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Research Article

Assessment of genetic diversity based on agro-morphological traits and ISSR molecular markers in einkorn wheat (*Triticum monococcum* ssp. *monococcum*) landrace populations from Turkey

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Abstract: The aim of this study is to investigate genetic diversity in 48 einkorn (*Triticum monococcum* L. ssp. *monococcum*) landraces grown in agricultural areas of Bolu and Kastamonu. Therefore, variation in seven agro-morphological traits was investigated. Agro-morphological traits such as leaf weight (mg), coleoptile length (cm), root number (n), root length (cm), fresh root weight (mg), and dry root weight (mg) were examined by the coefficient of variation, ANOVA, and principal components analysis. The highest coefficient of variation (%) was observed in fresh root weight (FRW = 52.09%), while the lowest was in leaf weight (LW = 8.9%). Principal Component Analysis (PCA) was calculated as 76.93% variation in two main components. For molecular characterization, data obtained with iSSR primers were analyzed with the population genetics analysis program PopGene (ver. 1.32). According to PopGene results, the mean number of alleles, the mean number of effective alleles, and average genetic diversity values were calculated as na = 2, nea = 1.33, and h = 0.13, respectively. Among the agro-morphological traits, germination power, root number, and coleoptile length appeared to be reliable traits. The results show that the use of morphological characters alone for genetic diversity in populations is not sufficient to determine the difference between populations and their genetic structure.

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1. INTRODUCTION

The *Triticum* genus is divided into three groups according to the number of chromosomes: diploids (*T. monococcum* L. ssp. *monococcum* 2n = 14, *AA*), tetraploids (*T. turgidum* L. 2n = 28, *AABB*), and hexaploids (*T. aestivum* L. 2n = 42, *AABBDD*). The name "einkorn," a single grain, comes from Germany. It is locally named "Iza" or "Siyez" in Türkiye. Einkorn spikes have single-grain and a husky structure (Aslan *et al*., 2018; Karagöz & Zencirci, 2005; Ünlü *et al*., 2018).

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In Türkiye, especially in the Western Black Sea Region (Sinop, Kastamonu, Samsun, and Bolu), *Triticum monococcum* L. ssp. *monococcum* is called IZA (einkorn) wheat and is consumed mostly as bulgur. There are 1.400 hectares of einkorn wheat cultivation in the Kastamonu region. While the production amount of einkorn wheat throughout Kastamonu is 3,500 tons, according to the data from the İhsangazi District Directorate of Food, Agriculture, and Livestock, İhsangazi had the highest production rate in 2013 with 6.750 decares, an einkorn wheat production of 1.687 tons, and an einkorn bulgur production of 470 tons. There are 1,050 Einkorn wheat farmers in the district, and the Einkorn Wheat Growers Association, which was established in İhsangazi, has approximately 100 members (Aslan *et al*., 2018; Ünal, 2002). Production in Seben and Bolu has also increased recently.

Løje *et al*. (2003) observed that ten Einkorn populations had a large amount of ash (2.3-2.8% DM), a variable level of proteins (10.03-26% DM), and glucan (0.29-0.71 % DM). It has a very low level of nutritional fiber (7.6 –9.9% KM), highly variable lysine levels (1.51–3.15 gram lysine 100 g^{-1} protein), low sedimentation, and a thin mixographer's curve compared to typical wheat cultivars (Abdel-Aal *et al*., 1997). The protein content is generally greater than that of rye and hard red wheat. The structure of amino acids is similar to common wheat (Troccoli & Codianni, 2005).

In the study of Abdel *et al*. (1997), the results show that einkorn has a high protein content but low gluten elongation ability. Cooked einkorn grains have a softer consistency, less white color, less stickiness, and a less fibrous structure than those of *spelta* and common wheat (Bavec & Bavec, 2006).

Based on previous studies, Zencirci *et al*. (2018) stated that the number of botanical varieties grown in Türkiye with all *Triticum* ssp. cultivated in Türkiye exceeds substantially the number of botanical wheat varieties cultivated in the other parts of the world. For instance, of the 73 botanical *T. turgidum* varieties recognized at the time, 48 were collected from Türkiye.

Guzy *et al*. (1989) identified a wide diversity in the number of spikelets per spike and the number of grains per spike in a series of diploids, tetraploids, and hexaploids. Sharma *et al*. (1984) compared 93 genotypes of einkorn entries with "Modoc" durum wheat and "Anza" bread wheat varieties. They compared several parameters, such as plant height (PH), grain weight, lysine content and protein ratio in flour, ear weight, earliness, height, etc., and they observed a wide genetic variation among the samples. Castagna *et al*. (1995) studied 21 *Triticum monococcum* L. ssp. *monococcum* populations from different locations and found that there were important genetic variations for ear emerging date, plant height (PH), grain yield, total biomass, and the number of ears per m^2. Empilli *et al*. (2000) examined 1,039 einkorn genotypes and reported that there was a wide variation for grain size, 13 genotypes with higher thousand kernel weight over 40 g, many genotypes with low SDS sedimentation, and eight genotypes with higher SDS sedimentation. Butnaru *et al*. (2003) characterized 37 local einkorn wheat genotypes collected from Romania and Hungary for six morphological characters and five agronomic characteristics and found that the number of seeds per spike and grain weight was diverse among genotypes. In the study of Özbek and Zencirci (2021), six einkorn (*Triticum monococcum* L. ssp. *monococcum*) landrace populations and two bread wheat (*Triticum aestivum* L.) populations were collected from agricultural areas in Bolu and Kastamonu provinces. They were characterized for genetic diversity by using 12 Intel simple sequence repeat primers. The genetic diversity was observed to be $h = 0.20$ in *T. monococcum* populations and $h = 0.14$ in *Triticum aestivum* populations. A dendrogram was constructed according to the genetic distance values by using the unweighted pair group method with the arithmetic mean method. *Triticum aestivum* and *Triticum monococcum* populations are clustered into different clusters (Zencirci *et al*. 2019). The number of sub-populations was

identified as the optimal value for $K = 7$. ISSR markers were successful in determining the genetic diversity and population structure within and between species.

This study aims to investigate the agro-morphologic and molecular characters in einkorn wheat (*Triticum monococcum* L. ssp. *monococcum*) from Türkiye. We investigated the agro-morphological characters in einkorn wheat, such as germinating power (GP), coleoptile length (CL), root count (RC), root length (RL), root fresh weight (RFW), root dry weight (RDW), and leaf weight (LW) in addition to the variation in molecular iSSR markers. The possibility of the development of more superior wheat varieties can be increased by determining the agro-morphological and molecular characteristics of wheat.

2. MATERIAL and METHODS

2.1. Materials

In this study, agro-morphological traits were investigated to determine the genetic diversity in 48 einkorn wheat (*Triticum monococcum* L. ssp. *monococcum*) local populations from Türkiye (Table 1). The seed samples were collected from the agricultural areas in İhsangazi (Kastamonu) and Seben (Bolu) in 2020.

Table 1. Using Einkorn populations in the study (abbreviations: population ID number N, registration number RN, and local name LN)

N	RN	Species Name	LN	Collection sites
1	10	*T. monococcum* ssp. *monococcum*	Einkorn	Kastamonu/Ihsangazi/ Uzunoğlu Mah.
2	11	*T. monococcum* ssp. *monococcum*	Einkorn	Kastamonu / İhsangazi / ÇayMah.
3	14	*T. monococcum* ssp. *monococcum*	Einkorn	Kastamonu / İhsangazi.
4	16	*T. monococcum* ssp. *monococcum*	Einkorn	Kastamonu / İhsangazi / Koçcugaz Köyü
5	29	*T. monococcum* ssp. *monococcum*	Einkorn	Kastamonu / Araç / Aliören Köyü
6	35	*T. monococcum* ssp. *monococcum*	IZA	Bolu / Seben / Musasofular Köyü
7	37	*T. monococcum* ssp. *monococcum*	IZA	Bolu / Seben / Musasofular Köyü Çıkışı
8	39	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/GüneyceKöyü.
9	44	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/YakuplarKöyü/Aynak Deresi Mevkii
10	43	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/ YakuplarKöyü.
11	45	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/YakuplarKöyü/Aynak Deresi Mevkii
12	47	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Musasofular Köyü.
13	48	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Haccağız Köyü/BeylikMevkii
14	49	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Musasofular Köyü
15	50	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Gerenözü Köyü
16	51	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Nimetli Köy
17	54	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Gerenözü Köyü
18	55	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Nimetli Köyü
19	56	*T. monococcum* ssp. *monococcum*	IZA	Bolu/HaccağızKöyü/Beylik Mah
20	57	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Musasofular Köyü/Akcumar Bölgesi
21	58	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Güneyce Köyü
22	59	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Güneyce Köyü
23	60	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Güneyce Köyü
24	B24	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Güneyce Köyü
25	B17	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Güneyce Köyü
26	B35	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Güneyce Köyü
27	B73	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Göynük/Çaylak Köyü
28	B63	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Göynük/Aşağı Kınık Köyü

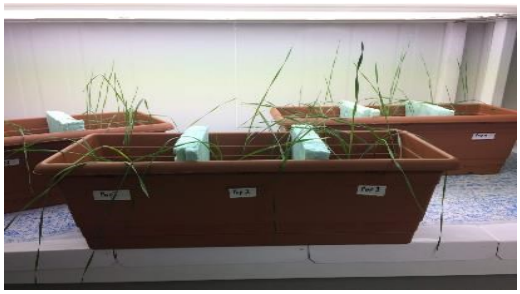
Table 1. *Continues.*

29	B71	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Göynük/Sarılar Köyü
30	B78	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Göynük/Aşağı Kınık Köyü
31	B61	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Göynük/Yukarı Kınık Köyü
32	B64	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Göynük/Yukarı Kınık Köyü
33	B69	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Göynük/Yukarı Kınık Köyü
34	B20	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Güneyce Köyü
35	B26	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Haccağız Köyü
36	B32	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Musasofular Köyü
37	B30	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Değirmenkaya Köy
38	B66	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Göynük/Yukarı Kınık Köyü.
39	B25	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Güneyce Köyü
40	B21	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Yağma Köyü
41	B65	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Göynük/Pelitçik Köyü
42	B33	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Güneyce Köyü
43	B19	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Güneyce Köyü
44	B18	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Güneyce Köyü
45	B16	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Güneyce Köyü
46	B23	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Yağma Köyü
47	B22	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Güneyce Köyü
48	B15	*T. monococcum* ssp. *monococcum*	IZA	Bolu/Seben/Güneyce Köyü

2.2. Methods

The agro-morphological traits were determined for germination power (GP), coleoptile length (CL), number of roots (NR), root length (RL), root fresh weight (RFW), root dry weight (RDW), and leaf weight (LW). Five seed samples from each population were planted in the soil and sterilized in the autoclave for 15 minutes at a temperature of 121 °C. Then, they were placed in the climate chamber, which was kept at constant climate conditions (Temperature 23° C ± 2, 16 hours day, 8 hours night) for 30 days. After 30 days, the plants were cut, and the leaves were weighed. The leaves were kept at -20 °C for molecular analyses of the populations (Figure 1).

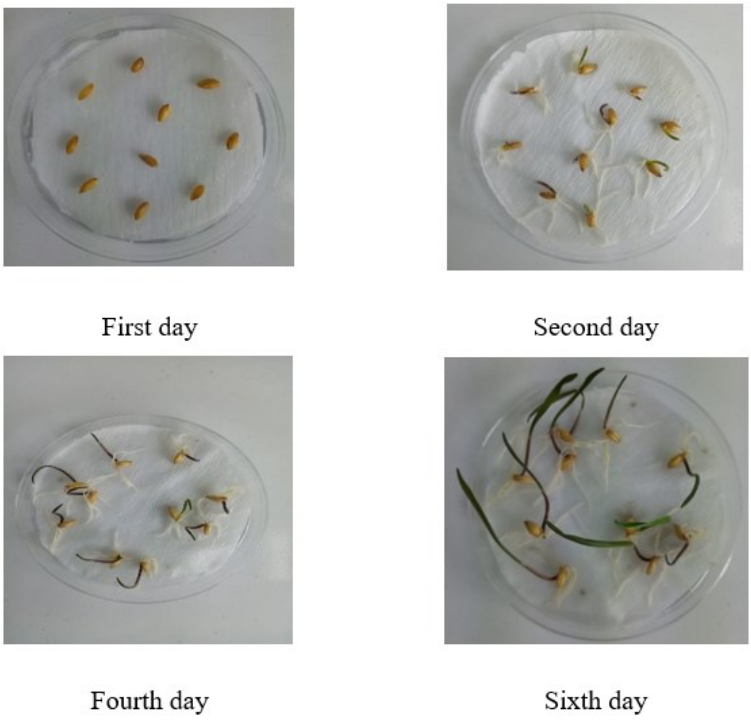
Figure 1. After 30 days, the plants.



The husks of 50 seeds in each population were peeled and cleaned for the measurement of germination power, coleoptile length, number of roots, root length, root fresh weight, and root dry weight. Then, the seeds of each population (50 seeds) were sterilized with the disinfection solution, which was prepared with two drops of Tween 20, 30 ml of bleach, and 70 ml of distilled water in total in a 500-ml glass beaker for 10 minutes. The floated seeds were then removed because they were considered dead seeds. Sterile conditions were achieved by placing three Petri plates, three filter papers, and 500 ml of water in a glass tube with a total volume of 1000 ml in a sterile room. Next, the sterilized seeds were placed in three Petri dishes in which

two filter papers were placed and moistened with 2 ml of water in the form of ten seeds. After that, the plates were placed inside the climate chamber with a constant climate condition (23±2˚C for 16 hours during the day and 8 hours at night). The germination process, which involved moistening each petri dish with 2 mL of water every day, took place after 8 days (Figure 2).

Figure 2. The images show the stages of the germination process between 1st-6th days.



After eight days from germination (Figure 3), the plantlets were cut, and data were taken for GP, CL, RN, RL, FRW, and DRW. The roots were cut, weighed in their fresh state, put in the incubator at 37 °C for four days, then weighed in their dry state.

Figure 3. After eight days of germination, the seed is harvested.

The aims of the molecular character's experiment were (i) to investigate genetic diversity in *Triticum monococcum* ssp. *monococcum* landrace populations, (ii) to investigate genetic diversity in *Triticum monococcum* ssp. *monococcum* landrace sub-populations, and (iii) to determine population structure and genetic differentiation among the populations and among the sub-populations in *Triticum monococcum* ssp. *monococcum* landrace populations grown in farmers' fields in Bolu and Kastamonu provinces in Türkiye.

DNA Isolation: The genomic DNA was extracted from the leaves of 1-1.5-month-old plants by the modification method as defined by Kidwell and Osborn (1992) as follows:

1. Collect more than one gramme of young leaf tissue from wholesome plants, freeze the samples, and keep at -20 °C in the desiccator.
2. Melt 250-300 mg lyophilized tissue into fine powder at room temperature and transfer tissue to the marked polypropylene pipe of 15 or 50 ml.
3. Add DNA buffer (5-10 ml) (approximately 1 ml per 30-50 mg tissue; the optimal ratio of tissue may vary with different plant species). Suspend the tissue in the buffer carefully and completely with gentle, rocking movements.
4. Incube with periodic mixing for 60 min at 55-60°C.
5. Add chloroform and isoamyl alcohol in equal proportions (24:1) and carefully and gently mix together. 1000-5000 g centrifuge, 20 ° C for 30-50 minutes.
6. Put the aqueous (upper) phase into the marked 50-ml tube using a large pipette and add 2,5 EtOH volumes to the tube (-20 °C) or 0,6-1 isopropanol volumes (-20 DC). Mix well until DNA rushes.
7. Choose the suitable procedure for washing, drying, and re-dissolving samples according to the state of precipitated DNA.

DNA Amplification: For DNA amplification, iSSR-PCR reactions were performed in a volume of 20 µL reaction mixture containing 1x Taq buffer (10×), 3 mM MgCl2 (25 mM), 200 µM dNTPs (10 mM each), 0.2 u of Taq DNA polymerase (5u/µL, Thermo), 0.2 µMISSR primer (10 pMol, Query, Alpha DNA), 1 µL template DNA (10–40 ng) in final concentration and distilled water was added up to 20 µL. PCR amplification was carried in a Thermo Scientific thermocycler PCR system.

The thermal program for DNA amplification was programmed as one cycle for 4 min at 94°C, 35 cycles for 45 s at 94°C, for 30 s at 58°C, and for 2 min at 72°C, followed by one cycle for 7 min at 72°C. The iSSR-PCR amplicons were run along with a 100-bp DNA molecular size marker (Thermo) on a 1.3% agarose gel (Sigma), and electrophoresis was carried out at 80 mA / 160 V for 2-2.5 h. Ethidium bromide (10 mg/mL) staining was used to visualize amplified fragments, and the pictures were taken under UV light (DNR bio-imaging system).

2.3. Statistical Analysis

2.3.1. *The Kaiser-Meyer-Olkin (KMO) test:*

To analyze the data on agro-morphological traits in each population, the Kaiser-Meyer-Olkin analysis (KMO) was applied to compare the populations. The KMO test is a metric test to estimate whether the information is suitable for factor analysis. The statistics were used to quantify the proportion of the difference between variables that is considered normal. The lower the percentage, the better the factor analysis (Goto *et al*., 2011).

The variance displayed the proportion of the deviation (dispersion) of the data collected by a statistical model. Often, a deviation is measured as a variance, so the explicit variance is used for more precise expression. The difference in fraction described by the main component is the ratio of the variance of the main component to the total variance (O'Grady, 1982). The estimate of the sample variances for all the individual variables is called the total variance (O'Grady, 1982). Bartlett's sphericity test, often performed before PCA or factor analysis, examines whether data come from a multivariate standard zero covariant distribution (Jackson, 1993).

2.3.2. *Coefficient of variation % (CV %)*

The coefficient of variation indicates the degree of variability in data as relative to the population mean. The CV % is the ratio of the standard deviation to the mean. A high coefficient of variation means a high distribution around the mean. Without units, measurement scales

allow comparison between distributions of non-comparable values. The lower the value of the coefficient of variation, the more accurate the prediction (Insee, 2016). If CV values are categorized according to ranges; CV<10 is very good, 10-20 is good, 20-30 is acceptable, and CV>30 is not acceptable (Insee, 2016).

2.3.3. *Analysis of variance (ANOVA)*

ANOVA is a type of statistical test used to determine if there is a statistically significant difference between two or more categorical groups by testing for differences in means using variance. Two hypotheses are proposed, in the null hypothesis (H_0), and the difference between the means of the groups is statistically significant. In the alternate hypothesis (H_0), when $p< \alpha$ (0.05) is calculated, it means that the means of some of the groups are unequal, and H_0 is rejected according to Steel and Torrie (1980).

2.3.4. *Principal component analysis (PCA)*

The principal component analysis distills the essence of the data into a few key components that explain the most variation in the data set. The principal components, which are based on the eigenvectors of the correlation matrix derived from the boron treatment data set of 48 einkorn wheat genotypes, were calculated by IBM-SPSS statistical software.

2.3.5. *Pearson's correlation*

The Pearson's correlation values were observed among the agro-morphological traits in einkorn wheat landraces populations. Pearson's correlation coefficients (r_P) were computed to relate the measures of metric agro-morphological traits by using SPSS statistical software (version 22 for Windows) according to Steel and Torrie (1980).

3. FINDINGS

3.1. Descriptive Statistics

According to descriptive statistics, the minimum values for agro-morphological traits ranged between 0.37 and 74.48 for the traits RL and LW, respectively, while the maximum values ranged between 1.70 and 426.90 for the traits CL and LW, respectively. The highest mean value was observed as 215.16 for LW, while the lowest mean value was observed as 1.06 for CL (Table 2). The standard deviation range was calculated between 0.30 and 68.89.

Table 2. The descriptive values observed in agro-morphological traits in Einkorn wheat landrace populations (abbreviations: N: Sample Number, CL: Coleoptile Length, RN: Root Number, RL: Root Length, GP: Germination Power, FRW: Fresh Root Weight, DRW: Dry Root Weight, LW: Leaf Weight)

	N	Minimum	Maximum	Mean	Std. Deviation
CL	48	0.40	1.70	1.06	0.30
RN	48	1.27	4.53	3.20	0.74
RL	48	0.37	3.41	1.75	0.67
GP	48	5.33	10.00	8.98	0.81
FRW	48	9.37	141.60	60.50	31.85
DRW	48	2.23	35.17	15.45	8.00
LW	48	74.48	426.90	215.16	68.89

3.2. Coefficient of Variation

The mean values for agro-morphological traits ranged between 1.06 and 215.16. According to the coefficient of variation values, the highest value was observed in FRW at 52.09%, while the lowest value was observed in LW at 8.9% (Table 3).

Table 3. Coefficient of variation in agro-morphological traits calculated in einkorn wheat populations (abbreviations: CL: Coleoptile Length, RN: Root Number, RL: Root Length, GP: Germination Power, FRW: Fresh Root Weight, DRW: Dry Root Weight, LW: Leaf Weight, N: Sample Number, M: Mean, SS: Squared Deviation, CV: Coefficient of Variation).

	N	M	SS	$\sigma^2 = SS/N$	$\sigma = \sqrt{\sigma^2}$	CV (%) = (σ/M) *100
CL	48	1.06	4.31	0.09	0.3	28.26
RN	48	3.2	26.06	0.54	0.74	23.02
RL	48	1.75	20.84	0.43	0.66	37.71
GP	48	8.98	31.03	0.65	0.80	8.95
FRW	48	60.50	47672.91	993.19	31.51	52.09
DRW	48	15.45	3007.20	62.65	7.92	51.23
LW	48	215.16	223075.05	4647.4	68.17	31.68

3.2.1. *One-way ANOVA*

According to one-way ANOVA, the measurement of agro-morphological traits of 48 *Triticum monococcum* L. ssp. *monococcum* landrace populations differed significantly between the groups; thus, the null hypothesis was rejected. The F values were 307.16 (at $p<0.05$ significance level) between the groups. When the results of the ANOVA were significant for the agro-morphological traits of 48 *Triticum monococcum* L. ssp. *monococcum* landrace populations, a post hoc test-Tukey's HSD was run to identify where the differences truly came from.

According to Tukey's HSD, the measurement of agro-morphological traits in 48 *Triticum monococcum* L. ssp. *monococcum* landrace populations was not homogeneous, and significant variations were determined between the groups. On the other hand, the variations within the groups were not significant. Significant differences were observed between FRW and CL, RN, RL, GP, and DRW as 60.04, 57.91, 59.35, 52.17, and 45.52, (at $p<0.05$ significance level), respectively, while between LW and CL, RN, RL, GP, FRW, and DRW, significant differences were observed as 215.52, 213.39, 214.83, 207.66, 155.48, and 201.00 (at $p<0.05$ significance level), respectively.

3.3. Pearson's Correlations among the Agro-Morphological Traits

The pearson's correlations among the agro-morphological traits were performed. The results indicated that there were significant correlations among the traits. The highest significant value was determined as 0.910 ($r = 0.00$ at $p <0.01$ significant level) between DRW and RL, while the lowest significant value was 0.296 ($r = 0.04$ at $p <0.05$ significant level) between LW and RL (Table 4).

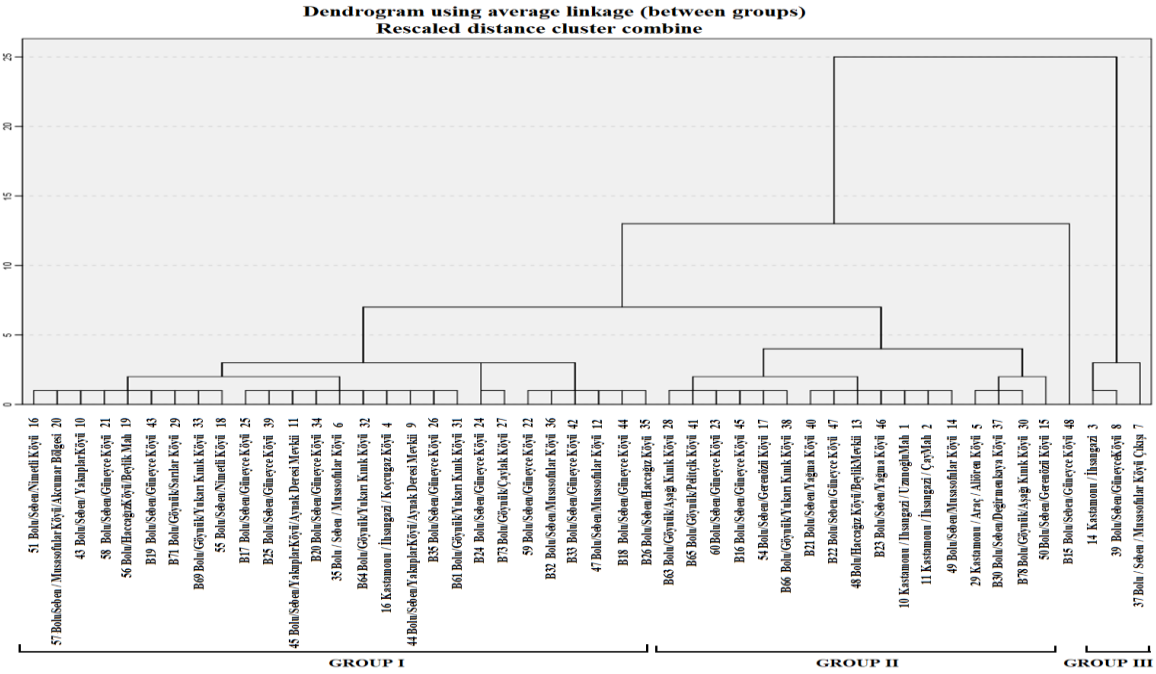
Table 4. Coefficient of variation in agro-morphological traits calculated in einkorn wheat populations (abbreviations: CL: Coleoptile Length, RN: Root Number, RL: Root Length, GP: Germination Power, FRW: Fresh Root Weight, DRW: Dry Root Weight, LW: Leaf Weight, N: Sample Number, M: Mean, SS: Squared Deviation, CV: Coefficient of Variation).

		CL	RN	RL	GP	FRW	DRW	LW
CL	r_P	1.00	-	-	-	-	-	-
	p	-	-	-	-	-	-	-
RN	r_P	0.815**	1.00	-	-	-	-	-
	p	0.00	-	-	-	-	-	-
RL	r_P	0.629**	0.695**	1.00	-	-	-	-
	p	0.00	0.00	-	-	-	-	-
GP	r_P	0.438**	0.561**	0.14	1.00	-	-	-
	p	0.00	0.00	0.33	-	-	-	-
FRW	r_P	0.356*	0.538**	0.870**	0.01	1.00	-	-
	p	0.01	0.00	0.00	0.92	-	-	-
DRW	r_P	0.571**	0.715**	**0.910****	0.13	0.832**	1.00	-
	p	0.00	0.00	0.00	0.39	0.00	-	-
LW	r_P	0.04	0.20	**0.296***	0.10	0.28	0.405**	1.00
	p	0.80	0.17	0.04	0.50	0.05	0.00	-
N		48	48	48	48	48	48	48

3.4. The Phylogenetic Relationships Among All Analyzed Populations According to Agro-Morphological Characters

A dendogram was constructed based on agro-morphological traits. The Einkorn wheat landrace populations were clustered into two main groups (Figure 4). The populations from the same area tended to be grouped into the same sub-groups in the first main group I. In the second main group, only three populations, one from Kastamonu and two from Bolu Provinces, were grouped as an out-group.

Figure 4. The dendrogram was constructed according to the between-group linkage method based on squared Euclidean distances, representing the phylogenetic relationship between 48 Turkish Einkorn wheat landrace populations.



3.5. Principal Component Analysis (PCA)

According to PCA results, the first two eigenvalues explain about 76.93% of the variance in the seven-dimensional data for morphometric data (Table 5). The first PC is the linear combination PC1 = 0.76 CL + 0.88 RN + 0.93 RL + 0.81 FRW + 0.92 DRW and 0.37 LW. It can be interpreted as a contrast between the CL and RN variables and the RL, FRW, DRW, and LW variables. For the second PC, the coefficients for the GP variable were small, PC2 = + 0.77 GP (Table 6). It can be interpreted as a weighted sum of vectors that point mostly in the direction of the GP, CL, and RN. In the component plot graph, the horizontal axis represents the PC1, and the vertical axis represents the PC2 (Figure 5).

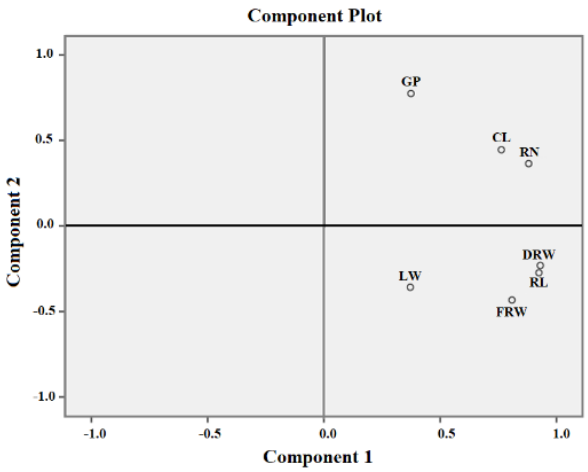
Table 5. Total variance explained by principal component analysis (PCA) performed by using data from CL, RN, RL, GP, FRW, DRW, and LW as variables according to the *Pearson* correlation (one-tailed) matrix with Eigenvalues, percentage of variance, and cumulative percentage of variance (C: component)

C	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.01	57.29	57.29	4.01	57.29	57.29
2	1.37	19.64	76.93	1.37	19.64	76.93
3	0.91	13.04	89.97			
4	0.40	5.75	95.73			
5	0.14	2.05	97.78			
6	0.10	1.47	99.25			
7	0.05	0.75	100.00			

Table 6. Component matrix of values, produced by PCA, of the variables (CL, RN, RL, GP, FRW, DRW, and LW) and their contribution to principal components (abbreviations: V: Variable, CL: Cleoptile Length, RN: Root Number, RL: Root Length, GP: Germination Power, FRW: Fresh Root Weight, DRW: Dry Root Weight, LW: Leaf Weight).

V	Components	
	1	2
CL	0.76	0.45
RN	0.88	0.36
RL	0.93	-0.23
GP	0.37	0.77
FRW	0.81	-0.43
DRW	0.92	-0.27
LW	0.37	-0.36

Figure 5. The component plot graph constructed by PCA represents the component 1 and component 2 values derived from 48 einkorn wheat landrace populations (abbreviations: CL: Cleoptile Length, RN: Root Number, RL: Root Length, GP: Germination Power, FRW: Fresh Root Weight, DRW: Dry Root Weight, LW: Leaf Weight)



3.6. The Statistical Analysis for Population Genetics

The iSSR-PCR amplification fragments were scored using TotalLab Image Quant software along with visual scores on the photographs of the gels. The raw information was translated into binary data: 1 for the current fragment and 0 for the missing fragment. Therefore, the data were diploid and dominant, and binary data were calculated using PopGen version 1.32 for population genetic analysis (Yeh *et al*., 1997). The mean number of alleles per locus (n_a), efficient alleles per locus (n_{ea}), and the mean value of genetic diversity (h) have been estimated for gene diversity estimates (Nei, 1973). The genetic distinction between populations was normally calculated by G_{ST} for mostly inherited DNA markers (Nei, 1973), which demonstrates the separation of genetic differences within and within populations. The gene flow (N_m) between populations of the sample was calculated using the G_{ST}value. Dendrograms built using an unweighted pair group approach with an arithmetic average (UPGMA) based on iSSR data were used to represent the phylogenetic relationships between populations or subpopulations.

3.6.1. *Genetic diversity estimates of einkorn wheat landrace populations*

The genetic diversity of einkorn wheat (*Triticum monococcum* L. ssp. *monococcum*) landrace populations was investigated by the inter-simple sequence repeats (iSSR) molecular marker system. In this study, 10 x 3 seeds for each population seed were analyzed using the iSSR primer UBC-826, which produced 30 polymorphic bands. The mean number of alleles, effective allele, and genetic diversity value at the locus level were observed to be 2, 1.33, and 0.13, respectively (Table 7). The highest number of alleles existed in Bolu-Seben population, while the lowest number was in Kastamonu-İhsangazi population. The highest number of effective alleles and genetic diversity values were observed as 1.29 and 0.18 in both Bolu-Seben / Güneyce and Bolu - Seben populations, respectively. The highest number of polymorphic bands was determined to be 22 (73%) in the Bolu-Seben population, while the lowest polymorphic band was determined to be 15 (50%) in the Kastamonu-İhsangazi population.

The total genetic diversity and the genetic diversity within the populations were identified as 0.21 and 0.17 at population levels, respectively (Table 8). The genetic differentiation among the populations was calculated at 0.21, while the gene flow between the populations was 1.91. The genetic distance between Population 1 and both Populations 2 and 3 was 0.06, while the genetic distance between Populations 2 and 3 was 0.12 (Table 9). Using iSSR data, the researchers were able to generate a dendrogram showing how different populations clustered. As a result, the Bolu population clustered away from the Kastamonu population (Figure 6).

Table 7. The total genetic diversity estimates among the Einkorn wheat landrace populations.

POP	Sample Size	n_a	n_{ea}	h	# PL	% PL
BOLU-SEBEN/GÜNEYCE	10	1.67	**1.29**	**0.18**	20	0.67
BOLU-SEBEN	10	**1.73**	**1.29**	**0.18**	**22**	**0.73**
KASTAMONU-İHSANGAZİ	10	**1.50**	**1.22**	**0.13**	**15**	**0.50**
MEAN	30	2	1.33	0.21	30	100

Table 8. The total genetic diversity and F statistics estimates among the Einkorn wheat landrace populations.

	Sample Size	H_T	H_s	G_{ST}	N_m
MEAN	30.00	0.21	0.17	0.21	1.91

Table 9. The genetic distance values among the Einkorn wheat landrace populations.

Pop ID	1	2	3
1	****		
2	0.06	****	
3	0.06	0.12	****

Figure 6. The dendrogram representing the phylogenetic relationships among einkorn wheat landrace populations.

3.6.2. *Genetic diversity estimates in the einkorn wheat landraces sub-populations*

The seed samples were collected from the local farmers in the region of Bolu-Seben and Kastamonu-İhsangazi. Bolu-Seben-Güneyce, Bolu-Seben, and Kastamonu-İhsangazi populations were divided into 4, 5, and 4 sub-populations, respectively. The genetic diversity estimates at the locus level were performed for 13 sub-populations. The mean number of alleles, effective alleles, genetic diversity value, and polymorphic locus number were observed as 2.00, 1.39, 0.24, and 30.00 (100%), respectively (Table 10). The highest numbers of alleles, effective alleles, genetic diversity values, and polymorphic locus numbers were determined to be 1.33, 1.21, 0.13, and 10 (33%) in the sub-population of Bolu/Seben/Güneyce (21/1), respectively. Additionally, the highest effective allele number of 1.21 was observed in the Bolu/Seben/Güneyce (23/2) and Bolu/Yakuplar populations, too. The lowest number of alleles, effective allele, genetic diversity value, and polymorphic locus number were identified as 1.13, 1.09, 0.06, and 4 (13%) in the sub-population of Bolu/Haccağız /Beylik region, respectively (Table 10).

Table 10. The total genetic diversity estimates among einkorn wheat landrace sub-populations.

POP		Sub-pop	Sample Size	n_a	n_{ea}	H	# PL	% PL
1	Bolu/Seben/Güneyce Köyü (8/1)	1	3	1.17	1.11	0.06	5.00	0.17
	Bolu/Seben/Güneyce Köyü (21/1)	2	3	1.33	1.21	0.13	10.00	0.33
	Bolu/Seben/Güneyce Köyü (22/1)	3	2	1.23	1.17	0.10	7.00	0.23
	Bolu/Seben/Güneyce Köyü (23/2)	4	2	1.30	1.21	0.12	9.00	0.30
2	Bolu/Yakuplar Köyü	5	2	1.30	1.21	0.12	9.00	0.30
	Bolu/ Haccağız Köyü/Beylik Mevkii	6	2	1.13	1.09	0.06	4.00	0.13
	Bolu/Seben/Musasofular Köyü	7	2	1.20	1.14	0.08	6.00	0.20
	Bolu/Seben/Gerenözü Köyü	8	2	1.17	1.12	0.07	5.00	0.17
	Bolu/Seben/Nimetli Köyü	9	2	1.27	1.19	0.11	8.00	0.27
3	Siyez-Kastamonu/İhsangazi/Uzunoğlu Mah.	10	3	1.23	1.15	0.09	7.00	0.23
	Siyez-Kastamonu/İhsangazi/Çay Mah.	11	3	1.20	1.16	0.09	6.00	0.20
	Siyez-Kastamonu/İhsangazi	12	2	1.17	1.12	0.07	6.00	0.20
	Siyez-Kastamonu/İhsangazi/Koçcuağzı Köyü	13	2	1.17	1.12	0.07	5.00	0.17
		Mean	30	2.00	1.39	0.24	30.00	1.00

At the sub-population level, the total genetic diversity and the genetic diversity within the sub-populations were 0.25 and 0.09, respectively, while the genetic differentiation and gene flow between populations were 0.63 and 0.29, respectively (Table 11).

Table 11. The total genetic diversity and G (*F*) statistics estimates among einkorn wheat landrace sub-populations.

Sample Size	H_T	H_S	G_{ST}	N_m
30	0.25	0.09	0.63	0.29

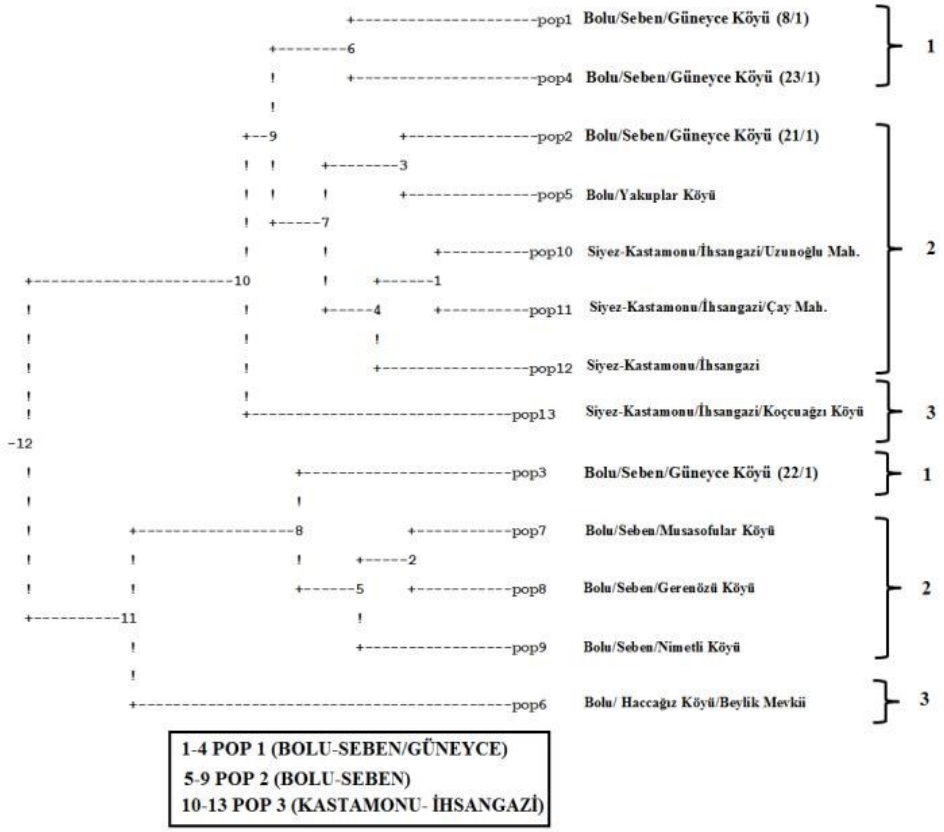
According to the genetic distance values, the highest genetic distance value was 0.49 between the sub-populations 4 and 6, while the lowest genetic distance value was 0.06 between the sub-populations 7 and 8, 10 and 11 (Table 12).

Table 12. The genetic distance values among the Einkorn wheat landrace sub-populations.

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13
1	****												
2	0.13	****											
3	0.29	0.12	****										
4	0.11	0.11	0.12	****									
5	0.15	0.08	0.22	0.15	****								
6	0.46	0.31	0.31	**0.49**	0.33	****							
7	0.27	0.17	0.14	0.24	0.21	0.18	****						
8	0.31	0.12	0.10	0.26	0.21	0.17	**0.06**	****					
9	0.33	0.17	0.14	0.27	0.21	0.22	0.11	0.07	****				
10	0.13	0.14	0.29	0.19	0.13	0.34	0.24	0.28	0.27	****			
11	0.08	0.09	0.23	0.15	0.09	0.37	0.21	0.25	0.24	**0.06**	****		
12	0.18	0.14	0.33	0.24	0.14	0.45	0.33	0.31	0.24	0.10	0.08	****	
13	0.14	0.17	0.35	0.19	0.17	0.42	0.31	0.33	0.33	0.16	0.15	0.10	****

A dendrogram constructed based on the genetic distance values, which were calculated according to iSSR data, indicated that the sub-populations were divided into sub-groups under two major groups. In general, in the first major group, Bolu-Seben/Güneyce and Kastamonu-İhsangazi populations were grouped together but in different subgroups, while in the second major group, Bolu-Seben populations were grouped. In the first major group, the population of Kastamonu-İhsangazi/Koçcuağzı was grouped as an outlier group, while in the second major group, the population of Bolu-Haccağız/Beylik region was grouped as an outlier group (Figure 7).

Figure 7. The dendrogram representing the phylogenetic relationships among einkorn wheat landraces and sub-populations.



4. DISCUSSION

According to the genetic diversity estimates, the average successful allele number and genetic diversity value were significantly high. One of the most important tools for determining genetic variation is the number of effective alleles. The number of successful alleles corresponded to the number of alleles contributing to genetic variation, roughly. The number of alleles included in the sample is very high, which shows that genetic variation is still very high. The extent of the genetic variation of self-pollinating plant species is very low, and the cross-pollination rate of the einkorn is less than 1 percent. However, the estimates of genetic variation observed in this study were quite high. One of the reasons was that einkorn wheat has been produced by local farmers for 10,000 years and passed down from generation to generation. Therefore, they harbor quite different gene/gene combinations in their gene pools, which may be the reason for the higher level of genetic diversity in their gene pools. According to the genetic diversity data observed at the locus level, Bolu populations had higher genetic diversity than Kastamonu populations. Considering the populations collected from both provinces and the number of samples analyzed, it made sense to have higher genetic diversity as the number of samples is higher in Bolu populations.

According to the genetic diversity data at the population level, the total genetic diversity was higher than the genetic diversity within the population. When the value of genetic differentiation among populations is > 15%, the genetic differentiation is substantially high. However, although the value of the genetic differentiation in this study appears to be high according to this criterion, it may not be that high compared to previous studies on einkorn wheat and other primitive landraces (Keskin *et al.*, 2015; Özbek *et al.*, 2011, 2012, 2013; Ozbek, 2021). The gene flow and genetic distance data also supported our study.

Three populations were divided into 13 sub-populations according to their gathering locations. In the sub-populations formed this way, the mean number of effective alleles and genetic diversity values were observed at very high levels. If the number of samples studied in sub-populations was large, estimates of genetic diversity would be observed at a higher level. While Bolu-Seben / Güneyce sub-populations showed higher values of genetic diversity values than other sub-populations, Kastamonu-İhsangazi sub-populations showed lower values of genetic diversity. The total genetic diversity values at the population level were quite high compared to the genetic diversity values at the locus level. On the other hand, the values of genetic diversity observed in subpopulations were quite low. Another remarkable result in sub-populations was that genetic differentiation was substantially high, whereas the level of gene flow was very low. Local farmers who produce local wheat exchange their seeds with neighboring farmers or farmers in other cities, buy local seeds from merchants, or mix them with their own seeds and plant them in their fields. This increases genetic diversity while eliminating the negative effects of seed depression. However, the high level of genetic differentiation observed in subpopulations in this study indicated that the villagers did not exchange their seeds or did so at very low levels. Villagers probably consume some of their harvested seeds while using some as seeds and sowing them in their fields for the next year. Since the same seeds are constantly used, genetic changes occur at different points in the genomes of the seeds in the hands of the villagers. The insufficient level of seed exchange and low cross-pollination levels due to selfing Three populations were divided into 13 sub-populations according to their gathering locations. In the sub-populations formed this way, the mean number of effective alleles and genetic diversity values were observed at very high levels. If the number of samples studied in sub-populations was large, estimates of genetic diversity would be observed at a higher level. While Bolu-Seben / Güneyce sub-populations showed higher values of genetic diversity values than those of other sub-populations, Kastamonu-İhsangazi sub-populations showed lower values of genetic diversity. The total genetic diversity values at the population level were quite high compared to the genetic diversity values at the locus level. On the other hand, the values of genetic diversity observed in subpopulations were quite low. Another remarkable result in sub-populations was that genetic differentiation was substantially high, whereas the level of gene flow was very low. Local farmers who produce local wheat exchange their seeds with neighboring farmers or farmers in other cities, buy local seeds from merchants, or mix them with their own seeds and plant them in their fields. This increases genetic diversity while eliminating the negative effects of seed depression. However, the high level of genetic differentiation observed in subpopulations in this study indicated that the villagers did not exchange their seeds or did so at very low levels. Villagers probably consume some of their harvested seeds while using some as seeds and sowing them in their fields for the next year. Since the same seeds are constantly used, genetic changes occur at different points in the genomes of the seeds in the hands of the villagers. The insufficient level of seed exchange and low cross-pollination levels due to selfing also cause low gene flow. As a result, genetic differentiation is increasing among the einkorn wheat populations and sub-populations. According to the genetic distance data, although, interestingly, the highest genetic distance is observed among Bolu sub-populations, the genetic distance data is consistent with other results in general. also cause low gene flow. As a result, genetic differentiation is increasing among the einkorn wheat populations and sub-populations. According to the genetic distance data, although, interestingly, the highest genetic distance is observed among Bolu sub-populations, the genetic distance data is consistent with other results in general.

The dendrogram constructed according to iSSR data for sub-populations is consistent with genetic distance and genetic differentiation values. When the analysis was done for only three populations, the Bolu and Kastamonu populations were grouped separately, while in the analyses of sub-populations, the Bolu-Seben/Güneyce sub-populations and the Kastamonu sub-

populations were clustered together but in different sub-groups in the first major group, while the Bolu-Seben sub-populations were clustered separately from the other groups. This is because einkorn wheat seeds have been distributed by Bolu Municipality to local farmers since 2014. Most likely, the einkorn wheat seeds distributed in the Bolu-Seben-Güneyce region originated from the einkorn wheat seeds grown in their fields. Apart from these, the eco-geographic (rainfall, humidity, temperature, daylight length, altitude, latitude, longitude, etc.) conditions in the regions where these populations were raised may have had a certain effect on genetic diversity and differentiation.

The genetic differentiation between the sub-populations was substantially higher (63%) than within populations (37%). The high level of genetic diversity determined in wheat landraces is related to some other functional factors. These factors might be, after the domestication process, domesticated wheat varieties that started to be cultivated by traditional farmers and the seeds have been sown for thousands of generations since then. The natural selection in the environment and farmers' interest in the wheat varieties they grew contributed to shaping the population structure. They also made selections on wheat they grow for their resistance to biotic and abiotic stress factors, and the amount of yield and yield stability in low-input agricultural systems (Ozbek, 2021; Zeven, 1999).

Inter-Retrotransposon Amplified Polymorphism (iRAP) markers indicated that Iranian diploid einkorn wheat (*Triticum monococcum* L. ssp. *monococcum*, *T. boeoticum* subsp. *boeoticum*, *T. boeoticum* subsp. *Thaoudar,* and *T. urartu*) had high genetic similarity due to high affinity and gene flow; however, *Triticum monococcum* L. ssp. *monococcum* was distinctively different from *T. boeoticum* and *T. urartu*, which were distant species to the other species studied (Eslami Farouji *et al*., 2015). Genetic diversity was investigated in 36 diploid wild einkorn wheats (*Triticum boeoticum*) by AFLP (Malaki *et al*., 2006), in diploid species belonging to the genus *Triticum* by RFLP (Le Corre & Bernard, 1995), and in 36 diploid wild einkorn wheats (*Triticum boeoticum*) from West Iran by RAPD, AFLP, and SSR markers (Naghavi *et al*., 2007). A larger collection of miRNAs and small RNA molecules was used for the analysis of *Triticum monococcum* L. ssp. *monococcum* plant samples grown under natural, drought, and salinity conditions. The appearance of 167 supposedly mature miRNA sequences belonging to 140 distinct miRNA families was suggested by next-generation technologies and bioinformatics analyses. In addition to a systematic study of scanned target genes within the *T. aestivum* L. genome, a comparative analysis was conducted to see target mirror genes that included the management of salt and drought (Ünlü *et al*., 2018). Ten cultivated einkorn (*Triticum monococcum* L. ssp. *monococcum*) landrace populations originating from Türkiye were investigated to determine the genetic diversity of high-molecular-weight (HMW) glutenin subunits and the gliadins. The cultivated einkorn populations in Türkiye displayed an enormous amount of genetic diversity for seed storage proteins of glutenin (H_e = 0.65) and gliadins (H_e = 0.17) (Keskin Şan *et al*., 2015; Ozbek, 2021).

5. CONCLUSION

The cultivated einkorn wheat [*Triticum monococcum* L. ssp. *monococcum* (2n = 2x = 14, A^mA^m)] and emmer wheat [*Triticum turgidum* ssp. *dicoccon* Schrank Thell. (2n = 4X = 28, *AABB*)] were the most popular crops until the early Bronze Age. Then, they started to be replaced by high-yielding and free threshing wheat varieties (*Triticum aestivum* L. 2n = 6X = 42, *AABBDD,* and *Triticum durum,* 2n = 4X = 28, *AABB*). Today, both species are relict and growing in Morocco, Tunisia, Italy, Spain, the Balkans, and Türkiye (Fritsch *et al*., 1996; Ozbek, 2021).

When reviewing the agro-morphological character averages, we found that one of the best characters was in population 27 of the IZA-Bolu/Göynük/Çaylak region for germination strength, root count, and root length. Germination power provides a better yield at harvest

because the population has better germination strength, the highest number of roots, and the best root length compared to other populations.

Population 21, IZA-Bolu/Seben/Güneyce Village, had the lowest mean coleoptile length, root count, root length, fresh root weight, and dry root weight.

The dendrograms presented show that some agro-morphological characteristics in certain populations do not correlate with those in other populations. For coleoptile length and root count, fresh root weight, and dry root weight, Population 21 had no relationship with the other populations. In population 27, the germination power and the root length did not correlate with each other. Population 15, on the other hand, did not correlate for fresh-root with the other populations, and Population 47 and Population 7 did not have a correlation for leaf weight with the other populations, and Population 1 did not correlate for germinating strength with the other population, and Population 35 did not correlate for coleoptile-length with the other populations.

Population 21 and Population 27 are the least related to the rest of the populations, which indicated that the characteristics of the other populations were different. While it has been found previously that the average Tukey HSD test also showed that Population 27 had the best characters, Population 21 had the worst characters.

Molecular markers have been used efficiently to represent phylogenetic relationships in plant species. An investigation of the nuclear and chloroplast genomes of diploid species using amplified fragment length polymorphism (AFLP) and simple sequence length polymorphism (SSLP) displayed that *T. urartu* was greatly differentiated from the other two *A-genome* species, and einkorn wheat had lower genetic diversity than that of *Aegilops* species (Mizumoto *et al*., 2002).

The wheat landraces have accumulated an enormous amount of genetic variation over thousands of years. The extent of variability, characterization, and partition of genetic diversity within a local germplasm collection are important criteria to determine the status of wheat landraces, particularly for future interests in their uses and for the improvement and efficient genetic diversity maintenance and utilization of plant species (Desheva *et al*., 2020).

Recently, people's interest in healthy foods has also increased interest in the cultivation of wheat landraces in Europe, and North African countries such as Morocco, Egypt, and Ethiopia, as well as in Türkiye. Nevertheless, it seems that einkorn wheat landraces are, today, growing only in Bolu, Çankırı, Çorum, Kayseri, Sinop, and Kastamonu provinces in Türkiye (Özberk *et al*., 2016).

The study found apparent differences between the populations in seven characters, and as can be noticed, there are differences between the populations in different cities in Türkiye. Accordingly, it is recommended that more extensive studies be carried out among the population on the most apparent characteristics through the germination of the population for a longer period until the crop ripens. In addition, they study the differences between the populations in terms of leaf length, PH, spike length, spike weight, number of seeds per spike, seed weight, and population study under different environmental conditions.

Also, Population 27 of IZA-Bolu/Göynük/ÇaylakKöyü is recommended to obtain the germination power of the crop and obtain productive power at harvest because the population has the highest germination power, the greatest number of roots, and the best root length compared to other populations.

Due to the presence of einkorn wheat in other countries, it is recommended to make a comparative study between the einkorn wheat in Türkiye and that in other countries for agro-morphological and molecular characteristics.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Suliman Zommita: Conception and design, sampling, data collection and analysis, evaluation of results, writing/editing the manuscript. **Ozlem Ozbek:** Conception and design, data analysis, evaluation of results, editing the manuscript, supervising. **Gulgez Gokce Yildiz:** Data collection, editing the manuscript. **Omer Can Unuvar:** Data collection and editing the manuscript. **Ercan Selcuk Unlu:** Conception and design, data collection, evaluation of results, and editing the manuscript. **Nusret Zencirci:** Supervising conception and design, editing the manuscript.

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