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Research Article

Mineral contents and antioxidant activities of Karakılçık durum wheat (*Triticum turgidum* subsp. *durum* (Desf.) Husn.) genotypes

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Abstract

The importance of antioxidants and minerals in food has increased with the growing awareness of consumers in recent years. Türkiye is rich in genetic diversity for wheat as it is located at the intersection of three floristic regions and is part of the Fertile Crescent. In this study, the antioxidant activity of Karakılçık durum wheat (*Triticum turgidum* subsp. *durum* (Desf.) Husn.) populations from 12 different regions of Türkiye was determined using the DPPH method. In addition, the contents of mineral nutrients (Ca, Cu, Fe, Mg, Mn, Zn) were determined in an ICP-OES device with milled wheat. Karakılçık genotypes were sowed in a field using an augmented trial design consisting of five rows and six blocks, with each block containing five control varieties. The result of the study was that the landrace Karakılçık genotypes exhibited antioxidant activity varying between 14.7-40.2 μmol TE g^{-1}. The Ca contents of landrace Karakılçık genotypes varied between 13.0-249.9 ppm, Cu contents 0.6-22.7 ppm, Fe contents 28.7-93.9 ppm, Mg contents 583.7-1194.0 ppm, Mn contents 31.4-87.7 ppm, and Zn contents 22.3-68.5 ppm. The results of the study reveal that Karakılçık landrace genotypes have large differences in antioxidant and mineral content, that these differences have the potential to be used in wheat breeding programs, and that wheat flour obtained from Karakılçık landrace genotypes is important.

Keywords: biodiversity; breeding; DPPH; genotypes; ICP-OES; nutrition

Introduction

Wheat was first cultivated in Türkiye and the Fertile Crescent region before being utilized all across the world. It has been a vital component of human nourishment since antiquity (Yiğit, 2019). Recent archaeological studies have shown that the finds made in this region support this hypothesis. The Anatolian geography, also known as the "granary", has significant potential as a genetic resource, with 23 wild and more than 400 cultivated wheat varieties (WWF, 2016). Cereal crops such as barley, rice, maize, and wheat are the most widely produced and consumed cereals in the world (Khodaei *et al*., 2021; Hekmatnia *et al*., 2022). However, with the increasing world population, meeting people's needs for proteins, fats, carbohydrates, vitamins, minerals, and other ingredients in their daily diets has accelerated studies such as research on

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functional foods to enhance the quality of nutrient content in recent years (Corbo *et al*., 2014; Macdiarmid *et al*., 2018). The fact that the world population will reach 9-10 billion people in 2050 has already led researchers to focus on functional foods to meet the nutritional needs of the increasing population. The fact that grains are durable products ensures that they can be stored and protected for a long time in certain periods. In addition, thanks to these features, it is considered by societies as a strategic product for events such as war, disease, and other disasters.

It is known that wheat is a good source because of the vitamins and minerals it contains, providing 20% of the energy needs of people (Braun *et al*., 2010). Moreover, cereals seem to have an important role in the prevention of chronic diseases due to the antioxidant substances and nutritional fibers they contain (Mpofu *et al*., 2006; Serpen *et al*., 2008). Agricultural production, which does not increase in parallel with population growth, causes malnutrition. Therefore, the challenge lies in agricultural production lagging behind population growth, resulting in malnutrition and fatalities in various parts of the world (Söderström *et al*., 2017). It can be said that the most applicable way to prevent this situation is to develop new varieties that are superior to others in terms of quality and quantity. In the development of new varieties, the breeder receives the greatest support from plant genetic resources, and these resources are a treasure for those who conduct this research.

Wheat has important potential with its mineral and antioxidant properties. Minerals are divided into macro- and microminerals. Those needed in large amounts for the human body are called macrominerals (calcium, phosphorus, sodium, potassium, chlorine, magnesium, and sulfur), and those required in small amounts are called microminerals (iron, iodine, copper, manganese, zinc, cobalt, molybdenum, and selenium) (Topçu *et al*., 2014). The distribution of minerals in wheat grain varies. Notably, minerals are more abundant in the bran layer of wheat than in the endosperm, underscoring the bran's significance (Ekinci and Ünal, 2002). This indicates that the bran layer has significant mineral content. There is also a global trend toward using natural substances in wheat as a source of antioxidants and functional ingredients. Natural antioxidants have attracted considerable attention for their potential nutritional and therapeutic effects. Antioxidants are defined as chemical substances that delay or slow the binding of oxidation, eliminate free radical damage in tissues, and therefore play a role in reducing the risk of many diseases, such as cancer and cardiovascular disease (Gulcin, 2020). Studies reveal that wheat is a natural source of nutritional antioxidants due to the phytochemicals it contains. Phenolic compounds in wheat are substances that carry one or more hydroxyl groups and have the ability to esterify with mono- and polysaccharides (Shahidi and Naczk, 1995; Duthie and Crozier, 2000). The amount and distribution of these substances can vary depending on plant variety, genotype, and ecology (Adom *et al*., 2005; Kim *et al*., 2006). In addition, the phenolic compounds found in wheat are generally benzoic acid and cinnamic acid derivatives, and these compounds are concentrated in the bran part of the wheat grain (Zhou and Yu, 2004; Li *et al*., 2008; Vaher *et al*., 2010). Numerous studies have investigated the mineral content of wheat (Gomez-Becerra *et al*., 2010; Hussain *et al*., 2010; Zhang *et al*., 2010; Hernandez-Espinosa *et al*., 2020).

In this context, our study aims to assess the antioxidant and mineral content of landrace Karakılçık durum wheat, *Triticum turgidum* subsp. *durum* (Desf.) Husn. genotypes collected from different regions of Türkiye and to compare them with conventionally produced wheat varieties.

Materials and Methods

Cultivation of Karakılçık

The main material of the study consisted of 126 landrace Karakılçık wheat populations collected from 12 provinces (Adana, Adıyaman, Antalya, Çanakkale, Diyarbakır, Hatay, Isparta, İzmir, Kilis, Manisa, Osmaniye, and Yozgat) in Türkiye. All obtained wheat populations were stored at the Türkiye Seed Gene Bank for the characterization of further studies (Table 1). The durum wheat cultivars 'Çeşit-1252', 'Eminbey',

'İmren', 'Kızıltan-91', and 'Kunduru-1149' were used as control varieties in the trials. The field trials were conducted at the Haymana-İkizce Research and Application Station of the Field Crops Central Research Institute in Ankara, Türkiye, during the 2020-2021 growing season. At sowing, N and P_2O_5 were used for fertilization at rates of 2.7 kg da^{-1} and 7 kg da^{-1}, respectively. The other nitrogen application to the plots was made in the spring at a dose of 5.3 kg.

Table 1. Provinces and districts where local Karakılçık genotypes were collected from Türkiye and Türkiye Seed Gene Bank Numbers

TGB No (Code)	Province	District	TGB No (Code)	Province	District
020705 (R1)	Antalya	Akseki	023090 (R24)	Antalya	Akseki
020753 (R2)	Diyarbakır	Dicle	023094 (R25)	Antalya	Akseki
020754 (R3)	Diyarbakır	Dicle	023097 (R26)	Adana	Kozan
020756 (R4)	Diyarbakır	Eğil	023104 (R27)	Osmaniye	Hasanbeyli
020766 (R5)	Diyarbakır	Eğil	023110 (R28)	Hatay	Yayladağı
022747 (R6)	Manisa	Akhisar	023112 (R29)	Hatay	Yayladağı
022750 (R7)	Manisa	Akhisar	023115 (R30)	Hatay	Altınözü
022781 (R8)	Çanakkale	Yenice	023116 (R31)	Hatay	Belen
022782 (R9)	Çanakkale	Yenice	023119 (R32)	Adana	Kozan
022783 (R10)	İzmir	Torbalı	023121 (R33)	Osmaniye	Düziçi
022807 (R11)	Çanakkale	Yenice	023123 (R34)	Osmaniye	Düziçi
023005 (R12)	Hatay	Hassa	023154 (R35)	Osmaniye	Hasanbeyli
023006 (R13)	Osmaniye	Düziçi	023218 (R36)	Diyarbakır	Ergani
023008 (R14)	Antalya	Korkuteli	023222 (R37)	Diyarbakır	Dicle
023010 (R15)	Antalya	Korkuteli	023230 (R38)	Diyarbakır	Lice
023017 (R16)	Antalya	Korkuteli	023231 (R39)	Diyarbakır	Eğil
023018 (R17)	Antalya	Korkuteli	023238 (R40)	Diyarbakır	Eğil
023021 (R18)	Antalya	Korkuteli	023240 (R41)	Adıyaman	Gerger
023023 (R19)	Antalya	Korkuteli	023245 (R42)	Diyarbakır	Eğil
023040 (R20)	Adana	Kozan	023837 (R43)	Manisa	Akhisar
023044 (R21)	Osmaniye	Hasanbeyli	023977 (R44)	Antalya	Akseki
023081 (R22)	Hatay	Hassa	024049 (R45)	Kilis	Musabeyli
023085 (R23)	Hatay	Altınözü			

For the management of weeds in the plots, the herbicide Atlantis Star WG 21.15 [active ingredients: 4.5% mesopleuron + 2.25% thiencarbazone-methyl + 0.9% iodosulfuron-methyl-sodium + 13.5% mefenpyrdiethyl (safener), BAYER] was used, and the experimental plots were hand-picked, and each plot was settled in 1m x 1.5 m. The result of the experiment was 45 genotypes selected from 126 genotypes according to different morphological characteristics, which formed the material for the studies.

Determination of antioxidant activity

The harvested wheat was first milled with a 500 μm sieve (Retsch ZM 200, Haan, Germany), and the obtained whole flour samples were weighed at 0.25 g, and placed in a falcon tube, and poured with 5 mL of pure methanol (CH_3OH, CAS Number: 67-56-1). The prepared samples were shaken in an orbital shaker (Thermomac DSL500) for 1.5 hours. Following this, the samples were then filtered on filter paper (Whatman No. 1). The filtered mixture was transferred to a separate Falcon tube, and an additional 5 mL of methanol was added to the remaining residue. The procedure described above was repeated again. Then the first filtrate and the second filtrate were combined and centrifuged at 3000 rpm for 10 minutes (Sigma-3V30). All samples were stored at 20 °C until analysis.

To determine the antioxidant activity, the method specified by Huang et al. (2005) was employed. From the aforementioned samples, 0.1 mL was taken and poured into 0.5 ml glass tubes, and 3.9 mL of DPPH (0.025

g/L CH_3OH) (2,2-diphenyl-1-picrylhydrazyl, CAS Number: 1898-66-4), a stable pink compound, was added to solution. Then, these samples were kept in a complete darkness environment at room temperature for 120 minutes. Subsequently, the samples were transferred to spectrophotometer tubes and read at 515 nm using a (VWR-UV-1600 PC) spectrophotometer. The control group was treated with pure CH_3OH. The experiment was conducted in duplicate. The inhibition rate of the DPPH radical was calculated using the following formula (Kumar *et al*., 2020):

% Inhibition=(Abs control-Abs sample)/Abs control ×100

Antioxidant activity results were calculated as percentage inhibition according to the above formula, but the results were given as Trolox equivalent antioxidant capacity (TEAC). To achieve this, readings were taken for Trolox concentrations of 5, 9, 14, 17, and 24 µM [6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich Chemical Co., St. Louis, MO, USA], and a regression curve ($y = 3.4505x + 1.8714$, $R^2 = 0.9986$) was generated (Figure 1).

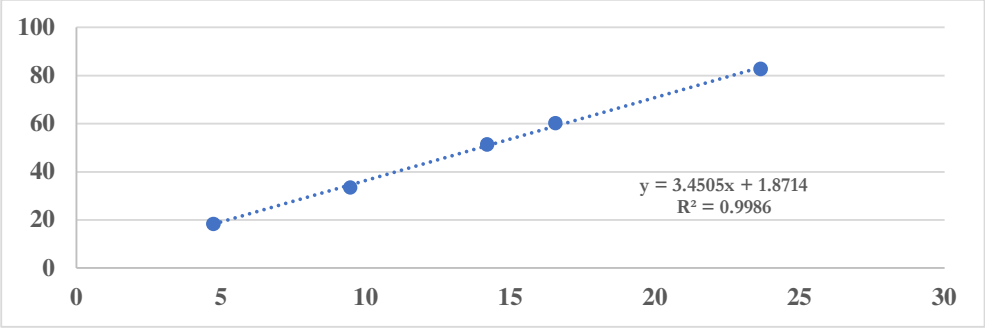

Figure 1. Regression curve graph created according to the DPPH method using different Trolox concentrations

Determination of mineral element contents

The method stated by Jones (2001) was used to determine the macro- and micro-element contents. According to this method, a 0.5 g flour sample prepared for analysis was, dried at 80 °C, passed through a 0.84 mm (20 mesh) sieve, weighed on a precision balance, and placed in a 50 mL incinerator. After that, 5.0 mL of HNO_3 was added to a fume hood with an automatic pipette. It was ensured that the flour sample was thoroughly wetted with acid by shaking it lightly. The combustion vessel, which was left in the block combustion unit at 125 °C for one hour, was removed and allowed to cool. Then, the incinerator, to which 3 mL of 30% hydrogen peroxide was added, was left in the block combustion unit at 125 °C. By adding hydrogen peroxide, the organic matter was completely burned, and the digest turned white. Then, the temperature of the block incinerator was reduced to 100 °C, the funnel was removed from the incinerator, and the digest was waited until near drying. To the incinerator, a 10 mL solution of hydrochloric acid (10%) was added, and the digest was dissolved by gently shaking. Subsequently, the digest in the incinerator was quantitatively transferred to a 50 mL measuring flask. An impression balloon was filled to a certain level with 10% hydrochloric acid and shaken. The prepared sample was analyzed using an ICP-OES device to determine the macro- and micronutrient content, specifically iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), magnesium (Mg), and calcium (Ca) in the landrace wheat genotypes. All measurements were conducted in triplicate.

Statistical analyses

In studies where antioxidant and mineral substance contents were determined, the first step involved testing the normal distribution of the obtained data. Subsequently, the data were subjected to analysis of variance, and Dunnet's test was used to evaluate whether or not the genotypes were in the same statistical group

as the control variety with the highest value. The MINITAB® 18.1 program was used for all statistical analyses (Mckenzie and Goldman, 2004).

Results

Antioxidant activity

Considering the varieties and genotypes used in the study, a wide variation in antioxidant activity was observed. Statistically significant differences were found between cultivars and genotypes (F=46.3; df=49. 99; P<0.01). In terms of antioxidant activity, the highest activity among the control cultivars was recorded as 38.4 μmol TEAC g^{-1} in Imren, and the lowest activity in the 'Eminbey' variety with 29.1 μmol TEAC g^{-1}. Among the genotypes, the highest activity was observed with 40.2 μmol TEAC g^{-1} in the R20-(3) genotype obtained from the Kozan district of Adana province, while the lowest antioxidant activity was determined in the R9-(6) genotype collected from the Yenice district of Çanakkale province with 14.7 μmol TEAC g^{-1} (Table 2). Following the statistical analysis, two genotypes were classified in the same statistical group as the 'İmren' control cultivar based on the results of the Dunnett test. Additionally, 'Çeşit-1252', one of the control cultivars, was grouped statistically with the 'İmren' cultivar, showing an activity of 34.8 μmol TEAC g^{-1}.

Table 2. Antioxidant activities (μmol TEAC g^{-1}) of Karakılçık durum wheat according to DPPH method

Variety/Genotypes	Antioxidant activity	Genotypes	Antioxidant activity
Çeşit-1252	34.8±0.4a	R21-(1)	31.8±0.4
Eminbey	29.1±1.7	R22-(1)	21.0±1.3
İmren	38.4±0.4a*	R23-(6)	26.1±0.9
Kızıltan-91	30.9±0.9	R24-(5)	27.6±0.4
Kunduru-1149	30.3±0.9	R25-(6)	24.3±0.0
R1-(1)	36.3±0.0a	R26-(7)	38.4±2.1a
R2-(6)	25.5±0.9	R27-(7)	35.7±0.0a
R3-(3)	26.4±0.4	R28-(1)	31.8±0.4
R4-(3)	28.2±0.4	R29-(3)	36.0±2.1a
R5-(2)	27.0±0.4	R30-(6)	29.1±1.7
R6-(5)	28.5±3.4	R31-(4)	30.0±1.3
R7-(5)	30.3±1.7	R32-(2)	36.0±1.3a
R8-(3)	15.3±0.0	R33-(2)	35.4±0.4a
R9-(6)	14.7±0.9	R34-(7)	36.6±1.3a
R10-(6)	27.3±1.7	R35-(2)	33.3±0.0
R11-(7)	24.6±0.4	R36-(4)	23.7±0.9
R12-(5)	22.2±0.4	R37-(5)	30.9±0.0
R13-(2)	31.8±3.8	R38-(1)	25.2±0.4
R14-(3)	27.9±2.6	R39-(2)	22.8±0.4
R15-(2)	33.6±1.3	R40-(7)	20.1±0.9
R16-(7)	31.8±0.4	R41-(7)	34.2±1.3
R17-(7)	16.2±1.3	R42-(2)	21.6±1.3
R18-(7)	25.8±2.1	R43-(3)	30.6±0.4
R19-(6)	24.6±0.4	R44-(3)	20.7±0.0
R20-(3)	40.2±1.3a	R45-(7)	32.4±0.4

[a] Means with the same letters were included in the same statistical group (Dunnet test, P<0.01). * It indicates the control variety with the highest mean.

Mineral element contents

When examining the zinc (Zn) contents of both cultivars and genotypes, statistically significant differences were observed (F=65.53; df=49.149; P<0.01). While the highest Zn content of control cultivars was 35.4 ppm in 'Kunduru', the lowest Zn content of control cultivars was determined to be 25.1 ppm in the 'Eminbey' cultivar. Among the genotypes used in the study, the highest zinc content was determined with 68.5

ppm in the R12-(5) genotype collected from Hassa district of Hatay province, while the lowest zinc content was detected in the R10-(6) genotype collected from İzmir and Torbalı district with 22.3 ppm (Table 3). After applying the Dunnett test, 20 genotypes were included in the same statistical group as 'Kunduru', one of the control cultivars. On the other hand, there are 21 genotypes that were higher than the zinc content of the 'Kunduru' cultivar, but not in the same statistical group as this control cultivar. In terms of zinc content, four genotypes had significantly lower zinc content than the 'Kunduru' variety. In terms of iron (Fe) content, statistically significant differences were observed among cultivars and genotypes (F=88.86; df=49.149; P<0.01). While the highest iron content was displayed in the 'Imren' variety with 42.0 ppm in control varieties, the variety with the lowest Fe content was the 'Eminbey' variety with 30.6 ppm. Among the genotypes, the highest iron content was measured at 93.9 ppm in the R12-(5) genotype collected from Hassa district of Hatay province (Table 3) and this genotype had a significantly higher iron content compared to the other genotypes. This genotype exhibited significantly higher iron content compared to the other genotypes, with 2.2 times more iron content than the 'Imren' variety used as a reference. While 19 of the genotypes used in the study were in the same statistical group as the control cultivar 'İmren', the remaining genotypes were in different statistical groups. The lowest iron content among the genotypes was found to be 28.7 ppm in the R10-(6) genotype collected from the Torbalı district of İzmir province.

Table 3. Zinc (Zn) and iron (Fe) contents (ppm) of Karakılçık genotypes

Varieties /Genotypes	Zn	Fe	Genotypes	Zn	Fe
Çeşit-1252	34.2±1.5a	35.0±0.2	R21-(1)	46.2±5.0	46.4±0.4a
Eminbey	25.1±0.6	30.6±0.5	R22-(1)	40.6±2.4	47.0±0.7
İmren	29.6±0.4	42.0±1.7a*	R23-(6)	37.1±2.9a	48.1±0.5
Kızıltan-91	26.5±2.3	33.4±0.3	R24-(5)	33.5±0.2a	49.5±1.6
Kunduru-1149	35.4±1.8a*	31.9±0.4	R25-(6)	35.4±0.5a	40.8±0.1a
R1-(1)	36.3±0.2a	39.4±2.5a	R26-(7)	46.6±2.1	34.5±0.6
R2-(6)	40.2±2.0	38.7±0.3a	R27-(7)	35.2±0.2a	51.0±1.6
R3-(3)	43.2±0.1	41.4±2.1a	R28-(1)	41.5±0.4	53.4±0.2
R4-(3)	37.8±2.0a	50.1±2.1	R29-(3)	34.2±2.1a	38.4±1.4a
R5-(2)	40.0±1.3	46.0±1.3a	R30-(6)	41.2±1.0	36.0±0.2
R6-(5)	32.3±0.0a	46.7±7.1	R31-(4)	27.5±0.4	34.6±0.6
R7-(5)	39.7±1.9	37.1±1.4	R32-(2)	43.7±0.6	35.9±0.4
R8-(3)	35.4±0.2a	54.3±1.9	R33-(2)	47.3±0.3	48.0±2.1
R9-(6)	42.5±0.3	49.7±0.2	R34-(7)	46.6±2.6	43.6±0.4a
R10-(6)	22.3±0.2	28.7±0.5	R35-(2)	32.1±0.5a	40.1±0.5a
R11-(7)	44.3±1.1	43.8±0.9a	R36-(4)	34.3±1.0a	42.0±1.9a
R12-(5)	68.5±2.9	93.9±0.2	R37-(5)	36.1±2.7a	41.5±3.0
R13-(2)	51.4±1.1	48.4±0.1	R38-(1)	42.0±1.0	47.2±1.3
R14-(3)	43.0±0.9	50.1±5.4	R39-(2)	36.8±1.6a	46.5±1.7
R15-(2)	36.5±1.0a	49.1±2.4	R40-(7)	33.9±1.9a	43.6±1.3
R16-(7)	43.6±0.8	45.7±2.2a	R41-(7)	36.4±1.0a	40.4±0.1a
R17-(7)	44.1±1.5	46.4±0.1a	R42-(2)	34.4±0.3a	40.4±0.4a
R18-(7)	41.6±2.1	43.0±0.4a	R43-(3)	38.4±1.8a	43.5±0.1a
R19-(6)	42.2±0.1	52.6±0.9	R44-(3)	38.4±0.9a	45.1±0.4a
R20-(3)	38.1±0.7a	30.7±1.4	R45-(7)	40.5±0.2	38.2±1.9a

[a] Means with the same letters were included in the same statistical group (Dunnet test, P<0.01). * It indicates the control variety with the highest mean.

Varieties and genotypes had significant variations in terms of magnesium (Mg) content, with statistically significant differences were observed between them (F=27.58; df=49.149; P<0.01). The highest Mg content among the control cultivars was determined to be 845.7 ppm in the 'Kızıltan' cultivar, while the Mg content of 'Eminbey' and 'Çeşit-1252' was determined to be 811.5 ppm and 768.7 ppm, respectively. These three cultivars were grouped statistically together. Among the genotypes, the highest Mg content with 1194.0 ppm was found in the R29-(3) genotype obtained from the Yayladağı district of Hatay province, but this genotype was not

statistically included in the same statistical group as the 'Kızıltan' control variety. While 26 of the genotypes were statistically grouped with 'Kızıltan', 15 of them were in different groups. The lowest Mg content among the genotypes was determined at 583.7 ppm in the R44-(3) genotype collected from the Akseki district of Antalya province (Table 4). Statistically significant differences were also identified in terms of manganese (Mn) content among cultivars and genotypes (F=102.62; df=49.149; P<0.01). While the highest Mn content was determined in the 'Kunduru' variety with 39.2 ppm among control varieties, the lowest Mn content was determined in the 'Eminbey' variety with 27.6 ppm. Among the genotypes, the highest Mn content, measuring 87.7 ppm, was detected in the 'R12-(5)' genotype collected from the Hassa district of Hatay province. This genotype was statistically grouped differently from the 'Kunduru' control cultivar. The lowest Mn content among the genotypes was recorded at 31.4 ppm in the R10-(6) genotype collected from the Torbalı district of İzmir province. In terms of Mn content, 18 genotypes were grouped statistically with 'Kunduru' one of the control cultivars, while 27 genotypes were in different statistical groups (Table 4).

Table 4. Magnesium (Mg) and manganese (Mn) contents (ppm) of Karakılçık genotypes

Varieties/Genotypes	Mg	Mn	Genotypes	Mg	Mn
Çeşit-1252	768.7±137.7a	34.1±4.0	R21-(1)	958.1±26.0	46.4±1.2
Eminbey	811.5±66.5a	27.6±1.3	R22-(1)	878.4±15.8a	52.3±0.1
İmren	683.2±135.8	33.0±1.8	R23-(6)	861.6±17.0a	48.4±2.2
Kızıltan-91	845.7±38.3a*	28.1±0.6	R24-(5)	754.6±6.0a	36.2±1.3a
Kunduru-1149	822.6±57.5a	39.2±0.3a*	R25-(6)	681.9±47.2	42.0±3.4a
R1-(1)	603.0±29.3	40.0±0.4a	R26-(7)	889.5±35.8a	53.5±2.4
R2-(6)	765.1±57.1a	45.5±2.4	R27-(7)	1003.8±8.3	55.6±0.4
R3-(3)	953.1±8.4a	45.9±3.8	R28-(1)	887.5±4.5a	63.3±1.5
R4-(3)	908.4±5.0a	52.8±1.6	R29-(3)	1194.0±54.0	49.8±0.1
R5-(2)	859.6±42.8a	42.2±1.5a	R30-(6)	913.9±54.6a	43.1±0.3a
R6-(5)	747.8±4.1a	39.7±0.1a	R31-(4)	1123.7±17.5	39.2±0.3a
R7-(5)	799.5±4.7a	50.2±1.0	R32-(2)	1032.0±9.0	50.9±1.2
R8-(3)	692.1±61.6	46.4±0.9	R33-(2)	875.8±17.4a	55.8±2.8
R9-(6)	728.1±13.2	53.7±0.1	R34-(7)	956.6±31.9a	52.1±0.0
R10-(6)	823.2±0.1a	31.4±0.1	R35-(2)	623.7±2.1	41.2±3.8a
R11-(7)	871.7±69.1a	49.8±1.9	R36-(4)	818.0±16.8a	40.6±1.6a
R12-(5)	877.0±4.9a	87.7±0.6	R37-(5)	706.5±16.7	36.5±0.8a
R13-(2)	997.7±66.2	64.0±1.3	R38-(1)	1053.8±59.3	50.3±0.7
R14-(3)	641.9±19.4	37.6±1.7a	R39-(2)	962.1±46.0	43.2±3.3a
R15-(2)	959.2±2.4	39.8±1.4a	R40-(7)	679.2±25.5	39.9±0.7a
R16-(7)	843.4±9.6a	47.9±0.4	R41-(7)	932.0±41.5a	42.9±0.2a
R17-(7)	874.7±23.8a	48.9±0.4	R42-(2)	851.3±17.2a	45.8±1.0
R18-(7)	853.9±27.2a	45.9±1.9	R43-(3)	745.7±41.3a	53.2±1.5
R19-(6)	813.9±27.8a	50.8±3.4	R44-(3)	583.7±7.1	38.0±2.1a
R20-(3)	984.3±3.7	35.4±0.4a	R45-(7)	826.5±35.2a	40.4±0.1a

[a] Means with the same letters were included in the same statistical group (Dunnet test, P<0.01). * It indicates the control variety with the highest mean.

When evaluating cultivars and genotypes in terms of copper (Cu) content, statistically significant differences were observed (F=286.76; df=48, 146; P<0.01). Among the control cultivars, the highest Cu content was recorded in the 'Eminbey' cultivar with 7.7 ppm, followed by the 'Kızıltan' cultivar with 6.9 ppm. These two cultivars were grouped together. Among the genotypes, the genotype with the highest Cu content was the R12-(5) genotype collected from Hassa district of Hatay province with 22.7 ppm, and this genotype was statistically included in the different statistical group with the 'Eminbey' control variety (Table 5). The lowest Cu content was observed at 0.6 ppm in the R33-(2) genotype. Varieties and genotypes also displayed statistically significant differences in terms of calcium (Ca) content (F=10.5; df=46.140; P<0.01). 'Çeşit-1252' had the highest Ca content (282.4 ppm) among control cultivars, while the lowest Ca content was determined in the Imren cultivar with 88.4 ppm. Among the genotypes, the highest Ca content was found with 249.9 ppm

in the R21-(1) genotype collected from Hasanbeyli district of Osmaniye province. This genotype was statistically in the same group as 'Çeşit-1252'. The lowest Ca content among the genotypes was determined to be 13.0 ppm in the R44-(3) genotype collected from the Akseki district of Antalya province (Table 5).

Table 5. Copper (Cu) and calcium (Ca) contents (ppm) of Karakılçık genotypes

Varieties/Genotypes	Cu	Ca	Genotypes	Cu	Ca
Çeşit-1252	3.7±0.9	282.4±133.5a*	R21-(1)	3.5±0.0	249.9±8.5a
Eminbey	7.7±0.2a*	98.5±69.3	R22-(1)	7.4±0.5a	184.4±1.5
İmren	3.5±0.1	88.4±97.4	R23-(6)	6.6±0.0	38.0±27.7
Kızıltan-91	6.9±0.0a	127.7±62.0	R24-(5)	4.1±0.2	87.1±26.8
Kunduru-1149	1.5±0.2	99.0±91.9	R25-(6)	3.6±0.1	81.1±9.4
R1-(1)	3.7±0.1	74.3±9.2	R26-(7)	1.3±0.2	243.3±32.3a
R2-(6)	2.1±0.0	142.6±13.8	R27-(7)	0.7±0.0	144.3±2.0
R3-(3)	1.1±0.2	116.5±3.5	R28-(1)	7.8±0.2a	127.5±23.6
R4-(3)	6.5±0.0	168.2±2.6	R29-(3)	5.1±0.0	215.7±3.0a
R5-(2)	4.0±0.2	68.1±20.1	R30-(6)	6.1±1.1	127.8±25.2
R6-(5)	4.4±0.0	141.6±51.0	R31-(4)	4.0±0.0	166.6±13.1
R7-(5)	6.7±0.1	78.5±20.8	R32-(2)	4.5±1.3	141.7±25.4
R8-(3)	3.3±0.2	43.8±7.5	R33-(2)	0.6±0.0	186.8±28.8
R9-(6)	4.5±0.1	84.3±0.4	R34-(7)	-	26.3±1.3
R10-(6)	2.0±0.2	213.4±18.9	R35-(2)	2.9±0.1	-
R11-(7)	1.6±0.4	129.5±4.1	R36-(4)	4.8±0.1	30.1±15.6
R12-(5)	22.7±0.5	78.7±7.2	R37-(5)	4.7±0.1	-
R13-(2)	3.3±0.1	180.3±7.3	R38-(1)	5.9±0.1	177.1±12.1
R14-(3)	4.6±0.2	57.7±13.7	R39-(2)	5.5±0.5	54.2±20.2
R15-(2)	4.6±0.2	94.6±9.5	R40-(7)	6.6±0.1	24.4±3.1
R16-(7)	5.3±0.2	136.0±17.8	R41-(7)	3.9±0.2	162.7±4.9
R17-(7)	6.2±0.4	103.4±12.1	R42-(2)	4.3±0.0	*-
R18-(7)	5.4±0.1	83.9±9.7	R43-(3)	6.3±0.1	77.8±10.9
R19-(6)	4.7±0.0	130.9±37.2	R44-(3)	2.5±0.1	13.0±2.9
R20-(3)	1.5±0.1	213.5±10.9a	R45-(7)	5.7±0.2	202.2±22.7a

[a] Means with the same letters were included in the same statistical group (Dunnet test, P<0.01). * It indicates the control variety with the highest mean.

When evaluating the parameters through cluster analysis, it was observed that the control varieties and study genotypes formed six clusters. 'Çeşit-1252' was in the first cluster, while the other control varieties were in the second cluster, which had the highest number of genotypes. The third cluster contained 10 genotypes, while the fourth, fifth, and sixth clusters each held five genotypes (Figure 2).

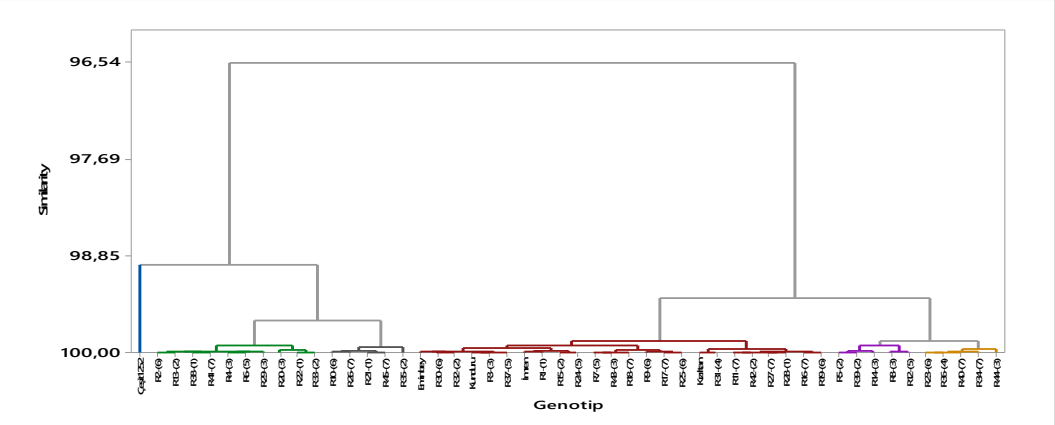

Figure 2. Cluster dendrogram of cultivars and genotypes in terms of examined characteristics

Discussion

Natural antioxidants found in foods have received great attention for their safety and potential nutritional and therapeutic effects in the last few decades. Studies have indicated that factors such as cultivar, genotype, and ecological conditions can substantially influence the antioxidant content of plants. In the case of wheat flour, antioxidant activity is primarily attributed to its phenolic compounds (Yu et al., 2004). In previous studies on the phytochemical profile of wheat, it has been reported that ferulic acid is found at high levels, and it also has components such as vanillic, syringic, and p-coumaric acid (Moore *et al*., 2006; Okarter *et al*., 2010). Although the phytochemical profiles of wheat were not investigated in this study, it is thought that the antioxidant activity originates from free or conjugated phenolic compounds. In addition, it was determined that the obtained genotypes had different antioxidant activities depending on the regions where they were collected. It was also determined that some genotypes showed higher antioxidant activity than conventionally produced cultivars. In addition, it has been revealed in previous studies that antioxidant activity may vary depending on the part of wheat used. In a previous study, it was concluded that the amount of phenolic substances in wheat decreases as you go from the bran to the aleuron layer, and accordingly, the antioxidant activity decreases. Researchers reported that the bran and aleuron layers are important for health (Beta *et al*., 2005). In a study conducted with durum wheat, they examined the antioxidant activities of durum wheat and reported that wheat has antioxidant activities comparable to red wine, tomato, or peach, which are known to be good antioxidants (Esposito *et al*., 2005). Wheat is the main component of human nutrition, especially in developing countries, and it is important to prevent chronic diseases such as whole grain consumption, cardiovascular diseases, and cancer (Stefoska-Needham *et al*., 2015). The landrace Karakılçık genotypes featured in our study exhibit substantial antioxidant potential, particularly when considering their integration into breeding programs aimed at creating new cultivars with heightened antioxidant activity.

The distribution of mineral substances in the wheat grain differs, and the amount of mineral substances generally increases as one goes out of the endosperm (Ekinci and Ünal, 2002). Many studies have been carried out on the mineral content of wheat or different parts of the wheat grain. In this study, Zn content varying between 22.3-68.5 ppm was determined in the carbuncle genotypes. In their study, Gomez-Becerra et al. (2010) revealed that genotype×environment interaction is important for the mineral substance content in the grain and that zinc element has the highest genotypic variation with a value of 19-145 mg kg^{-1} among the investigated nutrients. Although the results of our study show parallelism with the results of this study, the highest variation was determined in Mn content in our study. Zinc is the twenty-third most abundant element, making up about 0.02% of the earth's crust, and has a bluish-white metallic color. By its nature as a transition element on the periodic table, zinc has certain chemical properties that make it particularly useful and important in biological systems. Specifically, zinc can form strong but easily exchangeable and flexible complexes with organic molecules, thereby enabling nucleic acids to change the three-dimensional structure of special proteins and cellular membranes (Brown *et al*., 2001). Zinc is an essential element in both prenatal and postnatal periods of rapid growth and for tissues with rapid cellular differentiation and transformation, such as the immune system and gastrointestinal tract. Critical functions affected by zinc nutrition include pregnancy outcome, physical growth, susceptibility to infection, and neurobehavioral development (Brown *et al*., 2001). The Karakılçık genotypes examined in our study exhibited remarkable zinc content, underscoring their potential as a valuable dietary source.

Iron metabolism disorders are among the most common human diseases, and cover a wide spectrum of diseases with clinical manifestations ranging from anemia to iron overload and possibly neurodegenerative diseases (Briguglio *et al*., 2020). In this regard, it is important to consume foods with high iron content and to monitor the concentration in the human body. The iron content of the Karakılçık genotypes used in this study varied between 28.7 and 93.9 ppm. In previous studies, the iron content was determined at 32.2-33.3 mg/kg (Hussain *et al*., 2010), 39.2 mg kg^{-1} (Zhang *et al*., 2010), and 32.7-46.1 mg kg^{-1} (Hernandez-Espinosa *et al*.,

2020). Intriguingly, the landrace Karakılçık genotypes had a higher concentration of iron than the Fe content determined in previous studies. This suggests that secondary products derived from black pepper genotypes may harbor significant iron potential and could serve as a foundation for advanced wheat breeding initiatives.

The Mg content of the Karakılçık genotypes investigated in our study ranged from 583.7 to 1194.0 ppm. In a previous study, the B, Cu, Fe, Se, Mg, Zn, Ca, Mn, Mo, P, S, and K contents of 321 winter wheat genotypes were determined, and the Mg content was determined in the range of 1240-1300 mg kg^{-1} (Hussain *et al*., 2010). In a study conducted in China, the Fe, Zn, Mn, Cu, Ca, Mg, K, and P contents of different wheat genotypes were determined, and the Mg content was determined as 1519 mg kg^{-1} on average in 265 genotypes used in the study (Zhang *et al*., 2010). The results of the mentioned studies show parallelism with the Mg contents determined in our study. For manganese, our research identified content varying from 31.4 to 87.7 ppm in the genotypes. Previous studies by Hussain et al. (2010) and Zhang et al. (2010) reported Mn content ranging from 17.7 to 23.3 mg kg-1 and 48.8 mg kg-1, respectively, in their investigations on wheat. Our research findings are similar to the results of these researchers.

There is a substantial amount of information available about copper regarding its absorption, its biochemical role in the cell, and its excretion by various species. A 70 kg adult human body contains 80 to 120 mg of copper. The daily copper requirement for humans is estimated to be 2.5 mg per day. Daily consumption needs for human nutrition can vary based on geographical regions, and the copper content in cereals may fluctuate depending on the region of cultivation. In our study, we observed the Cu content of the Karakılçık genotypes ranging from 0.6 to 22.7 ppm. An earlier study reported, an average copper content of 265 wheat genotypes was determined to be 7.39 mg kg^{-1} (Zhang *et al*., 2010). While there is a degree of concurrence between this result and our findings, the Karakılçık genotypes, in general, exhibited higher copper content. Mineral content can indeed fluctuate based on genetic and environmental factors. The primary influencers of mineral content in wheat encompass variety, genotype, location, soil characteristics, cultivation practices, and climate conditions (Anglani, 1998). In another study, the B, Cu, Fe, Se, Mg, Zn, Ca, Mn, Mo, P, S, and K contents of 321 winter wheat genotypes were analyzed, and it was determined that ancient and primitive varieties had more mineral matter than modern wheat. It has also been reported that the place of production has a significant effect on the amount of mineral matter (Hussain *et al*., 2010). In our study, calcium content spanned from 13.0 to 249.9 ppm. A previous study determined calcium content in durum wheat to range between 383 and 388 mg kg^{-1} (Hussain *et al*., 2010). While there is similarity in terms of the upper limit of calcium content, a notable discrepancy is observed regarding the lowest Ca content, which can be attributed to genetic variability among local varieties.

Conclusions

Our research results show that landrace Karakılçık genotypes have significant potential in terms of antioxidant and mineral content. In addition, it was determined that they had higher values in terms of certain parameters compared to the varieties that were taken into the trial and produced conventionally. In this case, it is seen that two scenarios emerge for the upcoming process. First, it is possible to present the high-value Karakılçık populations directly to the producer and thus support the healthy nutrition of the consumer. Secondly, the use of Karakılçık genotypes as genotypes in wheat breeding programs and the development of new wheat varieties with high antioxidant potential and mineral substance content can contribute.

Authors' Contributions

FRA: Conceptualization, Methodology, Investigation, Writing-original draft, Supervision, MAB: Data curation, Writing-review & editing, Writing-original draft. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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