59.425 ± 13.216BC
Wheat (cultivar)	4667.500 ± 21.875B	529.556 ± 0B	476.986 ± 6.989A	67.300 ± 2.851B
Barley	2542.500 ± 71.875D	465.111 ± 5.555C	302.066 ± 1.549D	100.767 ± 10.276A
Oat	3337.292 ± 431.694C	577.704 ± 63.660A	346.962 ± 70.397C	48.711 ± 7.427C
Genotypes	AAC	ANT	FLV	
	mg 100 g <sup>-1</sup> DM	dualex index	dualex index	
Kose (landrace)	18.082 ± 0.644d	0.033 ± 0.006c	0.237 ± 0.0153e	
Kirik (landrace)	16.986 ± 0.781e	0.060 ± 0.010ab	0.427 ± 0.0230c	
Alparslan (cultivar)	16.712 ± 0.246e	0.073 ± 0.006ab	0.293 ± 0.0153d	
Beysehir barley	16.986 ± 0.233e	0.050 ± 0.000b	0.300 ± 0.0100d	
Seydisehir oat	20.411 ± 0.822c	0.073 ± 0.015a	0.607 ± 0.0400a	
Sari oat	22.055 ± 0.411b	0.050 ± 0.010b	0.503 ± 0.0420b	
Fetih oat	25.890 ± 1.369a	0.027 ± 0.006c	0.583 ± 0.0350a	
CV, %	2.040	17.427	7.118	
LSD	0.465	0.011	0.035	
Groups				
Wheat (landraces)	17.534 ± 0.877B	0.047 ± 0.0163B	0.332 ± 0.1050B	
Wheat (cultivar)	16.712 ± 0.246B	0.073 ± 0.0060A	0.293 ± 0.0150B	
Barley	16.986 ± 0.233B	0.050 ± 0B	0.300 ± 0.0100B	
Oat	22.785 ± 2.571A	0.050 ± 0.0220B	0.564 ± 0.0578A	

TPC: Total phenolic content; TFC: total flavonoid content; NBI: N balance index; AAC: ascorbic acid content; ANT: total anthocyanin content; FLV: total flavonol content.

There is nonsignificant difference between values with the same letter in the same column. Values are given with standard deviation (n = 3).

Indeed, Niroula et al. (2021) formulated an equivalent for each of these two methods (DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity, FRAP: ferric reducing antioxidant power). According to the equivalents, the ratio of TAC, FRAP/DPPH was found to be 2.09 in the microgreen analyzed at the end of 8 d in the 8/16 h (dark/light) period. FRAP/DPPH ratios of another researcher (Chomchan et al., 2016) were around 7.66 in ricegrass juice and 6.79 in wheatgrass juice. From here, it is understood that the rates emerging according to the methods are closely related to the analyzed materials (grain, sprout, grass juice, microgreen, herbal piece, etc.) In this respect, the research findings are lower than TAC obtained from buckwheat, barley, wheat and rye by the DPPH method (Djordjevic et al., 2011), while wheat, oats, barley and buckwheat (Mikulajova et al., 2007) and 7-d germinated corn (Niroula et al., 2019b). Although the lowest TAC was found in barley (2542.500 ± 71.875 mg TE g<sup>-1</sup> FRAP) in our study, Mikulajova et al. (2007) determined this amount 6 times higher in barley than oats and wheat. Current results were higher than data of Chomchan et al. (2016) on ricegrass and wheatgrass juices (35.62 ± 0.03 and 37.45 ± 0.98 mg TE g<sup>-1</sup> DM extract) respectively, with the similar analysis (FRAP) method. The TAC capacities of six broccoli genotypes were determined between 20.1-34.6 μM g<sup>-1</sup> in the immature and commercial periods by Bhandari et al. (2019). It was determined that there was an increase of 62% between the two periods.

Table 2 shows TPC contents of local wheat varieties and other cereals; they vary between 465.111 and 634.556 mg GAE 100 g<sup>-1</sup> DM. The highest TPC was obtained from 'Fetih' oat, while the lowest was obtained from the 'Beysehir' barley. The TPC of Kirik and Kose landraces took place in the middle groups following each other. According to the averages based on the groups, the highest TPC was obtained from oat and local wheat varieties in the same group, and the lowest (465.111 mg GAE 100 g<sup>-1</sup> DM) was from barley.

The TPC obtained in the research is lower than Djordjevic et al. (2011), who investigated the extracts of buckwheat, barley, wheat, rye and oat grains, it is higher than Mikulajova et al. (2007). Furthermore, TPC values were higher than results obtained from control group of Falcinelli et al. (2017), einkorn wheat sprouts (1.00 mg GAE g<sup>-1</sup> FW) and from the 7-d corn sprouts (200 mg 100 g<sup>-1</sup> DM) of Niroula et al. (2019b), and it is similar to wheat sprouts (500 mg 100 g<sup>-1</sup> DM) of the same researcher. The TPC values are higher than Xu et al. (2009) results based on the 48 h germination of *Avena nuda* L. (2.62 ± 0.1 mg GAE g<sup>-1</sup> DM) and the average results obtained by Kilincer and Demir (2019) of germinated wheat, barley and oats (1378.38, 1445.45 and 1720.76 µg GAE g<sup>-1</sup> DM, respectively) by days. The fact that germination and sprouting are highly affected by different temperature, humidity and light conditions, this causes change in the TPC which makes it difficult to measure (Niroula et al., 2019b). The TPC of six broccoli genotypes were determined between 416.4 and 53.7 mg GAE 100 g<sup>-1</sup> in commercial periods by Bhandari et al. (2019). Similarly, Joaquín-Ramos et al. (2020) found TPC in leaves, stems, roots and flowers of *Barkleyanthus salicifolius* between 6.82 and 48.21 mg QE g<sup>-1</sup> dried extract and the highest in leaf.

Total TFC of wheat landraces and cereal genotypes examined in the study vary between 258.893 and 476.986 mg QE 100 g<sup>-1</sup> DM. The highest TFC was obtained from 'Alparslan' and the lowest was from 'Seydisehir'. The TFC of Kirik and Kose landraces followed each other behind the highest group. According to the averages based on the species, the highest TFC was obtained from the 'Alparslan' (476.986 mg QE 100 g<sup>-1</sup> DM) and the lowest (302.066 mg QE 100 g<sup>-1</sup> DM) from barley (Table 2). The TFC of six broccoli genotypes were determined between 1.88 and 9.10 mg 100 g<sup>-1</sup> DW in the immature and commercial periods by Bhandari et al. (2019), and it was determined that there was an 80% increase between the two periods. Similarly, Joaquín-Ramos et al. (2020) determined TFC in leaves, stems, roots and flowers of *Barkleyanthus salicifolius* between 10.60 and 31.98 mg GAE g<sup>-1</sup> DE and the highest determined in leaf.

The NBI of wheat landraces and cereals examined in the study were between 42.500 and 100.767 dual index. The highest group NBI was 'Beysehir' barley, while the lowest group was 'Seydisehir', 'Fetih' and Kirik. Kose was one below the highest group. According to the average based of the genotypes, the highest NBI were obtained from the barley (100.767 dual index) and the lowest from the oat cultivars. In this respect, it is seen that there is more than twice difference between the NBI averages of barley and oat cultivars. It has been observed that the 'Beysehir' barley has 25% more NBI than Kose wheat landrace and 50% more than from the other genotypes.

The AAC varied between 16.712 and 25.890 mg 100 g<sup>-1</sup> DM. The highest AAC was obtained from the 'Fetih' oat, while 'Alparslan' wheat, Kirik landrace and 'Beysehir' barley were in the lowest group. Kose landrace is also located in the upper group of the lowest. According to the averages based on the groups, the highest AAC were taken from the oat varieties (22.785 mg 100 g<sup>-1</sup> DM) and the other cereal groups were in the lowest group. The AAC of six broccoli genotypes were determined between 228.0 and 502.3 mg 100 g<sup>-1</sup> in immature and commercial periods by Bhandari et al. (2019), and it was determined 50% increase between the two periods.

The ANT contents were between 0.027 and 0.073 dual index. The highest ANT was obtained from 'Seydisehir' oat, while the lowest was obtained from the 'Fetih' oat and Kose wheat landrace. The ANT level of the Kirik landrace is in the upper group together with 'Alparslan'. According to the averages based on species, the highest ANT was taken from 'Alparslan' (0.073 dual index), while the other wheat genotypes were in the lowest group.

The FLV levels were between 0.427 and 0.607 dual index. The highest FLV was obtained from 'Seydisehir' and 'Fetih' oats and the lowest from the Kose landrace. The FLV content of the Kirik wheat landrace was in the middle group. According to the averages based on groups, the highest FLV amounts were obtained from oat varieties (0.564 dual index), while the remaining cereals were found together in the low group (Table 2).

It was determined that 'Alparslan' wheat was ahead of the other groups in terms of ANT on a group base, and oat in terms of AAC and FLV. It was revealed that the NBI, AAC, ANT and FLV contents of the wheat landraces based on genotypes and groups are low, while the contents of TAC, TPC and FLV are high.

In the control group of wheat microgreens study of Islam et al. (2019), while the amount of ANT was 36.61 ± 3.62 µg mL<sup>-1</sup>, TPC was lower (39.22 ± 0.25 µg mL<sup>-1</sup>) and FLV and AAC (135.51 ± 2.21 and 25.14 ± 0.55 µg mL<sup>-1</sup>, respectively) were higher. In another study, ANT content of maize ranged 646-1052 mg cyanidin 3-glucoside kg<sup>-1</sup> (Urias-Lugo et al., 2015). Xiao et al. (2012) in 25 commercial plant micro-sprouts (20.4 ± 0.5-131.6 ± 2.9 mg 100 g<sup>-1</sup> FW), and ricegrass (0.243 ± 0.002 mg g<sup>-1</sup> FW) and wheatgrass (0.487 ± 0.020 mg g<sup>-1</sup> FW) AAC by Chomchan et al. (2016) were found similar to this research.

In the application of five LED light doses to tatsoi, red pak-choi and basil ‘Sweet Genovese’ (Vastakaite et al., 2015), determined AAC were higher than our research in all plants, while total TPC were low and total ANT were determined between  $0.34 \pm 0.15$  and  $1.14 \pm 0.04$  mg g<sup>-1</sup> FW. In the same study, FLV ( $0.18 \pm 0.03$ - $0.89 \pm 0.24$  dualex index) and TAC ( $2.06 \pm 0.10$ - $4.82 \pm 0.08$  μmol g<sup>-1</sup> FW, DPPH) values in all plants were determined similarly to our study, while tatsoi was low and basil ‘Sweet Genovese’ was high. In another study under growing greenhouse conditions, in the measurements made with the dualex device (Matysiak and Kowalski, 2019) on the leaves of two basil varieties (day 45), lamb’s lettuce and garden rocket (day 30), according to our research, while FLV indexes are similar to basil varieties and garden rocket, but higher than lamb’s lettuce ( $0.22$ - $0.63$ ,  $0.11$ - $0.46$  and  $0.02$ - $0.11$  dualex index, respectively). The ANT index measured in the green-leaved basil variety (could not be measured in other plant) was higher than our research ( $0.40$ - $0.42$  dualex index). In the same research, while dualex device was successful in measuring chlorophyll and FLV in leaves, it was determined that ANT measurements were only possible in green leaf of plant.

### Chlorophyll and carotenoid content

According to the means of two wheat landraces and some cereal microgreens on the basis of genotypes and groups chlorophyll measurement with dualex device (CHLdx), TCHL, CHLa, CHLb and CAR contents and pigment ratios of the CHL (a/b), CHLdx/CAR, TCHL/CAR and TCHL/CHLdx are shown in Table 3. Accordingly, the differences between all examined traits according to means on the genotypes and groups were significant.

**Table 3. Pigment contents and their ratios of some of wheat landraces and cereals.**

Genotypes	CHLdx**	TCHL	CHLa	CHLb	
	μg cm <sup>2</sup>	μg g <sup>-1</sup> FW	μg g <sup>-1</sup> FW	μg g <sup>-1</sup> FW	
Kose (landrace)	15.900 ± 0.655e	22.734 ± 0.141f	17.374 ± 0.011f	5.361 ± 0.129c	
Kirik (landrace)	20.300 ± 1.600d	35.438 ± 0.047a	27.989 ± 0.594a	7.450 ± 0.120a	
Alparslan (cultivar)	18.867 ± 1.289d	32.933 ± 0.785b	25.621 ± 1.103b	7.312 ± 0.318a	
Beysehir barley	30.100 ± 0.435a	30.285 ± 0.410c	22.997 ± 0.430c	7.288 ± 0.020a	
Seydisehir oat	25.133 ± 3.214c	27.003 ± 0.187e	20.278 ± 0.040e	6.725 ± 0.227b	
Sari oat	28.833 ± 2.000ab	28.243 ± 0.401d	21.020 ± 0.283d	7.224 ± 0.118a	
Fetih oat	26.803 ± 0.268bc	16.184 ± 0.275g	12.410 ± 0.028g	3.773 ± 5.773d	
CV, %	6.712	0.919	1.942	2.822	
LSD	1.853	0.294	0.477	0.211	
Groups					
Wheat (landraces)	18.100 ± 2.646C	29.087 ± 6.965C	22.681 ± 5.826B	6.406 ± 1.149B	
Wheat (cultivar)	18.867 ± 1.289C	32.933 ± 0.785A	25.621 ± 1.103A	7.312 ± 0.318A	
Barley	30.100 ± 0.435A	30.285 ± 0.410B	22.997 ± 0.430B	7.288 ± 0.020A	
Oat	26.923 ± 2.485B	23.810 ± 5.749D	17.903 ± 4.134C	5.907 ± 1.620C	
Genotypes	CAR	CHLa/CHLb	CHLdx/CAR	TCHL/CAR	TCHL/CHLdx
	μg g <sup>-1</sup> FW				
Kose (landrace)	4.185 ± 0.032e	3.242 ± 0.076	5.431 ± 0.008	3.799 ± 0.175	1.432 ± 0.066
Kirik (landrace)	6.203 ± 0.085a	3.759 ± 0.140	5.713 ± 0.002	3.272 ± 0.237	1.752 ± 0.130
Alparslan (cultivar)	5.934 ± 0.118b	3.513 ± 0.304	5.549 ± 0.021	3.177 ± 0.155	1.749 ± 0.077
Beysehir barley	5.428 ± 0.093c	3.156 ± 0.067	5.580 ± 0.020	5.547 ± 0.118	1.006 ± 0.019
Seydisehir oat	4.639 ± 0.022d	3.018 ± 0.108	5.821 ± 0.067	5.416 ± 0.671	1.085 ± 1.947
Sari oat	4.710 ± 0.051d	2.910 ± 0.008	5.996 ± 0.019	6.120 ± 0.395	0.982 ± 0.064
Fetih oat	2.814 ± 0.004f	3.289 ± 0.008	5.752 ± 0.001	9.526 ± 0.082	0.604 ± 0.005
CV, %	1.045				
LSD	0.059				
Groups					
Wheat (landraces)	5.194 ± 1.106C	3.500 ± 0.300	5.572 ± 0.154	3.536 ± 0.343	1.592 ± 0.0198
Wheat (cultivar)	5.934 ± 0.118A	3.513 ± 0.304	5.549 ± 0.021	3.177 ± 0.155	1.749 ± 0.077
Barley	5.428 ± 0.093B	3.156 ± 0.067	5.580 ± 0.020	5.547 ± 0.118	1.006 ± 0.019
Oat	4.054 ± 0.931D	3.072 ± 0.177	5.856 ± 0.114	7.021 ± 1.943	0.890 ± 0.232

There is nonsignificant difference between values with the same letter in the same column. The values for the properties are given with the standard deviation (n = 3).

CHLdx: Chlorophyll (measured with dualex device); TCHL: total chlorophyll; CHLa: chlorophyll a; CHLb: chlorophyll b; CAR: total carotenoid content.

The CHLdx amounts of the cereal genotypes varied between 15.900 and 30.100  $\mu\text{g cm}^{-2}$ . The highest CHLdx was obtained from 'Beysehir' barley and the lowest from Kose wheat landrace. The CHLdx content of the Kirik wheat landrace was also close to the lowest group. According to the averages based on the groups, CHLdx values were obtained from the highest barley (30.100  $\mu\text{g cm}^{-2}$ ) and the lowest wheat landraces and cultivar were in the same group.

The TCHL amounts of the landraces and other cereals varied between 16.184 and 35.438  $\mu\text{g g}^{-1}$  FW. The highest TCHL was obtained from Kirik landrace and the lowest was obtained from 'Fetih' oat. Although Kose landrace is one above the lowest group, it contains around 72% more TCHL than the 'Fetih' oat. According to the averages based on the groups, the highest TCHL contents were obtained from wheat cultivar (32.933  $\mu\text{g g}^{-1}$  FW) and the lowest from wheat landraces.

CHLa contents of wheat landraces and cereals were between 12.410 and 27.989  $\mu\text{g g}^{-1}$  FW. The highest CHLa was obtained from Kirik wheat landrace and the lowest from 'Fetih' oat. The CHLa content of Kose wheat landrace was placed one above the lowest group. TCHL and CHLa group rankings of all examined material were formed in the same way. According to the means based on the groups, CHLa contents were obtained the highest from wheat cultivar (25.621  $\mu\text{g g}^{-1}$  FW) and the lowest from the oats (17.903  $\mu\text{g g}^{-1}$  FW). CHLb amounts were between 3.773 and 7.450  $\mu\text{g g}^{-1}$  FW. The highest CHLb was obtained from Kirik wheat landrace, 'Alparslan' wheat, 'Beysehir' barley and 'Sari' oat, while the lowest was obtained from the 'Fetih' oat. The level of CHLb of Kose wheat landrace was in the middle group. According to the averages based on the groups, the highest CHLb contents were obtained from wheat and barley cultivars in the same group, and the lowest from oat cultivars (5.907  $\mu\text{g g}^{-1}$  FW).

The CAR contents of wheat landraces and cereals varied between 2.814 and 6.203  $\mu\text{g g}^{-1}$  FW. The highest CAR was obtained from Kirik landrace, and the lowest was obtained from 'Fetih' oat. Although Kose wheat landrace (4.186  $\mu\text{g g}^{-1}$  FW) was in the group close to the lowest level, it was seen that it contains 67% more CAR than 'Fetih' oat. According to the averages based on the groups, the CAR amounts were measured the highest from wheat cultivar (5.934  $\mu\text{g g}^{-1}$  FW) and the lowest from landraces (5.194  $\mu\text{g g}^{-1}$  FW).

When microgreen pigments were examined according to the genotypes and groups, it was understood that the CHLdx values of barley and oats were close to twice that of wheat. According to the cultivars, TCHL values were close to each other except for Kose wheat landrace and 'Fetih' oat. Kirik wheat landrace had high values in terms of pigments (TCHL, CHLa, CHLb, CAR except for CHLdx). Kirik landrace was similar to the 'Alparslan' wheat. All pigment values of Kose wheat landrace were low (except CAR content). Various methods and devices can be used for CHL measurements. For example, Cerovic et al. (2012) made the measurements with three types of leaf clip (in kiwi, grapevine, wheat and maize) chlorophyll meters (Dualex-4 Scientific, SPAD-502 and CCM-200 by Opti-Sciences). The CHLdx value (21/32  $\mu\text{g cm}^{-2}$  dualex index) determined for four plant species (kiwi, grapevine, wheat and maize) in their research is similar to the values in our study. Likewise, Samuoliene et al. (2017) measured CHL in mustard and beet ( $15.03 \pm 2.09$  and  $20.84 \pm 3.52$  dualex values, respectively) using chlorophyll meter (Dualex-4), and their findings were similar to our study, while the CHL value ( $13.04 \pm 8.52$  dualex value) in parsley was lower than our study. The CHLdx values in our study were similar to the values of green-leaved basil, lamb's lettuce and garden rocket measured with the same device (Matysiak and Kowalski, 2019), but lower than the values of purple-leaved basil. Likewise, CHL values (26 and 29 dualex indexes, respectively) obtained by Hosseini et al. (2019) from studying 14 d maize sprouts using (Dualex-V4) under deficient of Mg and Mg control dose, were similar to our study measurements made in barley and oat cultivars.

TCHL amount of freshly squeezed and frozen juices varied between 36.9 and 141.8  $\text{mg L}^{-1}$  (Skoczylas et al., 2018). The TCHL amounts obtained in our study were obtained with the data obtained from 7 and 10 d microgreens of barley and wheat (Niroula et al., 2019a) and (Ozkose et al., 2016) wheat cultivars ( $25.0 \pm 0.5$ - $42.1 \pm 0.8$   $\text{mg 100 mL}^{-1}$ ) is similar. Our results are higher than TCHL amounts determined by Islam et al. (2019) in the control group of wheat microgreens ( $18.48 \pm 0.53$   $\mu\text{g mL}^{-1}$ ). Conversely, our findings are lower than TCHL in milled barley sprouts ( $600.6 \pm 19.2$   $\text{mg 100 g}^{-1}$ ) examined by Meng et al. (2017). Our TCHL findings are similar to results of studies on chicory (175-370  $\text{mg 100 g}^{-1}$  DM) (Znidarcic et al., 2011), but are lower than spinach and cabbage ( $780 \pm 160$  and  $820 \pm 100$   $\text{mg 100 g}^{-1}$  DM, respectively) (Gedi et al., 2017).

The amounts of CHLa obtained in our study were formed between the amounts determined by Niroula et al. (2019a) in the 7 and 10 d microgreens of wheat and barley. On the other hand, our results are higher than the CHLa values ( $11.00 \pm 0.31$   $\mu\text{g mL}^{-1}$ ) determined in the control group microgreens of wheat under salt stress (Islam et al., 2019). However, our CHLb results are similar to Islam et al. (2019) wheat microgreens control group ( $7.48 \pm 0.22$   $\mu\text{g mL}^{-1}$ ). In contrast, our CHLb contents are lower than the values of Niroula et al. (2019a) who measured in 7 d-old wheat and barley microsprouts ( $85.87 \pm 4.48$  and  $80.09 \pm 4.87$   $\text{mg 100 g}^{-1}$  DM, respectively).



The CAR determined in wheatgrass juice by Skoczylas et al. (2018) varied between 38.6 and 105.0 mg L<sup>-1</sup>), our CARs were lower than wheat and barley microgreens reported by Niroula et al. (2019a) and than rice and wheat grasses (0.063 ± 0.002 and 0.051 ± 0.012 mg g<sup>-1</sup> FW, respectively) reported by Chomchan et al. (2016).

### Pigment ratios

The rates of the pigments of the microgreens are given in Table 3. According to their group-based rates; CHLa/CHLb ratios were 3.500 for wheat landraces, 3.513 for wheat cultivar, 3.156 for barley and 3.072 for oat cultivars. The closeness of CHLa/CHLb ratios of averages on group basis is remarkable. CHLdx/CAR ratios were 5.572 in wheat landraces, 5.549 in wheat, 5.580 in barley and 5.856 in oat cultivars. It is seen that the CHLdx/CAR ratio of the averages is close to each other. TCHL/CAR ratios were 3.536 in wheat landraces, 3.177 in wheat, 5.547 in barley and 7.021 in oat cultivars. The TCHL/CAR ratios of the group averages were close to each other in wheat cultivar, approximately 40% more than wheat landraces in barley and 50% more in oats. TCHL/CHLdx ratio were also formed as 1.592 in wheat landrace, 1.749 in wheat, 1.006 in barley and 0.890 in oat cultivars. It is observed that TCHL/CHLdx ratio are relatively low in barley and oats.

Although the CHLa/CHLb ratio of microgreens produced the indoor area under daylight are lower than those produced by sunlight in the open area, it has been determined that their bioavailability is higher (Niroula et al., 2019a). In our research, CHLa/CHLb and TCHL/CAR ratios obtained from wheat and barley cultivars were determined lower than results of Niroula et al. (2019a). However, our CHLa/CHLb values are higher than Shah et al. (2017) taken under greenhouse conditions on wheatgrass 2.47 ± 0.38 and from Wakeham (2013) taken in the laboratory (2.07). On the other hand, it is similar to values 3.14 (Wakeham, 2013) and 2.9-3.7 (Skoczylas et al., 2018) from wheatgrass grown in open land and 3.2-3.35 from barleygrass (Januskaitiene and Klepeckas, 2015). However, the TCHL/CAR ratio is accepted as an indicator of environmental stress on plant and damage to photosynthetic apparatus affecting the aging of plant (Filimon et al., 2016).

### Relationships between properties (Pearson correlation)

The relationships of pigment properties of microgreen material examined in the research were analyzed by Pearson Correlation method (Table 4). Nonsignificant correlation was found between TAC and bioactive compounds. For this reason, a correlation table was not created.

According to the correlation results, it was seen that there were a negative and nonsignificant relationship CHLdx × TCHL, CHLa × CAT, and a positive but nonsignificant relationship CHL × CHLb. It was understood that there was a positive and significant relationship TCHL × CHLa (r = 0.996\*\*\*), TCHL × CHLb (r = 0.946\*\*) and TCHL × CAR (r = 0.991\*\*\*). Similarly, it was seen that there is a significant positive relationship between pigments CHLa × CHLb (r = 0.915\*\*) and CHLa × CAR (r = 0.993\*\*\*). Likewise, it was determined that there was a positive and significant relationship CHLb × CAR (r = 0.993\*\*\*).

Niroula et al. (2019a) determined that in addition to the significant relationship CHLa × TCHL, as in our study, there are also significant correlations CHLa × CHLb and CHLa × CAR. Similarly, Kopsell et al. (2005) determined that there is a positive correlation TCHL × CAR of eight sweet basil cultivars produced in a greenhouse and open field. Likewise, Ndukwe et al. (2016) found significant CHLa × CHLb, TCHL × CAR correlations between 10 maize varieties. Besides, it has been determined a significant negative correlation CHLa × CHLb in beet microgreens (Samuoliene et al., 2017). Bhandari et al. (2019) found correlations among TAC, TPC strong positive with ascorbic acid content (0.674\*\*\*) and TFC (0.571\*\*) in immature and commercial periods of in six broccoli genotypes.

**Table 4. Correlations between pigments.**

Genotypes	CHLdx	TCHL	CHLa	CHLb	CAR
CHLdx		-0.144	-0.194	0.053	-0.222
TCHL			0.996***	0.946**	0.991***
CHLa				0.915**	0.993***
CHLb					0.993***
CAR					

CHLdx: Chlorophyll (measured with Dualex device); TCHL: total chlorophyll; CHLa: chlorophyll a; CHLb: chlorophyll b; CAR: total carotenoid content.

## CONCLUSIONS

Kose and Kirik are wheat landraces extensively used in the countryside of the Eastern Anatolia Region where the continental climate is dominant. These landraces are also considered valuable breeding material. In this research, these landraces were evaluated in terms of pigment, antioxidant and bioactive compounds together with cereals.

The total antioxidant capacity (TAC) of Kose and Kirik wheat landraces were more than twice the other (except 'Alparslan' wheat). It was determined that the analyzed wheat landraces had high anthocyanin, phenolic and flavonoid contents based on genotypes and groups, while the N balance index, ascorbic acid, anthocyanin and flavonol contents were low. Kirik wheat landrace was rich in other pigments (total chlorophyll, chlorophyll a, chlorophyll b and carotenoids) other than chlorophyll. All pigment values of Kose wheat landrace are low. It has been determined on a group basis that have high ascorbic acid and flavonol of oat cultivars, N balance index of barley, and anthocyanin content of wheat 'Alparslan'. The chlorophyll values of barley and oats are close to twice that of wheat.

The ratios of chlorophyll a (CHLa)/chlorophyll b (CHLb) and total chlorophyll (TCHL measured by dualex)/carotenoid (CAR) was close to each other based on the group, with the highest TCHL/CAR in oats (7.021) and the lowest in wheat. According to the correlation results of the pigment contents, there were very significant and positive correlations TCHL  $\times$  CHLa, CHLb  $\times$  CAR, very significant and positive correlations CHLa  $\times$  CHLb and CAR  $\times$  CHLa.

Finally, it was determined that TAC and total phenolic content (TPC) of landraces examined in the study were high. Kirik wheat also had high CAR, CHLb and CHLa. The TFC and ANT in 'Alparslan' wheat, NBI in 'Beysehir' barley and FLV and AAC in oats were high.

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