T.R.
BOLU ABANT İZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES

ASSESSMENT OF GENETIC DIVERSITY BASED ON AGRO-MORPHOLOGICAL TRAITS AND ISSR MOLECULAR MARKERS IN EINKORN WHEAT (*Triticum monococcum* ssp. *Monococcum*) LANDRACES POPULATIONS FROM TURKEY

MASTER OF SCIENCE

SULIMAN HISAIN MOHAMED ZOMMITA

BOLU, HAZİRAN- 2021

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APPROVAL OF THE THESIS

ASSESSMENT OF GENETIC DIVERSITY BASED ON AGRO-MORPHOLOGICAL TRAITS AND ISSR MOLECULAR MARKERS IN EINKORN WHEAT (*Triticum monococcum* ssp. *Monococcum*) LANDRACES POPULATIONS FROM TURKEY submitted by **SULIMAN HISAIN MOHAMED ZOMMITA** and defended before the Examining Committe Members listed below in partial fulfillment of the requirements for the degree of **Master of Science**i n **Department of Biology, Institute of Graduate Studies of Bolu Abant Izzet Baysal University in 23.06.2021** by

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SULIMAN HISAIN MOHAMED ZOMMITA

ABSTRACT

ASSESSMENT OF GENETIC DIVERSITY BASED ON AGRO-MORPHOLOGICAL TRAITS AND ISSR MOLECULAR MARKERS IN EINKORN WHEAT (*Triticum monococcum* ssp. *Monococcum*) LANDRACES POPULATIONS FROM TURKEY
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In this study, it was aimed to investigate genetic diversity, population genetic structure, and genetic differentiation in 48 einkorn landraces wheat (*Triticum monococcum* ssp. *monococcum*) populations grown in farmer fields in Bolu and Kastamonu. For this purpose, seven agro-morphological traits and morphological variation were investigated, while genetic variation was investigated at the molecular level with ISSR molecular markers. As agro-morphological traits, leaf weight (mg), coleoptile length (cm), root number (n), root length (cm), fresh root weight (mg), dry root weight (mg) were examined. Genetic variation was evaluated in the obtained morphometric data by using coefficient of variation, ANOVA and principal coordinates analysis statistical analysis methods. According to coefficient of variation values, the highest value was observed in FRW as 52.09%, while the lowest value was observed in LW as 8.9%. According to *Pearson*'s correlation values between agro-morphological traits, the highest significant correlation value was 0.910 (p <0.01 significant level r = 0.00), the lowest significant correlation value was 0.296 (p <0.05 significant level r = 0.04) between LW and RL. PCA results revealed the variation in the two main components as 76.93%. 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 n_a = 2, n_{ea} = 1.33 and h = 0.13, respectively. The highest number of polymorphic bands were determined as 22 (73%) in Bolu-Seben population, while the lowest polymorphic band was determined as 15 (50%) in Kastamonu-İhsangazi population. Genetic differentiation between populations (63%) was observed at significantly higher levels as well as within populations (37%). It was observed that the level of genetic diversity in einkorn populations was still quite high. Among the agro-morphological traits, germination power, root number and coleoptile length appeared to be reliable traits. It was determined that the use of morphological characters alone for genetic diversity in populations is not sufficient to determine the differentiation between populations and population genetic structure.

KEYWORDS: Einkorn Wheat, *Triticum monococcum* ssp. *monococcum*, ISSR, wheat landraces, Agro-Morphological traits.

ÖZET

TÜRKİYE'NİN YEREL EINKORN BUĞDAY (*Triticum monococcum* ssp. *monococcum*) POPULASYONLARINDA GENETİK ÇEŞİTLİLİĞİN ARGRO-MORFOLOJİK ÖZELLİKLERE VE ISSR MOLEKÜLER BELİRTEÇLERİNE DAYALI OLARAK DEĞERLENDİRİLMESİ

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Bu çalışmada, Bolu ve Kastamonu'da çiftçi tarlalarında yetişen 48 einkorn yerel buğday (*Triticum monococcum* ssp. *monococcum*) popülasyonunda genetik çeşitlilik, popülasyon genetik yapısı ve genetik farklılaşmanın araştırılması hedeflendi. Bunun için yedi agro-morfolojik özellik ile morfolojik varyasyon araştırılırken ISSR moleküler markerleri ile genetik çeşitlilik moleküler düzeyde araştırıldı. Agro-morfolojik karakterler olarak yaprak ağırlığı (mg), koleoptil uzunluğu (cm), kök sayısı (n), kök uzunluğu (cm), taze kök ağırlığı (mg), kuru kök ağırlığı (mg) incelendi. Elde edilen morfometrik verilerde genetik varyasyon varyasyon katsayısı, ANOVA ve temel koordinatlar analizi istatistik analiz yöntemleri ile değerlendirildi. Varyasyon katsayısı değerlerine göre en yüksek değer %52,09 ile FRW'de, en düşük değer ise %8,9 ile LW'de gözlenmiştir. Agro-morfolojik özellikler arasındaki *Pearson* korelasyon sonuçlarına göre en yüksek anlamlı korelasyon değeri 0,910 (p <0,01 anlamlı düzeyde r = 0,00) olarak DRW ile RL arasında, en düşük anlamlı korelasyon değeri 0,296 (p <0,05 anlamlı düzeyde r = 0,04) olarak LW ile RL arasında belirlendi. PCA sonuçları iki temel bileşende varyasyonu %76,93 olarak açıkladı. Moleküler karakterizasyon için ISSR primerleri ile elde edilen veriler popülasyon genetiği analiz programı olan PopGene (ver. 1.32) ile analiz edildi. PopGene sonuçlarına göre ortalama alel sayısı, etkili alel sayısı ve genetik çeşitlilik değerleri sırasıyla n_a = 2, n_{ea} = 1.33 ve h = 0.13 olarak hesaplandı. En yüksek polimorfik bant sayısı 22 (%73) ile Bolu-Seben popülasyonunda, en düşük polimorfik bant ise 15 (%50) ile Kastamonu-İhsangazi popülasyonunda belirlendi. Popülasyonlar arasındaki genetik farklılaşma (% 63), popülasyonlar içinde (% 37) olduğu kadar önemli ölçüde yüksek düzeyde gözlendi. Einkorn popülasyonlarında genetik çeşitlilik düzeyinin hala oldukça yüksek olduğu gözlendi. Agro-morfolojik özellikler arasında çimlenme gücü, kök sayısı ve coleoptil uzunluğu güvenilir özellikler olarak ortaya çıktı. Populasyonlarda genetik çeşitlilik için morfolojik karakterlerin tek başına kullanılması popülasyonlar arasındaki farklılaşmayı ve popülasyon genetik yapısını belirlemek için yeterli olmadığı belirlendi.

ANAHTAR KELİMELER: Einkorn buğday, *Triticum monococcum* ssp. *monococcum,* Yerel buğday, ISSR, Agro-Morfolojik Özellikler.

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LIST OF ABBREVIATIONS AND SYMBOLS

LW **:** Leaf Weight (mg)

CL **:** Coleoptile Length (cm)

RC **:** Root Count (n)

RL **:** Root Length (cm)

FRW **:** Fresh Root Weight (mg)

DRW **:** Dry Root Weight (mg)

GP **:** Germination Power (%)

KMO **:** Kaiser-Meyer-Olkin Test

CV **:** Coefficient of Variation

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1. INTRODUCTION

1.1 Wheat

Wheat (*Triticum* L.) meets some of the calories and protein required for human nutrition, and it is the basic foodstuff of approximately in 40 countries which make up 35% of the world population. It is the most cultivated cereal both in Turkey and in the world. Its annual production in the world is around 785 million tons (FAOSTAT, 2019).

Wheat production in Turkey was 20.6 million tons on 7.6 million hectares with a yield per hectare was 2.81,5 kg per hectare in 2019. Seventeen million tons is approximately for bread and the remaining 3.6 million tons is for durum wheat. In parallel with the increase in the population in Turkey, it is needed to increase the wheat production (Tüik, 2019).

The nutritional needs have been met with high yielding and high-quality cultivars improved by conventional plant breeding programs. However, the world agriculture faced with serious problems due to the shifting biotic and abiotic environmental pressures. Modern cultivars have become more homogeneous for genetic structures, and landraces, though they are more diverse, contain lower genetic diversity than their transitional and wild relative forms (Newton et al., 2011).

Wild species, transitional forms, and landraces are gene repositories with a wide genetic basis and most likely constitute an important resource for eliminating the expected future problems in cultivated plants or providing new characteristics to plants (Ozkan et al., 2007; Pickersgill, 2000).

While the cultivation of spelt wheat is not practiced in Turkey, emmer and einkorn has a very old historical cultivation background in Turkey. In Çayönü excavation, wheat and emmer were found dated back 10,000 years (Pickersgill, 2000).

The genetic variation in einkorn wheat is of great importance for both durum and bread and durum wheat breeding programs (Aslan et al., 2018; Karagöz & Zencirci, 2005; Ünlü et al., 2018). The bread genotypes and grains are behaved differently for the characteristics under drought stress (Aslan et al., 2018). These wheat types are named "Kavlıca" in Eastern Anatolia as well as "Gacer" in Kayseri (Ozkan et al., 2007).

Einkorn wheat has, however, again become the center of attention for farmers and consumers around the world in recent years. The reason for this is its highly nutritious properties (Tüik, 2019). Many studies have shown that einkorn wheat is better than red hard wheat cultivars for protein, beta carotene, crude oil, phosphorus content (Abdel-Aal et al., 1997), and the 3-5 times higher amount of riboflavin and lutein. In addition to these, einkorn wheat is known to be rich in microelements such as Fe, Zn, and Mn (Pehlivan Karakas et al., 2021; Ünlü et al., 2018; Zhao et al., 2009).

In addition, the overall vitamin E content of wheat grass is higher than that of emmer, durum, and bread (Pehlivan Karakas et al., 2021). There is no ideal demand for the standard of seed to be sold and produced again according to Yaman et al (2019). As a result, the farmers grow unicorns, and are appreciated using conventional home consumption techniques, as in subsistence agriculture (traditional type). The popularity of einkorn and emmer wheat is growing while public health issues are rising. Acreage of these wheats in Turkey can also be expanded to satisfy the market demand depending on the demand.

In the different parts of Turkey, the grains still occur and are usually known as siyez, IZA, and gernik (emmer) by local people. The processing area and overall production of hulled wheat in Turkey are not statistically available (Yaman et al., 2019).

Einkorn plant is about one meter tall depending on mostly humid conditions. In dry conditions, the plant may have lower height. The cultivated einkorn spikelet has a single grain. Peeled grains are usually small (21.2-37.4 mg / grain) and have a soft endosperm texture (hardness index between -7.3 and 27.2) (Løje et al., 2003).

1.1.1 History of wheat in Turkey and in the World

Twelve thousand years ago, nomadic peoples started to increase in numbers and needed more food than ready found food in nature thanks to the more favorable climatic conditions that followed the long ice age in the ancient world. Among them, those living in the area we call Fertile Crescent (Figure 1.1) today were more fortunate than others. Because this region is the center, where wild ancestors of many grains, especially wheat and barley emerged, and, then, provided high nutritious wheat and barley grains to the lives and diets (Westoby et al., 2002).

Figure1.1. The map of Fertile Crescent

From the first day of their existence, people moved from the nomadic hunter-gatherer life style they have maintained for thousands of years to settled-productive life because planting and harvesting required a long stay in the same place. Probably, both ways of life existed together for a long time, but eventually, about 10,000 years ago, the first human farming villages on earth began to appear in southeastern Anatolia and northern Syria (Heun, 1997).

Can Hasan Hacılar and Çatalhöyük excavations revealed einkorn samples from 7,000-8,000 years ago in Turkey. Many other studies also stated that these two species were among the first cultivated plants (Zohary, D. & Hopf, 1988).

Abu Hurairah in Syria and Turkey Çayönü, Cafer Höyük archaeological sites such as Nevalı Çori are among the first agricultural villages. In the next 1,500 years, wheat agriculture spread to the south (e.g., Beidha in the Jordan Valley), east (Jarmo and Ali Kosh in Iran), and west (Aşıklı Höyük, Can Hasan III and

Çatalhöyük in Central Anatolia). The earliest cultured wheat samples in Europe were those obtained from Greece and dated back to 5,900 BC (Nesbitt, 1998).

According to the researches and findings in the region, the predecessor of wheat, a significant cultivated plant with hundreds of genetic variants, was grown in Göbekli Tepe for the first time in 12,000 years back (Zencirci, 2015).

The wheat remains regularly unearthed from settlements belonging to this period, which is called Neolithic (Neolithic Age), point to the indispensable place of wheat for people in the early times when agriculture, surplus product, settled life, and social values started to emerge. In the period of political structures and great states that started to be seen in Anatolia later, the economic and cultural importance of wheat can be observed from the large amount of wheat stocks and religious scenes they carved on the rocks.

For example, wheat silos with a capacity of 4,200–5,900 tons were found in Hattusha, the capital of the Hittites near Çorum, which founded the oldest and the first empire in Anatolia, dating from 13 century BC. İvriz Rock Relief near Konya, also belonging to the Hittites, points to the social and religious importance of wheat. Grain silos and wheat remains unearthed near the Urartian temple and palace dating back to 800 -700 BC in Patnos of Van indicate that similar traditions have continued in Anatolia for thousands of years. Wheat has preserved its importance in all civilizations in Anatolia until today (Abbo et al., 2015).

Wheat has as well affected human life economically and culturally, while man has exaggerated the evolution of wheat. There were two types of wheat planted in the first agricultural villages: emmer (*Triticum dicoccum* Schrank.) and einkorn (*Triticum monococcum* ssp.*monococcum*). These were species with a slightly larger grain than their wild ancestors, but, like wild ones, had semi-brittle stems.

In later periods, two types emerged, which were coarse-grained, tall and without husks, and therefore much easier to process: common wheat (*Triticum aestivum* L.) anddurum wheat (*Triticum durum* Desf.). These genetic and physical changes experienced by wheat were the result of artificial and natural selection pressure, which starts with the selection of wheat that serves to their own purposes for bread. Bread and durum wheat are two types that are widely cultivated all over the world today.

In some high regions of Turkey, although in very limited quantities, einkorn and emmer occur mostly as animal feed. In other parts of the world, there are other

wheat varieties or subspecies in limited quantities, suitable for local climate and soil conditions. In addition, there are wheat varieties spelta (*Triticum spelta*) in Europe, which were widely cultivated in the past, but today mostly replaced by durum and common wheat.

Turkey is the center of genetic diversity of wild wheat species (Aegilops ssp.) as mentioned before. The Middle East and its neighboring Mediterranean region and West Asia are the area where 22 wild wheat species spread. However, Turkey is the geograpical place, where 14 species are most densely found together (Van Slageren, 1994).

Wild wheat species, which we can encounter in every corner of Turkey, are of great importance both in studies on the improvement, spread and evolution of wheat. Studies conducted on today's wild and primitive einkorn wheat samples have shown that Karacadağ region in Diyarbakır is where the agriculture of einkorn wheat was started. This study shows the need for extensive research on wheat availability and diversity in Turkey. Thus, it will be possible to exercise the great potential of Turkey in the history for development and improvement of wheat today (Nesbitt, 1998).

1.1.2 Wheat Species

1.1.2.1 Einkorn Wheat

It is known as “Iza/Siyez” wheat in Turkey. The name einkorn, a single grain wheat speices, comes from German. It is also called small hulled (*T. monococcum*, syn: *Tr. monococcum* ssp. *monococcum*, *T. vulgare bidens* Alef., *Niviera monococcum* Ser.), (Bavec & Bavec, 2006).

The origin of einkorn (*Triticum monococcum* ssp. *monococcum*) especially between the Euphrates and Tigris, is the near southeast Turkey. Primitive hulled (*T. beoticum, T. aegilopoides* - single grain wheats; *T. thaoudar and T. urartu*- double grain wheats), and *Triticum monococcum* ssp. *monococcum* (einkorn) genetically have AA structure. Einkorn began to be harvested in the 16,000-15,000 Chipped Stone Age (Stallknecht, Gilbertson & Ranney, 1996).

The collection of these grains continued until the beginning of the twentieth century. In the Caucasus Mountains, in the Mediterranean and in northwestern Europe, a grain was planted according to the historical ranking. It is the first cereal grown in the Balkans. It is the most important grain grown in barren soils and

moderate environments in the Middle East and Southwest Europe. In the 1970's it was only cultivated in a few European regions (Bavec & Bavec, 2006).

Today, its products were manufactured Turkey, has been limited to small regions in India and America. Einkorn organic farming is also popular in the small areas, mainly in Italy, Australia, Switzerland, Germany, and Slovenia.

In its zenith, it was mainly cooked with water or milk and consumed in the form of porridge or simply cooked. This type of use does not require fermentation. This process was developed by the Egyptians, transformed into bread making and put into practice. More recently, it is preferred in animal nutrition and is even still consumed as feed. Therefore, no effort was made to bake einkorn bread before history and a similar trend has probably continued in more recent times. As a result, it was not considered suitable for bakery products due to its sticky dough and weak rheological properties until recently. However, despite the dough processing difficulties, some einkorn bread like bread wheat could be obtained with loaf volume and characteristics (Degidio & Vallega, 1994).

It is known as siyez (IZA) or einkorn in Turkey. It was cultivated by the Hittites and Phrygians. The name Siyez has taken its present form due to the word "zız", in Hittite language. Einkorn wheat is a different type from the wheat that Europeans call "Emmer" or "Speltatoides" and *Triticum dicoccum* Schrank Type Gernik (*Triticum dicoccum* Schrank) wheat grown in Anatolia (Wikitrend, 2011).

Einkorn has attractive agronomic and quality characteristics as the first cultivated diploid wheat. Drought and salinity are the most drastic environmental variables that have a major impact on crop yield and quality. However, plant safety mechanisms may be used to overcome these stresses. One of the most conserved mechanisms is the posttranscriptional shift in gene expression by microRNAs (miRNAs). miRNAs participated in the treatment of salt and drought stress (Ünlü et al., 2018).

Figure 1.2. Spike and enlarged grain in Einkorn

In Turkey, especially in Sinop, around Kastamonu, Western Black Sea Region, Samsun, and Bolu *Triticum monococcum* ssp. *monococcum* is called IZA (einkorn) wheat and utilized mostly for bulgur. Einkorn bulgur is a product obtained by drying einkorn wheat, whose spikes are single-grain and has a husky structure, after boiling and splitting in stone mills using completely traditional methods (Zencirci, 2015). There is 1.400 hectares of einkorn wheat cultivation area in Kastamonu region. While the production amount of Einkorn wheat throughout Kastamonu is 3,500 tons, the production of einkorn bulgur is approximately 700 tons; According to the data of İhsangazi District Directorate of Food, Agriculture and Livestock, İhsangazi has the highest production rate in 2013 with 6.750 decares, Einkorn wheat production 1.687 tons and einkorn bulgur production 470 tons. 92% of the all grains produced are wheat.43% of the only wheat is Einkorn wheat. There are 1,050 Einkorn wheat growers in the district, and the Einkorn Wheat Growers Association, which was established in İhsangazi, has approximately 100 members (Aslan et al., 2018; Ünal, 2002).

Einkorn bulgur is of great importance for Ihsangazi region, where it is intensively produced. The district was specialized in the production of einkorn bulgur and it was ensured that the name İhsangazi was associated with einkorn bulgur. The fact that Ihsangazi region is unsuitable for the cultivation of other agricultural products has made einkorn wheat, which is resistant to drought and inefficient soil, as the most important agricultural product there. While the local people consider a significant portion of the einkorn wheat they cultivate as animal feed, the other part is processed into bulgur by traditional methods (Ünlü et al., 2018).

The most important feature of einkorn bulgur in İhsangazi is that it is produced using completely traditional and natural methods. Einkorn bulgur is a very nutritious and healthy food. İhsangazi Sepetçioğlu and einkorn Bulgur Festival are organized with the first harvest of the year in order to promote the einkorn bulgur grown in the district.

Demand for einkorn bulgur from inside and outside the region is quite high, and it is important to support einkorn bulgur in the district and to increase its production. While bread is produced from einkorn wheat flour from time to time in İhsangazi, preparations are made for regular production in line with consumer demands (Ünal, 2002).

Slow Food launched by the Youth Food Movement working in Istanbul as a result of ‘Slow Food Foundation for Biodiversity', declared the first Slow Food Presidia of einkorn bulgur in Turkey. Presidium means a product that is unique to a certain region, essential to protect and must be supported in order to make it sustainable. In this sense, it represented Turkey in Turkey's first presidium product einkorn bulgur in Torino, Italy in 2012, held in the city of Terra Madre-Salone Del Gusto festival and presented to the world public. Later, "Traditional Einkorn Bulgur Producer Certificate" was given to Einkorn Bulgur Producers Association. In addition, an application has been made for Kastamonu einkorn wheat and bulgur to be a geographically marked product and the examinations came successful.

After the harvest, einkorn is first passed through the sieve. It is separated from foreign materials such as stone and soil by sieving process. Wheat pieces that are not suitable for being bulgur are separated as animal feed. Later, it boiled in a cauldron over a wood fire, laid in an open area and dried. The dried wheat is sieved and subjected to re-sorting process. In the first stage of the mill, the millstones are opened, extracted from the husk and subjected to the screening process. The sifting process is continued when the husks of all the grains are removed. In the second stage, the millstones are brought closer to each other and the splitting process is performed. Large grains are for puddings, small grains are for soup. The bulgur of einkorn pilaf is very rich in B complex vitamins, has an important place in today's nutrition understanding, has a very low glycemic index, and microbial and enzymatic reactions are almost non-existent (Degidio & Vallega, 1994).

The protein content varies between 10% and 26%. This worth is generally greater than rye, hard wheat and ordinary red wheat. The structure of amino acid is like common weeds (Troccoli & Codianni, 2005).

The evaluation and use of einkorn and emmer in the western Black Sea was calculated by Yaman et al. (Yaman et al., 2019). Although 78.0% of farmers cultivated einkorn grain, 22.0% cultivated emmer wheat. Due to the difficulty in harvesting and handling, most farmers (86.0%) have not sold or traded hulled wheat.

Løje et al. (2003) observed that 10 types of einkorn had a large amount of ash (2.3% -2.8% DM), a variable level of proteins (10.3% -19.5% DM), and glucan (0.29 percent -0.71 percent DM). It has very poor nutritional fibre (7.6% –9.9% KM), highly variable lysine levels (1.51–3.15 gram lysena 100 g^{-1} protein), low

sedimentationç and a thin mixographer's curve in comparison with typical wheat cultivars (Abdel-Aal et al., 1997).

Most grained samples showed a high decrease in number (average 362 seconds) (average 1185 BU), (Løje et al., 2003). Abdel et al. (1997), in another study, concluded that although einkorn had high protein content had low gluten elongation ability. Cooked einkorn grains have softer consistency and less white color, less stickiness, less fibrous structure than *Triticum spelta* and common wheat.

The high gelatination, viscosity and high protein content makes for the use of cooked cereals as pudding, soups and foods, as well as the poor dietary fibre content, strong consensus and the perfect taste (Bavec & Bavec, 2006).

Einkorn grain has high protein and lipid than bread and durum wheat. It has some important trace elements such as zinc and iron in high concentrations. Low beta-amylase and lipoxygenase activities in einkorn limit the reduction during food processing by preserving the nutritional value. On the other hand, compared to common wheat, einkorn contains less dietary fiber, insoluble bonded polyphenol, and high polyphenol oxidase activity. Nowadays, developments in low-impact agriculture, and more use of organic and functional goods, emphasises the idea that einkorn can be a good candidate for the consumption of human beings, particularly in developing new and unique products like bakeries, baby foods and high levels of dietary fibre products, carotenoidsç and tocol. It is not appropriate for patients with celliac disease even if it induces a lower toxicity reaction than other Triticum ssp. Trends in the consumption of functional foods, particularly special or new foods with a superior food value in human nutrition, which play a major role today in developing this cereal form (Brandolini & Hidalgo, 2011; Ünlü et al., 2018).

1.1.2.2 Emmer Wheat

It is known as gernik or çatalsiyez in Turkey. *T. dicoccum* Schrank which means two-grain wheat in German, is called emmer and its origin goes back to the near east (Nevo et al., 2002).

Emmer is called by many names: *T. turgidum (L.) Thell.* ssp. *dicoccum* (Schrank) Schübl., *T. farrum* Bayle-Barrele, *T. amyleum* Seringe, *T. zea* Wagini, *Spelta amylea* Seringe, *T. volgense* (Flaskb.) Nevski, *T. vulgare dicoccun* Alef., *T. sativum dicoccum* Hack., and *T. ispahanium* Helsot). The tetraploid and the hexaploid varieties are two grain wheat. Two prevailing ears are the ear. Each of

them is fitted with sophisticated grains. The long and firm husk is the characteristic. The stem is fragile and the spikelets fall off easily when harvested fully mature (Bavec & Bavec, 2006).

Triticum dicoccum Schrank Type Gernik (*Triticum dicoccum* Schrank) wheat grown in Anatolia is an exceptional species where is grown smoothly in an area of approximately 6,50 hectares in Kastamonu district, İhsangazi, where people can benefit from bulgur, wheat and animals from its stalks (Wikitrend, 2011).

Extensive research has shown the remains of primitive *Triticum dicoccum* Schrank in early settlements from the late Stone Age (17,000 BC) to the late Mesolithic Age and the early Neolithic (Stone Age) (10000 BC) to the Bronze Age (10,000 BC). It existed between 1,000 and 10,000 BC in the Near East and Far East, North Africa, and Europe (Zohary & Hopf, 1988). Emmer production has been taken under protection in the south Russia, in India, and in the isolated regions of Abyssinia (Pickersgill, 2000).

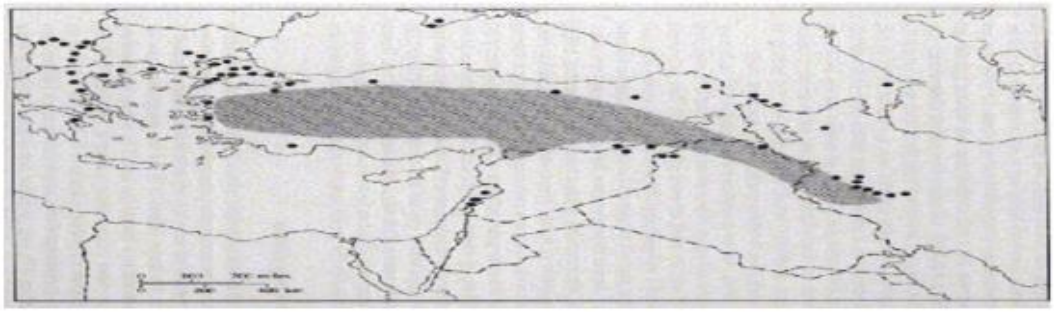


Figure 1.3. Distribution of *Triticum dicoccum* Schrank (emmer) wheat

Troccoli and Codianni (2005) determined the wheat type which can grow the best in insufficient or inefficient soils and found that the most economically can be cultivated one is *Triticum dicoccum Schrank.* It has a lower grain yield than durum wheat in continuous field cultivation trials, but *Phalaris arundinacea* and *Avenafatua* have higher yields than einkorn and spelt (Troccoli & Codianni, 2005).

Troccoli and Codianni (2005) stated in his study that increasing the rate of nitrogenous fertilizers decreases the yield. In a similar study, Castagna et al. (1995) found that increasing the nitrogenous fertilizers does not affect the yield. These studies are important for reducing inputs in production systems such as in organic agriculture.

The grain yield in emmer varies from 200 kg / hectare to 4,000 kg / hectare and there is a strong correlation between climatic conditions (location) and grain yield. The estimated grain yield is between 45% and 75% in spring wheat (Cubadda & Marconi, 2002).

Cubadda and Marconi (2002) stated that good loaves can be produced from emmer flour and its breads made from whole grain flour have an intense texture and mild taste compared to breads made from triticale or rye. It is also stated that the texture of the loaves and emmer bread is different from the common wheat. Emmer is an ancient wheat variety that has been grown in Anatolia for centuries. It is mostly grown around Kars province and is known as farro and wild wheat. Farro wheat started to disappear in the early 2000s due to its difficult harvest, because of lack of government support, difficult to separate from the shell of the grain, and poor flour for good bread alone in the early 2000s. Today it gained importance with the efforts of some non-governmental organizations and farmers.

It was supported by the Environment Fund / Small Grants Program and it, thus, found the opportunity for a national recognition. The increasing interest in healthy and safe products for a long and energetic life has played a significant role for the production of such products. Today, Kablıca (emmer) wheat is cultivated by 400 farmers in approximately 12 provinces and around 600 tons are produced. Traditionally, bulgur, cabbage wrap, and milk soup is consumed in noodles, pastries, and bread flour (Zengin, 2015).

1.1.2.3 Spelt Wheat

Spelt (*Triticum spelta*) is also called dinkel, dinkel wheat, and German wheat. There are two controversial hypotheses about its origin. According to the first hypothesis, the first samples of *Triticum spelta* (spelled) were found in Iran. The second hypothesis divided its origin into two regions as North East Europe and Iran (Stallknecht, Gilbertson & Ranney, 1996).

Figure 1.4. Einkorn, emmer and Khorasan wheat from left (Grausgruber et al., 2005).

Common wheats, including bread wheat (*Triticum aestium* L. ssp. *vulgare*) and hard wheat (*Triticum durum Desf*.) have not been cultivated in high amounts over the centuries, hulled wheat, however, has been highly significant in human life. At the end of the 18th century, *Triticum spelta* (spelled) was produced in regions in the vicinity of Asia (Afghanistan, Pakistan, Iraq, Iran, Turkey) and several European countries. Production has, in time, declined, even in Europe. In the last years of the 20th century, *Triticum spelta* began to proliferate in the mountains and hills in the centers of European countries. It is a very suitable plant for organic farming because it is easy to grow and adaptable. Due to the increase in consumer awareness, it has become very important for bread making where organic agriculture is common. Traders, wheat traders, and wheat producers in Canada and Central America show great interest in *Triticum spelta* today (Bavec & Bavec, 2006).

Triticum spelta (spelled) belongs to the family Poacea, as genus *Triticum* ssp.and as a group to the glumes group. It is more resistant to diseases and infections than other wheat speices. In addition, its genotypes show various levels of resistance against diseases. For example, Hubel variety is very strong against fungi (*Puccini striiformis, P. recondita, and Eptorisa nodorum*) but it is not similarly resistant to powdery mildew (*Erysiphe graminis*), another fungal disease.

It is a plant with roots and stems 1.4-1.7 meters long with hollow structure. The leaves are long, narrower and less hairy than *Triticum aestivum* or no hair at all. Its seeds are fragile. It is like *Agropyron repens*, whose ear is a weed. Spikelets have six flowers and after self-pollination, these flowers release two seeds. These seeds are enveloped in the shell and are known as husks after harvest. Husks are wrapped tightly in the seed and cannot be removed after harvest, so the spike stalk is broken. In some varieties, the spike awn is attached to the husk. Husks surrounded by spikelets have short, thick, rough projections in the middle. Husks can make up 25% to 45% of the whole spike (Bavec & Bavec, 2006).

It is frost tolerant and more resistant to the long winter seasons at lower temperatures than most ordinary wheat. It can be grown up to 1,200 metres above sea level. It can be adapted to bad conditions and cultivated in all kinds of soil. Consequently, it is appropriate for large-scale processing. Suitable pH in soil is 6-7. It is highly resistant to viruses and due to excess nitrogen in the soil. Therefore,

either to prior legumes or potential excess mineralization is very suitable for its organic farming. After all, all grain goods can be seeded.

In the second or third year, a less labour intensive crop can be planted for a minimum of three years. It can be planted after maize, beets, and lettuce. It is grown in Europe in the winter and is mostly grown in America and Canada in the spring. In temperate climates, relative to other typical wheats, it can be seeded during its regular season. They are harvested in the middle of summer until they are fully ripe.

It should not be expected to fully mature like other common wheats for harvest. The dark stalk indicates that it is overripe, it is difficult to harvest, and causes a lot of loss. Harvest should be done at night or in the morning when it is moist. It cannot be stored like wheat. The reason is stated that it is insect prone and deterioration. It should be stored together with the hull in dried warehouses. The spelled seed should contain less than 15% moisture (Bavec & Bavec, 2006).

In general, when we compare its approximate chemical composition with other common winter wheat grains, its protein content is quite high. The approximate chemical composition and nutritional value depends on its genotype and environmental conditions, sometimes these values may be like common wheat or low (Ranhotra et al., 1996).

Konvalina et al. (2010) stated that the average nitrogen content in *Triticum spelta* was 0.5% higher than common wheat grains and dietary fiber is 0.35% higher. There are significant differences between fat content and dietary fiber content in spelt varieties. In a study on the oil content of *Triticum spelta* wheat, it was observed that the oil content in the grains varied between 0.8% and 4.7%, while the total dietary fiber in dry matter varied between 10.5% and 14.9%. The ratio of mono-unsaturated fatty acid in the grain in the varieties is 21.5%, this rate is 13.7% in common wheat and in triticale (Ranhotra et al., 1996).

Table 1.1. Approximate Composition of Spring and Winter *Triticum spelta* (seeds with 10% moisture)

Compound	**Marconi et al.**	**Ranhotro et al.**	**Abdel et al.**
Number of varieties	5	15	5 (Summer)
Ash	1.71	1.8	1.6

Fiber	11.4	9.5	9.0
Oil	4.0	1.5	2.0
Carbohydrate	58.7	60.4	59.3
Protein	14.2	16.7	13.9

Source: Abdel-Aal et al. (1997); Ranhotra et al. (1996); Marconi et al. (1999)

The differences in dehulled *Triticum spelta* protein contents due to genetic effects in crude protein, total protein and nonessential amino acid, particularly glutamic acid and proline are significantly higher (Chrenkova, Čerešňáková, Sommer, Galova & Král'ová, 2000).

The amino acid composition of *Triticum spelta* is like common wheat. The amount of lysine amino acid is very limited in both common wheat and *Triticum spelta*. The lysine content among *Triticum spelta* varieties ranges from 2.35 (Marconi et al. 2000) to 3.96 (Chrenkova, Čerešňáková, Sommer, Galova & Král'ová, 2000).

The reason for the high lysine content in some *Triticum spelta* (spelled) products is that *Triticum spelta* (spelt) wheat is less refined and *Triticum spelta* (spelled) wheat is used as whole flour (Chrenkova, M., Čerešňáková, Z., Sommer, A., Galova, Z., & Král'ová, 2000).

Grela (1996) reported that the average phosphorus content in four *Triticum spelta* samples was 427 mg / kg and had higher values for microelements (especially Cu, Mn, Zn) than common wheat. In another study, it was found that *Triticum spelta* species contain higher levels of phosphorus than common wheat and emmer as well (Abdel-Aal et al., 1997).

When looked at the vitamin content of *Triticum spelta* (Abdel-Aal et al., 1997) found some B vitamin values (thiamine, niacin) were higher in *Triticum spelta* than common wheat. There are different results, too. Grela (1996) found that vitamin E activity was 143% higher than common wheat, lower tocopherol content in Marconi et al. (1999).

1.1.3 Some Agro-Morphological Characters in Einkorn Wheat

Zencirci et al. (2018) stated that Gökgöl reported that the number of botanical varieties grown in Turkey with all the *Triticum* sp. cultivated in Turkey exceeds substantially the number of botanical varieties cultivated in other parts of

the world. For instance, of the 73 botanical *T. turgidum* varieties recognised at the time, 48 were collected from Turkey.

Guzy et al. (1989) identified a wide diversity for number of spikes per spike and number of grains per spike in a series of diploids, tetraploids and hexaploids.

Sharma (1984) compared 93 genotypes of einkorn with 'Modoc' durum wheat and 'Anza' bread wheat varieties for plant height, grain weight, protein ratio in flour and lysine content, ear weight, and earliness. They reported that they had a wide genetic variation for those characters and earlier and shorter genotypes than bread and durum wheat ones.

Castagna et al. (1995) studied 21 *Triticum monococcum* ssp. *monococcum* population from different locations and found that there were important genetic variations for earing date, plant height, 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) have characterized 37 local einkorn wheat genotypes collected in Romania and Hungary for eleven agro-morphological characteristics (six morphological characters and five agronomic characteristics) and found that number of seeds per spike and grain weight were diverse in genotypes.

1.1.3.1 Leaf Weight (LW, mg)

Leaf weight is one of the simplest growth components to calculate. It is is one of the few morphological features of plants that display significant changes within a single day.

The indirect calculation of several basic leaf processes can be leaf weigth (mg), leaf-water relationships, photosynthesis and growth potential (Dong, 2011).

The quantity of light is absorbed by the flower and the direction of carbon dioxide (CO^2) diffusion through its tissues controls at least partially dependent on its weight and thickness. Negative relationships were observed between leaf weight and photosynthesis and growth rates (Westoby et al., 2002).

Thus, leaf weight is also used as a control method for the development or environmental performance of species and/or cultivars. Larger leaves usually have

higher densities of chlorophyll per unit area and are hence more photo-synthetic than thinner pads (Fageria et al., 2006).

Davidson (2014) has indicated that high tissue N levels lead to very sugary development, high water content, however, low dry content, very small plants, fast photosynthesis.

1.1.3.2 Germinating Power (GP, %)

The start of seed development into a seedling is referred to as germination. GP is the percentage of emerged seeds at 14 days after planted. Both seeds need to germinate with water, oxygen and the exact temperature (Aslan et al., 2018). Wheat germinates just above 4°C and, then, shoots. Though high temperatures limit germination, the germination is accelerated by lower temperatures of 2°C, 8-10°C optimum and 20-22°C maximum. Germination and growth are typically pre-emergence regulated by soil temperature. The root temperature in the soil influences root growth and root count. Barley, oats, rye, and wheat roots grow shorter at low temperatures (15° - 25°C) whereas at higher temperatures (such as 50°C) grow faster. It also affects shooting and root ratio. A successful setting up of a stand and further crop production are guaranteed by successful seed germination (Coolbear et al., 1984).

While good wheat seeds germinate between 90-100% under laboratory conditions, the highest germination rate is 90% and average 85% under field conditions. For maximum yield in wheat production, there should be between 220 and 550 seedlings per square meter depending on the tillering ability. 100 plants per square meter decrease grain yield.

Seed selection in agricultur al production has a significant effect on yield. It is stated that seed size is strong seedling development, and this affects the yield positively (Caballero et al., 2009).

1.1.3.3 Coleoptile Length (CL - cm)

The coleoptile is the first root part of the plant and, during germination. The first leaf would push the soil and appear black, while the coleoptile is shorter than the seed profound (Aslan et al., 2018).

The longer the ground surface takes, the more delicate is the ground cross. The more fragile it becomes. For effective growth and early plant vitality, the

coleoptile is important. Drought may lead to an increase of the coleoptile length and inhibit the depth of the sowing (Aslan et al., 2018; Hong & Honggang, 2012).

When seeded deeply, the frequency of wheat with long coleoptiles is higher. When deeply planted, but later, wheat plants with short coleoptiles appear and have no germination power. Deep sowing allows farmers to take advantage of soil humidity in the dry topsoil and is a preference farmer take into account in Australia (Jackson, 1993).

The elongating base organs, for example coleoptile and the first internode, will enter the shoots from deep ground in some genotypes. Closer sowing tends to reduce seed removal from birds and rats and avoid the phytotoxicity of such herbicides that are pre-emergent. Smaller relatively low growth rates in seedy coleoptian plants have slower leaf development, which leads to smaller leafy areas in early seasons, reduced weed productivity and the water damage by evaporating the soils' surface, thus decreasing the consistency, biomass and yield of the use of plant waters (López-Castañeda & Richards, 1994).

Deep placement of the seeds and reduced elongation of coleoptiles in the mainly hot soil can have a potentially devastating effect on stand construction. Seed size was reported to have little effect on barley coleoptiles, but not in wheat or oats.

Surveys in the fields of Australian farms show when short coleoptiles are sown deeper than 5 cm, the yield is decreased by at least 10 percent. Reduced set-up and lower sowing grain yields for shorter coleoptile wheats are recorded. There was a substantial varying population of the recombinant inbred line population, natural distribution and transgressive segregation under field conditions for the coleoptile length (Singh et al., 2014).

Coleoptile length selection typically occurs in either a managed greenhouse or in deep plantation in field plots.

1.1.3.4 Root Count (RC - count)

The number of roots in a single wheat grain is the root count. In the radical structure of wheat are two major forms of root, seminal (emerging) and nodal (post-embryonic). As the first node, seminal roots are fully responsible for the seedlings in nutrients and water (Aslan et al., 2018). Roots are, therefore, essential for seedling and early planting establishment, which also influence the competitiveness

of weeds. Nodal roots on the other hand are shooted and develop soon after tillering, particularly during the growth of wheat (Manske & Vlek, 2002).

The roots continue functionally working in the reproductive period despite their early establishment and can go deep by up to 2 m in length. It was also shown that nutrient consumption is comparable with nodal roots in wheat and contributes to their output potential in low soil humidity, where nodal roots can not grow (Sebastian et al., 2016).

Due to its importance, root characteristics like number and angle were linked to water-limiting adaptive reactions. The angle of roots has been related to an increase in depth in soil exploration in the drought, when topsoil moisture is reduced (Golan et al., 2018).

Any quantitative trait loci (QTL) root variations in wheat germplasm have been determined. However, many QTLs are established in large areas to confuse their genetic dissection and limit their use in reproduction. Only one root counts gene in wheat has been found, compared to other cereals such as rice and maize, for example (Atkinson et al., 2019).

1.1.3.5 Root Length (RL - cm)

Roots act as boundaries for complex soil media between plants. The root length is the below-ground analogue. In addition to the anchorage of the plant to the soil medium (Aslan et al., 2018; Khan et al., 2016), the plants are also provided access to nutrient and water consumption by root. Roots are also essential for symbiosis with beneficial rhizosphere microbes and are used as storage organs. Roots in preserving plant health are also important. Many environmental factors interfere with soils and contribute to the heterogeneous space and time in the land (Smith & De Smet, 2012).

This root system spatial distribution in the soil is called architecture of the root system (RSA). RSA generally defines the structural and morphological root structure. For plant growth, the roots length is critical as it determines the plant's ability to access broad heterogenous edaphic resources successfully. Thus, roots length has a direct effect on grain production (Meister et al., 2014).

The radical first appears in wheat grain and is covered by a layer known as the coleorhiza. The coleoptile emerges and grows quickly after the roots have spread much further. Sowing a specific roots length would then have a significant

effect on the early seed production and productivity of the seedling during the later stages of growth (Richard et al., 2015).

1.1.3.6 Root Fresh Weight (RFW - g) and Root Dry Weight (RDW - g)

The capacity of plants to consume water could be measured by fresh root weight (Aslan et al., 2018). The relationship between shoot and roots is interdependent. The shoot provides carbohydrate to roots which, in turn, provide water and shooting nutrients; its functional balance differs from one species to another.

The rooting habits of water and nutrient uptake are different for plants. Sheng and Sheng (Sheng & Hunt, 1991) discovered that they infiltrated easily in the beginning of the season, whereas the root systems of certain cultivars of winter wheat (*Triticum aestivum* L.). Wheat is closely connected with rye (*Secale cereale* L.) and triticale (x wittmack) and lacks specific features, like the early spring vigour. However, the variation in rooting properties between the three species and the significance of the diverse rooting features in the determination of water consumption and productivity is not enough knowledge.

1.1.4 Some Molecular Characters in Einkorn Wheat

As with all living species, plant species also owe the continuity of the lineage to their adaptability under changing environmental conditions. Therefore, knowing the genetic diversity of the plant species studied is essential for a sustainable agriculture. The genetic diversity is also utilized today's wheat breeding studies. Molecular determination of genetic diversity in wheat was practiced via cytological, isoenzyme, and various DNA markers / DNA sequences. The genetic diversity in einkorn, as well, is of great importance and may serve in diploid, bread, and durum wheat breeding programs (Fayaz et al., 2018).

Today, the rate of genetic variation in wheat breeding is gradually decreasing. As a result of this, wheat breeders prioritize using biotic and abiotic stress resistant wild wheat species or their more primitive forms in wheat breeding programs. Therefore, in the light o above mentioned stated reasons, characterizing einkorn wheat genotypes as agro-morphological characters and molecular characters is very important.

Knüpffer (2009) reported that there is a total of 5,367 black accessories in 54 gene banks. Although this genetic material is quite scarce, only a small part of these accessories could be analyzed phenotypically and molecularly.

Seifolahpour et al. (2017) examined 252 wild einkorn populations (*Triticum boeoticum* Boiss.) from the Zagros Mountains for morphological, agricultural, and phenological characteristics and found a wide variation for plant height and thousand kernel weight.

Karahan et al. (2019) studied 64 einkorn wheat genotypes from different origins and determined their plant height, upper internode length, spike length, spike weight, and spikelet number per spike under Çukurova (Turkey) conditions for two years. They reported a larger variation for grain number per spike and spike yield.

1.1.4.1 Genetic Transformation

Einkorn is one of the world's oldest cereal crops grown. This species is especially appealing to use as a diploid model to understand the genomics and proteomics of Triticeae due to its high genetic polymorphism, low ploidy (2n = 2x =14, Anim), and low genome size (~ 5.7 GB). But einkorn, for the application of modern biotechnology, like transgenesis is still a recalcitrant monocotyledon plant.

The efficient genetic transformation of the seed using bio-mediated DNA delivery is recorded in the study (Miroshnichenko et al., 2018). The rice actin promotor (act 1) and the selective bar gene (bialaphos immune gene) of the maize ubiquitine promoter with plasmid containing the study GFP (green fluorescent protein) (ubi1) bombarded the non-immature embryo-derived tissue of the sparrow of the insert. For effective transformation, it was important to adjust the different parameters, including gas pressure, microcarrier, and tissue production phases. Tissues of the bombarded unknown regeneration plants are recalcitrant, but certain improvements in the culture medium have shown that transgenic events are more efficient. Independent transgenic plants at frequencies ranging from 0.0 to 0.6 percent were produced in different experiments. Molecular studies, marker gene expression, and treatment for herbicides have shown that gfp / bar genes are stably incorporated into the entry genome. In Mendelian and non-Mendelian fashion, the transgene was differentiated by several insertions as a dominant locus. Transgenic herbicide-resistant fertile homozygous populations of T1-T2 have been selected.

1.1.4.2 The Molecular Structure

Molecular markers are defined as a DNA fragment or biochemical substance belonging to a gene region in the genome. Molecular markers take their source from DNA in the plant's own cells. It gives close to 100% results in determining the diversity in plant populations and the relationships between these individuals. Molecular markers are used to identify individuals in gene resources and to prevent duplications, determination of kinship relationships, mapping, and selection. Molecular markers are not affected by environmental factors. Nucleus and organelle genomes such as chloroplast and mitochondria with different inheritance can be studied. Since less pleiotrophic, they reflect genetic changes more and different characters from each rootstock can be detected. Moreover, they have advantages such as detecting their genetic origin and obtaining an infinite number of molecular markers.

Dograr et al. (2000), in a study, who conducted to determine the genetic diversity between 5 winter pasta varieties and 7 advanced lines using seven SSR primers, found that seven seven SSR were homozygous in all varieties, WMS6 primer produced two bands in all varieties, all genotypes could be separated using seven SSR primers, alleles. They found that all genotypes could be distinguished from each other by using only three SSR primers (WMS6, WMS30 and WMS120) by reporting that the number of polymorphism information varied between 0.609 and 0.872.

Empilli et al. (2000) examined 1,344 genotypes of *T. monococcum* ssp. *monococcum*, *T. monococcum* ssp. *boeoticum*, *T. monococcum* ssp *sinskajae* subspecies for a total of 17 morphological and quality characters such as earring time, plant height, 1000 grain weight, and SDS sedimentation value. They reported that *T. monococcum* ssp. *monococcum* genotypes with good agronomic features were determined here with wider variations.

Cao et al. (1999), using ten RAPD marker primers to determine interspecies and intra-species genetic diversity in 69 *T. spelta* and 32 *T. macha* wheat varieties, calculated Jaccard genetic similarity coefficient and performed cluster analysis. They reported that two species were in accordance with their geographical origins and that *T. macha* had more genetic diversity than *T. spelta*. They also added that RAPD markers could be used easily in the identification of wheat gene resources and in identifying duplicate samples in gene banks.

Chabane et al. (1999), in their study using the AFLP marker, investigated the genetic variation between six *T. urartu* genotypes collected from northern (Aleppo) and southern (Sweida) regions of Syria and 12 Turkic genotypes collected from other regions. They found that AFLP primers produced a total of 176 bands, with band lengths of 50 and reported that six genotypes collected from northern and southern regions formed a separate group from the other genotypes in the cluster analysis. Their conclusion was the genetic diversity in *T. urartu* differed by region.

Cao et al. (1999) investigated the usability of RAPD technique in reclassifying incorrectly classified wheat samples. They analyzed control genotypes with RAPD markers and found that 12 genotypes that were morphologically classified as *T. macha* or *T. vavilovii*are misclassified. They reported that five out of twelve genotypes examined were *T. turgidum* ssp *dicoccum,* one was *T. timophevii* ssp *timophevii* and six were *T. monococcum* ssp. *monococcum.*

Dograr et al. (2000), in a study on five winter pasta varieties and seven forward lines using seven SSR primers, found that seven SSR loci were homozygous in all varieties. WMS6 primer produced two bands in all varieties; all genotypes could be separated using seven SSR primers. They found that all genotypes could be distinguished from each other by using only three SSR primers (WMS6, WMS30, and WMS120), stating that the number of polymorphism information ranged between 0.609 and 0.872.

Liu and Wendel (2001), in their study investigating the applicability of iSSR markers in cotton plants, found that the heritability level of ISSRs in cotton is high and that ISSR markers can be used in the study of both intra-species and interspecies genetic relationships in the cotton plant (Ozbek Ö., 2021).

Rodriguez-Quijano et al. (2004) reported that all the einkorn genotypes examined had the Wx-A1a allele of Chinese Spring, a bread wheat variety, in their study examining waxy proteins in 39 einkorn wheat (*T.monococcum* ssp. *monococcum*), and reported that the amylase content in the genotypes varied between 22% and 35%.

Salimi et al. (2005) conducted a field survey to determine the distribution area of *T. urartu,* A genome donor, in Iran. They found for the first time that *T. urartu* existed in different regions of Iran. They reported that different sub-varieties were also defined.

Góral et al. (2015), in their study examining genetic diversity using RAPD and iSSR markers in cytoplasmic male sterile five triticale genotypes and three triticale varieties with the cytoplasm of *Triticum timopheevi*, applied 34 RAPD and 10 ISSR primers, and both DNA markers showed a low level of polymorphism,

Fernandez et al. (2002), examined the phylogenetic relationships of 16 barley cultivars from different countries using RAPD and iSSR markers, and ten RAPD primers produced 125, ten iSSR primers produced 228 bands, and the RAPD primer named S10 and the ISSR primers no. 811, 820, 835, and 881 were examined. They reported that RAPD and iSSR markers are DNA markers and can be used in DNA fingerprint analysis, indicating that they can easily distinguish all varieties and that the varieties are grouped as summer/winter and six-row / two-row as a result of cluster analysis using RAPD and ISSR data.

The progenitor of cultivated wheat is the wild emmer (*Triticum turgidum* ssp. *dicoccoides*). Turkey is the largest centre of wheat and plays a crucial part in the distribution of different crops across the continents. As the domestication core of wheat, Karacadağ is regarded the cneter and hundreds of landraces are still there popular. The genetic diversity and phylogenetic relationship were studied through the use of the markers iPBS-retrotransposons, using a total of 29 wild emmer cultivars, four cultured emmer wheat (*T. turgidum* ssp. *dicoccum*) and five hard wheat cultivars (*T. turgidum* ssp. *durum*).87.85 percent and 0.660, respectively, were mean polymorphism and polymorphic information value (PIC). Mean efficient allle numbers (1.961), the information index of Shannon (0.682) and gene variability (0.489) have shown great differences in frequency (Arystanbekkyzy et al., 2019).

Pujar et al. (1999), with 63 tetraploid wheat genotypes in their study, pre-screened 100 ISSR primers in seven selected genotypes; 15 ISSR primers were selected as a result of preliminary analysis and produced 134 bands in total, of which 129 were polymorphic. The total number of bands obtained per primer and the number of polymorphic bands was respectively, 8.93 and 8.60, and as a result of cluster analysis with iSSR data, four different groups were formed. ISSRs are found that could be used successfully to determine a genetic variation within durum genotypes/cultivars.

2. AIM AND SCOPE OF THE STUDY

Wheat meets a part of primary calorie and protein required for human nutrition and is the basic food material of approximately in 40 countries which make up 35% of the world population. Depending on the changing consumption habits of people and developing technology, wheat products have diversified, and consumer demands have also changed in time.

The most common consumption forms of wheat are flour, bread, pasta, semolina, biscuit, bulgur, and vermicelli. The products outside of these traditional products in the world and in Turkey are sweets, starches etc.

Triticum is divided into three groups according to the number of chromosomes: diploid (2n = 14), tetraploid (2n = 28), and hexaploid (2n = 42). Cultured wheat is one diploid: *T. monococcum* ssp. *monococcum* (2n = 14, AA), two tetraploids: *T. turgidum* ssp. *dicoccoides* (2n = 28, AABB) and *T. timopheevii* (2n = 28, AAGG), and a hexaploid wheat: *T. aestivum* L. (2n = 42, AABBDD). These species can be classified differently according to their usage areas as well. Hexaploid wheat is extensively in making bread, baklava, pies, and biscuits while tetraploid wheat is in pasta and bulgur. Diploid wheat, on the other hand, is used in bulgur and rarely pasta, and some bread today.

This study aims to investigate the "Agro morphologic and molecular characters in einkorn wheat (*Triticum monococcum* ssp. *monococcum*) from Turkey". This can be achieved by studying the agro-morphological characters in einkorn wheat which contains; Germinating Power, Coleoptile Length, Root Count, Root Length, Root Fresh Weight (RFW), Root Dry Weight (RDW), Leaf Weight and iSSR marker supported molecular characters as well. Determining t the agro-morphological and molecular characters of wheat, which is among the most produced and consumed plants in the world, would prepare the basis for the development of more superior wheat varieties.

3. MATERIAL AND METHODS

3.1 Seed Samples

The seed samples were collected from İhsangazi (Kastamonu) and Seben (Bolu) by researcher in november 2020 (Table 3.1).

Table 3.1. The registration number (RN), the species name, and the name of the collection sites (Abbreviation: N number)

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

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

3.2 The Experimental Design of the Analyses of Agro-Morphological Characters

3.2.1 The First Experiment

First, to weigh the leaves, the soil has been sterilized in a microwave for 15 minutes at a temperature of 121°C. Then, five seeds samples for each population were planted and then they were placed in the climate room, which had a constant climate conditions (temperature 23° C ± 2, 16 hours day, 8 hours night) for 30 days. The plants were watered every day (Figure 3.1).

Figure 3.1. Planting five seed samples in each population

After 30 days, the plants were cut and the leaves were weighed and recorded. The leaves were kept at -20 °C (or -70 °C?) for molecular analyses of the populations (Figure 3.2).

Figure3.2. The plants grown after 30 days

3.2.2 The Second Experiement

To measure germination power, coleoptile length, number of roots, root length, root fresh weight, and root dry weight, the husks of 50 seeds in each population were peeled and cleaned, and three replicates of 10 seeds germinated in petri dishes.

Then, the seeds of each population (50 seeds) were sterilized by washing with the dezenfection solution, which was prepared with two drops of Tween 20, 30 ml of bleach, and 70 ml of distilled water in total 100 ml water in a 500 ml glass beaker for 10 minutes on a stirrer device.

After that, the floated seeds were removed since they are considered dead seeds. Three petri dishes, filter paper and 500 ml of water have been put in a glass tube with a capacity of 1000 ml. Then, they all put centered inside the sterile cabin.

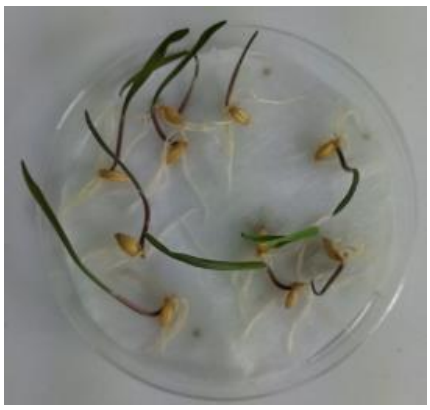
Next, the seeds washed with sterilization solution were placed in three petri dishes in which two filter papers placed and moistened with 2 ml of water in the form of ten seeds.

After that, the plates were placed inside the climate room with a constant climate conditions (23±2˚C, 16 hours during the day, 8 hours at night). The germination process, moistioning with 2 ml of water to each petri dish everyday, took place after 8 days, (Figure 3.3).

Fist day

Second day.

Fourth day



Sixth day

Figure 3.3. The images of the stages of germination processes between 1-6th days.

After eight days of germination (Figure 3.4), the plantlets have been cut and data have been taken for germination power, coleoptile length, root number, root length, weight of fresh roots, root dry weight.

Figure 3.4. After eight days of germination when harvested.

The roots were cut and weighed in the fresh state, and put them in the drying device (incubator) at 37 °C for four days, then, weighed them in the dry state.

To analyze the data of agro-morphological characters in each population, a statistical analysis is applied to obtain a comparison between the populations. The statistical analyses used in this study were; KMO test is a metric to understand how the information is suitable for factor analysis. KMO testing is a measurement to understand the data. Test the adequacy of the sampling for each model predictor. The statistics are used to quantify the proportion of difference between variables that might be normal. The lower the percentage the better the factor analysis (Goto et al., 2011).

Explained variance calculates the proportion of the deviation (dispersion) of the data collected by a statistical model. Often deviation is measured as variance, so the explicit variance is used for more precise expression. The difference in fraction described by a 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 prior to PCA or factor analysis, examines whether data comes from a multivariate standard zero covariant distribution (Jackson, 1993).

3.2.2.1 Statistical Analysis

Coefficient of variation (CV)

The coefficient of variation indicates the degree of variability of data in a sample relative to the population mean. The coefficient of variation (CV) is the ratio

of the standard deviation to the mean. A high coefficient of variation means a high distribution around the mean. It is usually expressed as a percentage. 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.

Analysis of variance (ANOVA)

ANOVA helps to understand how different groups respond, with a null hypothesis for the test that the means of the different groups are equal. If there is a statistically significant result, then it means that the two populations are unequal.

Principal component analysis (PCA)

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

Pearson's correlation

Pearson's correlation coefficients (r_P) were computed to relate the measures of metric agro-morphological traits by using SPSS version 22 for Windows according to Steel and Torrie (1980).

3.3 The Molecular Analyses

This was the experiment to study the molecular structure in each population and compare them for the partial difference between populations.

The aims of the molecular characters 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, (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 Turkey.

3.3.1 DNA Isolation

The genomic DNA has been extracted by the modification method as defined by Kidwell and Osborn (Kidwell & Osborn, 1992) from the leaves of 1-1.5-month-old plants. Steps in the procedure

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 movement.

4. Incube with periodic mixing for 60 min at 55-60°C.

5. Add chloroform/isoamyl alcohol in equal proportion (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 volume (- 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.

3.3.2 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 100 bp DNA molecular size marker (Thermo) on 1.3% agarose gel (Sigma), and electrophoresis were carried out at 80 mA / 160 V for 2-2.5 h. Ethidium bromide (10 mg/mL) staining is used to visualize amplified fragments and the pictures were taken under UV light (DNR bio-imaging system).

4. RESULTS AND DISCUSSIONS

In this section, the findings obtained in the thesis are explained in comparison with the results of previous studies, and the possible reasons for the relationships or differences between them are specified.

4.1 Descriptive Statistics

According to descriptive statistics, the minimum values for agro-morphological traits ranged beteen 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 mean highest mean value was observed as 215.16 for LW, while the lowest mean value was observed as 1.06 for CL (Table 4.1)

Table 4.1. The descriptive values observed in agro-mophological traits in einkorn wheat landraces populations (Abbreviations: N: Sample Number, CL: Cleoptile_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

4.2 Coefficient of Variation

The mean values for agro-morphological traits ranged between 1.06 and 215.16. According to coefficient of variation values, the highest value was observed in FRW as 52.09%, while the lowest value was observed in LW as 8.9% (Table 4.2).

Table 4.2. Coefficient of variation in agro-morphological traits calculated in einkorn wheat populations (Abbreviations: CL: Cleopatile_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 (%) = $(\sigma/M)*100$
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

The CV values indicated that the GP trait has the most reliable CV value <10 and stable trait. The traits CL and RN had CV values in the range of 10-20 and these were acceptable values, while the traits RL, FRW, DRW and LW had CV values higher than >30, these infers that CV values of these traits were not acceptable and reliable. However, CV value of LW was slightly over 30, it might be acceptable in the acceptable range. Nevertheless, the traits RL, FRW, and DRW were less reliable and stable traits according to CV values.

4.3 The Agro-Morphological Characters Statistical Analysis

4.3.1 Differences Among Populations

Analysis of variance (ANOVA) is a collection of statistical models and their associated estimation procedures used to analyze the differences among means. Analysis of variance (ANOVA) was used to test differences between 48 sub-populations. *F* values, which is the *F* value in one way ANOVA to answer the question "Is the variance between the means of two populations significantly different?" The *F* value in the ANOVA test also determines the *p* value (Table 4.3) and was discriminated by Tukey HSD, (Table 8.1 in appendix). There was statistically higher significant difference at $p > 0.05$ level in mean scores for 48 einkorn wheat sub-populations for leaf weight, coleoptile length, roots counts, and root length 6.027, 18.775, 10.271, and 12.472, respectively ($p<0.001$). The effect sizes, calculated using teta squared, were 0.60, 0.39, 0.26, and 0.30, respectively, indicated a larger effect (Table 4.3).

There was statistically significant difference at $p <0.01$ level in mean scores of 48 einkorn populations for dry root weight, and germination power 2.098 and

2.063, respectively ($p<0.01$). The effect sizes, calculated using eta squared, were 0.51 and 0.50 respectively, indicated a larger effect. There was no statistically significant difference in mean scores for 48 populations of wheat for fresh roots weight 1.398 ($p = 0.084$).

Table 4.3. F values in ANOVA for leaf weight and coleoptile length of 48 wheat populations

Sources of variance	DF*	LW*	CL	RC	RL	FRW	DRW	GP
48 wheat populations	47	6.027^{***}	18.775^{***}	10.271^{***}	12.472^{***}	1.398^{ns}	2.098^{**}	2.063^{**}

* LW= Leaf Weight (mg); CL= Coleoptile Length (cm); RC= Root Count (n); RL= Root Length (cm); FRW= Fresh Root Weight (mg); DRW= Dry Root Weight (mg); GP= Germination Power (%).

DF = degrees of freedom

*** Significant at 0.001 probability level, ** significant at 0.01 probability level, ns not significant.

Population 7 showed the heaviest leaf weight, while population 27 showed the lightest ($p<0.05$). Populations 30 and 34 showed the shortest coleoptile length, while population 21 showed the highest ($p<0.05$). Populations 5 and 27 showed the highest roots counts, while population 21 showed the lowest ($p<0.05$). Population 27 showed the longest root length while population 21 showed the shortest ($p<0.05$). Populations 5 and 7 showed the heaviest dry roots weight, while population 21 showed the ightest ($p<0.05$). Population 27 showed the highest GP while populations 3, 4, 7, 35, and 44 showed the lowest ($p<0.05$).

By analysis of variance (ANOVA) there was a statistically higher difference $p<0.01$ in mean scores for 48 einkorn sub-populations (*Triticum monococcum* ssp. *monococcum*) for leaf weight (6.027), coleoptile length (18.775), roots counts (10.271), and root lengths (12.472). This indicated significant differences between the sub-populations. There was a statistically significant difference in the mean scores of 48 einkorn populations for dry root weights (2.098) and germination power (2.063), indicated significant differences. Here, a statistically significant difference in mean scores for 48 einkorn sub-populations for fresh roots weight (1.398, P = 0.084, is considered by the presence of differences between populations (Table 4.3).

Tukey HSD analysis of variance differentiated the mean values obtained in the study (Table 8.1 in appendix).

When all the population characters were assessed together, there were various groups within the population, as found by studying the difference between populations. Population 7 (IZA - Bolu / Seben / Musasofular Köyü Çıkışı) had the heaviest leaf weight = 426.90 g while Population 27 (IZA – Bolu / Göynük / Çaylak Köyü) had the lightest (141.40) ($p < 0.05$).

Population 27 (IZA – Bolu / Göynük / Çaylak Köyü) showed the highest germination power = 5.33 while Populations 3, 4, 7, 35, and 44 had the lowest 10.00 ($p < 0.05$).

Population 30 (IZA-Bolu / Göynük / Aşağı Kınık Köyü) and 34 (IZA – Bolu / Seben / Güneyce Köyü) showed the longest coleoptile of 1.76 and 1.77, respectively. While Population 21 had the shortest coleoptile length = 0.49 ($p < 0.05$).

Population 5 (Siyez- Kastamonu / Araç / Aliören Köyü) and 34 (IZA – Bolu / Seben / Güneyce Köyü) and 27 (IZA – Bolu / Göynük / Çaylak Köyü) and 34 (IZA – Bolu / Seben / Güneyce Köyü) showed the highest roots counts = 4.60 and 4.70, respectively. While Population 21 had the lowest root count (1.80), ($p < 0.05$).

Population 27 showed longer root length (5.27) while Population 21 had shorter root length (0.54), ($p < 0.05$).

Population 15 showed the heaviest fresh root weight = 141.60 while Population 21 had the lightest fresh root weight = 9.37, ($p < 0.05$).

Population 5 and 7 showed the heaviest dry root weight = 34.53, 35.17 while Population 21 had the lightest dry root weight = 2.23, ($p < 0.05$).

Here it can be noticed by studying these agro-morphological characters for averages that one of the best characters was in the Population 27 of IZA - Bolu / Göynük / Çaylak Köyü for germination power, root count, and root length.

While the Population 21, IZA - Bolu / Seben / GüneyceKöyü, is the worst for coleoptile length, root count, root length, fresh root weight, and dry root weight, (Table 8.1 in appendix).

The best population is seemed to be the Population 27 of IZA - Bolu / Göynük / ÇaylakKöyü.

4.3.2 Pearson's Correlations Among the Agro-Morphological Traits

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 determined as 0.296 (r = 0.04 at p <0.05 significant level) between LW and RL (Table 4.4).

Table 4.4. The Pearson's correlation values observed among the agro-morphological traits in einkorn wheat landraces populations (Abbreviations: N: Sample Number, CL: Cleoptile_Length, RN: Root Number, RL: Root_Length, GP: Germination_Power, FRW: Fresh_Root_Weight, DRW: Dry_Root_Weight, LW: Leaf Weight)

		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

** Correlation is significant at p< 0.01 level (2-tailed) * Correlation is significant at p< 0.05 level (2-tailed).

4.4 The Phylogenetic Relationships Between All Analyzed Populations According to Agro-Morphological Characters

A dendrogram was constructed based on the values obtained in agro-morphological traits. The einkorn wheat landraces populations were clustered into two main groups (Figure 4.1). 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 population one from Kastamonu and two from Bolu were grouped as out-group.

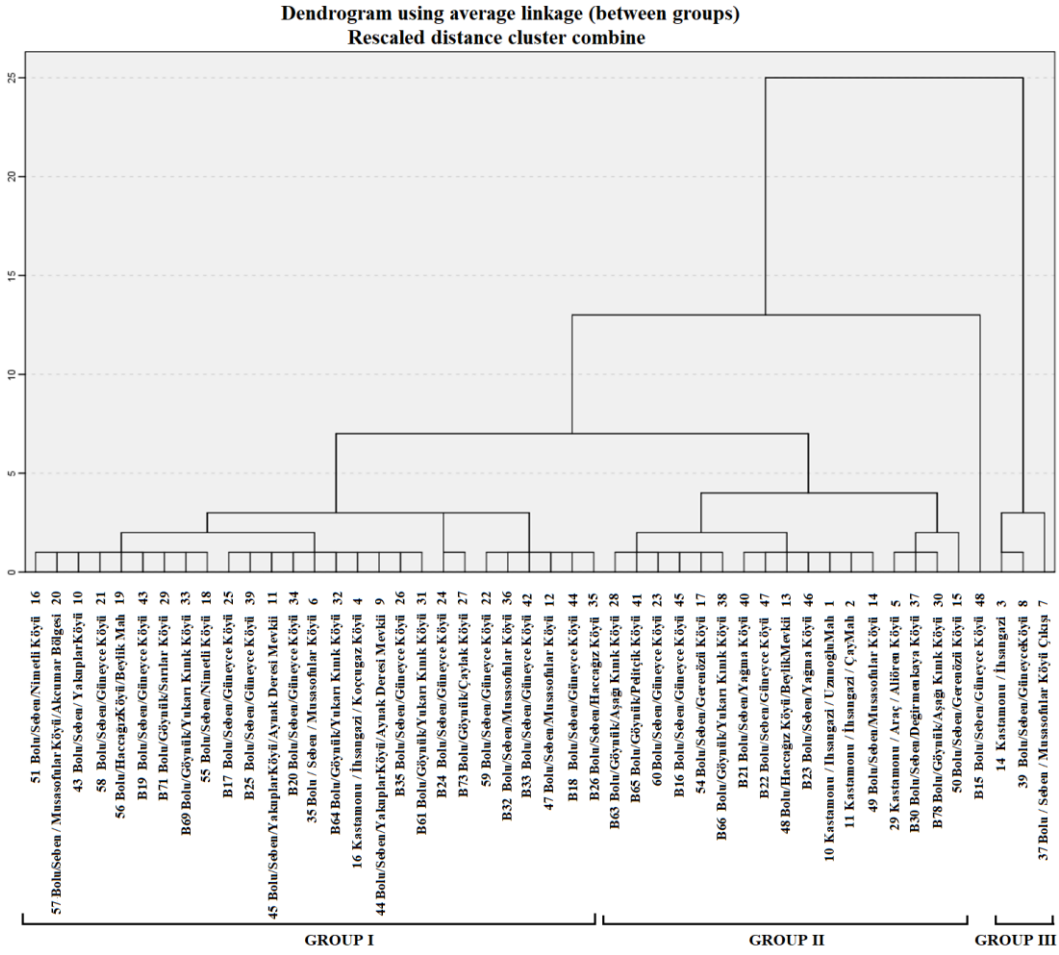


Figure 4.1. Dendrogram, constructed according to the between group linkages method based on squared Euclidean distances, representing phylogenetic relationship between 48 Turkish einkorn wheat landrace populations

The dendrogram, constructed according to morphometric values revealed that the einkorn landraces populations were clustered randomly and only three populations clustered as an outgroup in the second group. The results showed that morphometric measurements of agro-morphological traits were not sufficient to explain the differentiation between einkorn populations. The analyses of more agro-morphological traits and molecular analyses might reveal the phylogenetic relationships better and reliable.

Three major populations were analyzed in the study. However, each population was formed with seed samples collected from different villagers. These

results might be related to the ability of landraces populations to adapt well to their environments they are grown. The einkorn wheat is the only domesticated diploid wheat and a self-compatible plant species. It is considered that domestication process causes to reduction in agro-morphological characters as reported from previous studies (Reif et al., 2005; Xue et al., 2016), in contrast to previous studies, a high level of agro-morphological characters was observed among einkorn populations in this study and this is consistent with Ozbek and Zencirci (Ozbek Ö., 2021). This might be also due to *T. mononcoccum* ssp. *monoccocum* was devoid of breeding bottlenecks by modern wheat breeders and was, therefore, conserved great agro-morphological characters that was present during its domestication (Ozkan et al., 2007).

4.5 Principal Component Analysis (PCA) of Agro-Morphological Characters

According to PCA results, it can be seen that the first two eigenvalues explain about 76.93% of the variance in the seven-dimensional data for morphometric data (Table 4.5). 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 RL, FRW, DRW and LW variable (Table 2.6). For the second PC, the coefficients for the GP variable were small PC2 = + 0.77 GP. It can be interpreted as 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 4.2).

Table 4.5. Total variance explained by principal component analysis (PCA) performed by using data of CL, RN, RL, GP, FRW, DRW, and LW as variables according to *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 4.6. 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

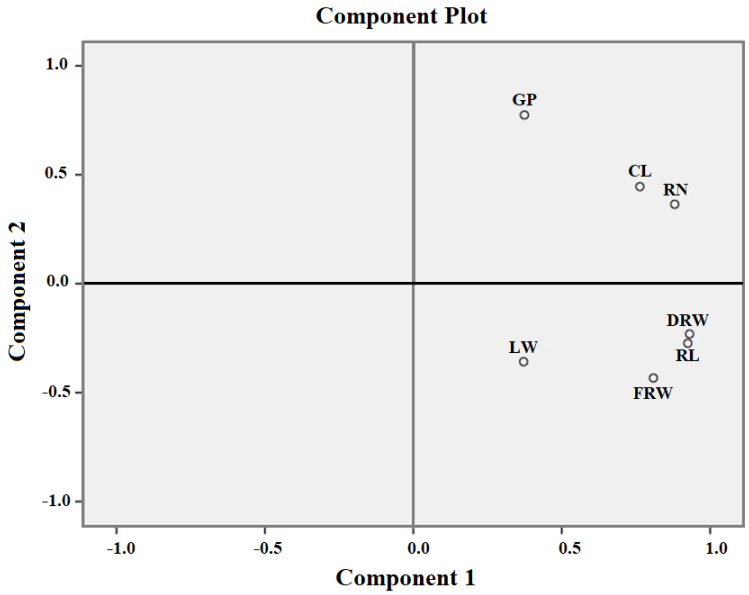


Figure 4.2. The component plot graph constructed by PCA represent the components 1 and 2 values derived from 48 einkorn wheat landraces populations (Abbreviations: CL: Cleoptile_Length, RN: Root Number, RL: Root_Length, GP: Germination_Power, FRW: Fresh_Root_Weight, DRW: Dry_Root_Weight, LW: Leaf Weight)

PCA results displayed those two components explain the variation in the acceptable percentage. The CL and RN variables were contrast to RL, FRW, DRW and LW variables, that in the first component CL and RN on the reliable site and contributed to the variation with GP in the second component. Overall results of PCA and CV were consistent.

4.6 The Statistical Analysis for Population Genetics

The ISSR-PCR amplification fragments were scored by 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 ver. 1.32 for populations genetic analysis (Yeh, F.C., Yang, R.C., Boyle, T., Ye, Z.H., Mao, 1997). The mean number of allels per locus (n_a), efficient allels per locus (n_{ea}) and the mean value of genetic diversity (h) had been estimated for gene diversity estimates (Nei, 1973). The genetic distinction between populations was normally calculated by $G_{ST}$for mostly herited 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. Dendograms built using unweighted pair group approach with arithmetical average (UPGMA) based on ISSR data were used to represent the phylogenetic relationships between populations or subpopulations.

4.6.1 Genetic Diversity Estimates of Einkorn Wheat Landrace Populations

The genetic diversity of einkorn wheat (*Triticum monococcum* ssp. *monococcum*) landrace populations was investigated by inter simple sequence repeats (iSSR) molecular marker system. In this study, 10 X 3 seeds for each population seeds were analyzed by using iSSR primer UBC-826, which produced 30 polymorphic bands. The mean number of alleles, effective allele, and genetic diversity value at locus level were observed as 2, 1.33, and 0.13, respectively (Table 4.4). The highest number of alleles existed in Bolu-Seben population, while the lowest number was in Kastamonu-İhsangazi population. The highest number of effective allele and genetic diversity value were observed as 1.29 and 0.18 in both Bolu-Seben / Güneyce and Bolu - Seben populations, respectively. The highest

number of polymorphic bands were determined as 22 (73%) in Bolu-Seben population, while the lowest polymorphic band was determined as 15 (50%) in Kastamonu-İhsangazi population (Table 4.4).

The total genetic diversity and the genetic diversity within the populations were identified as 0.21 and 0.17 at population levels, respectively (Table 4.5). The genetic differentiation among the populations was calculated as 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 4.6). A dendrogram was constructed based on the genetic distance values was calculated according to iSSR data indicated that the Bolu populations grouped together apart from the Kastamonu population (Figure 4.8).

Table 4.7. 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 4.8. The total genetic diversity and *F* statistics estimates among the einkorn wheat landraces populations

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

Table 4.9. The genetic distance values among the einkorn wheat landraces populations

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

Figure 4.3. The dendrogram representing the phylogenetic relationships among einkorn wheat landrace populations

4.6.2 Genetic Diversity Estimates in the Einkorn Wheat Landraces Sub-Populations

The seed samples were collected from the local farmers in the villages of Bolu-Seben and Kastamonu-İhsangazi districts. The Bolu-Seben-Güneyce, Bolu-Seben, and Kastamonu-İhsangazi populations were divided into 4, 5, and 4 sub-populations, respectively. The genetic diversity estimates at locus level were performed for 13 sub-populations. The mean number of alleles, effective allele, genetic diversity value, and polymorphic locus number were observed as 2.00, 1.39, 0.24, and 30.00 (100%) respectively (Table 4.7). The highest number of allele, effective allele, genetic diversity value, and polymorphic locus number were determined as 1.33, 1.21, 0.13, and 10 (33%) in the sub-population of Bolu/Seben/Güneyce Köyü (21/1), respectively. Additionally, the highest effective allele number 0.21 was observed in Bolu / Seben / Güneyce Köyü (23/2) and Bolu / Yakuplar Köyü populations, too. The lowest number of allele, 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 Köyü / Beylik Mevkii, respectively (Table 4.7).

Table 4.10. 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 sub-populations 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 4.8).

Table 4.11. 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-population 4 and 6, while the lowest genetic distance value was 0.06 between the sub- populations 7 and 8, 10 and 11 (Table 4.9).

Table 4.12. The genetic distance values among the einkorn wheat landraces 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 was constructed based on the genetic distance values which was calculated according to iSSR data indicated that the sub-populations grouped into two major groups and which were also divided into sub-groups. In generally, in the first major group, Bolu-Seben / Güneyce and Kastamonu-İhsangazi populations grouped together but in different subgroups, while in the second major groups, Bolu-Seben populations were grouped together. In the first major group, the population Kastamonu-İhsangazi / Koçcuağzı Köyü was grouped as an outlier group, while in the second major group, the population Bolu-Haccağız/Beylik Mevkii was grouped as an outlier group (Figure 4.4).

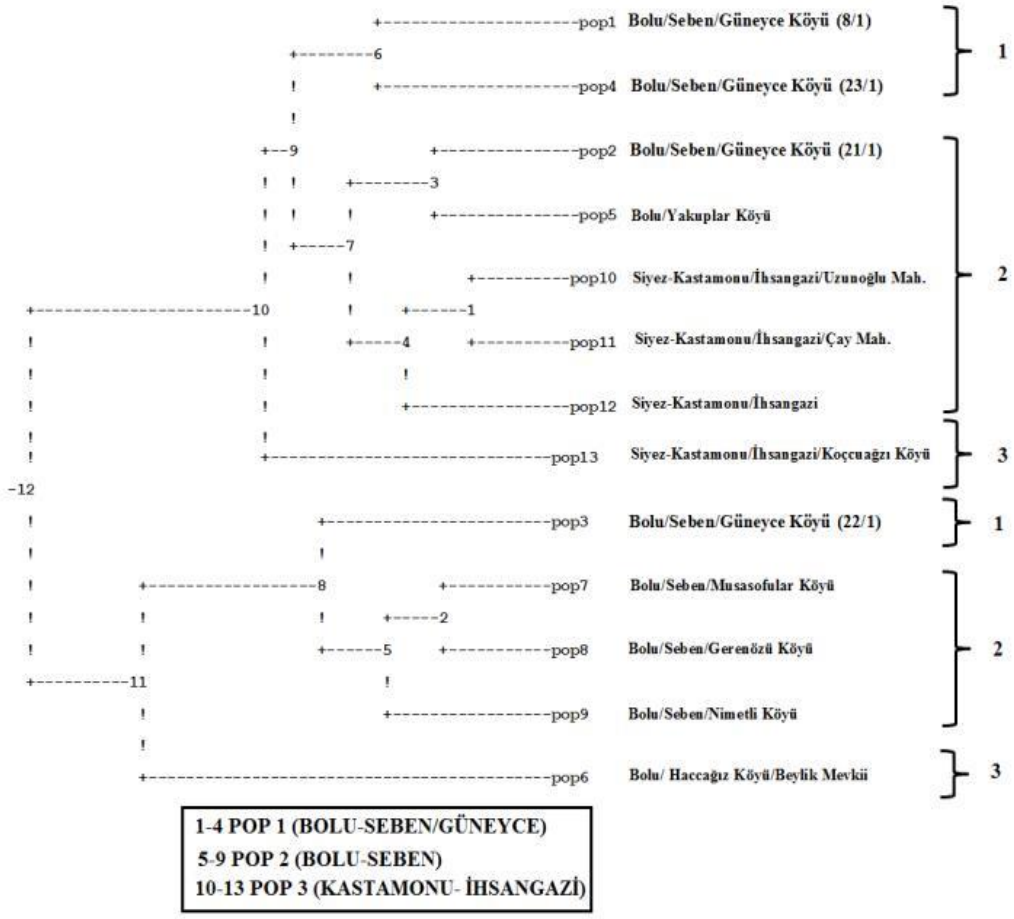


Figure 4.4. The dendrogram representing the phylogenetic relationships among einkorn wheat landraces sub-populations

5. THE DISCUSSION

The average successful allele number and genetic diversity value were significantly high according to the genetic diversity estimates. One of the most important tools for determining genetic variation is the amount of effective allles. The number of successful alleles corresponded to the number of allels contributing to genetic variation roughly equal. The number of allles included in the sample is very high, which shows that genetic variation is still very high. The extent of 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 Şan et al., 2015; Ozbek Ö., 2021; Ö Özbek et al., 2013; Özlem Özbek et al., 2011, 2012). The gene flow and genetic distance data also supported our study.

Therefore, 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. In fact, if the number of samples studied in sub-populations were many, 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 are quite high compared to the genetic diversity values at the locus level. On the other hand, the values of genetic diversity observed in sub-populations 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, farmers in other cities, or 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 sub-populations 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 level due to selfing also cause low gene flow. As a result, the genetic differentiation is increasing among the einkorn wheat populations and sub-populations. According to the genetic distance data, although it is interesting that the highest genetic distance is observed among Bolu sub-populations, the genetic distance data are 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 weredone for only three populations, Bolu and Kastamonu populations were grouped separately, while in the analyses of sub-populations, the Bolu-Seben / Güneyce sub-populations and 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 group. This is because einkorn wheat seeds have been distributed by Bolu Municipality to local villagers since 2014. Probably, the einkorn wheat seeds distributed in Bolu-Seben-Güneyce villages may most likely have originated from the einkorn wheat seeds grown in their own fields. Apart from these, the eco-geographic (rainfall, humidity, temperature, daylight length, altitude, latitude, and 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%) as well as within populations (37%). The high level of genetic diversity determined in wheat landraces related to some other functional factors. These factors might be, after the domestication process, domesticated wheat varieties 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' personal interest on wheat varieties they grown contributed to shape the population structure. They also made selections on wheat they grow for their resistance to biotic and abiotic stress factors, and amount of yield and yield stability in low input agricultural system (Ozbek Ö., 2021; Zeven, 1999).

Inter-Retrotransposon Amplified Polymorphism (iRAP) markers indicated that Iranian diploid einkorn wheat (*T. monococcum*, *T. boeoticum* subsp. *boeoticum*, *T. boeoticum* subsp. *thaoudar* and *T. urartu*) had high genetic similarity due to a high affinity and gene flow, however, *T. monococcum* ssp. *monococcum* was distinctively different from both *T. boeoticum* and *T. urartu*, which were the distant species to other species studied (Eslami Farouji et al., 2015). Genetic diversity was investigated in 36 diploid wild einkorn wheat (*Triticum boeoticum*) by AFLP (Malaki, M., Naghavi, M. R., Alizadeh, H., Potki, P., Kazemi, M., Pirseyedi, S. M., ... &FAKHR, 2006), in diploid species belong to genus Triticum by RFLP (Le Corre & Bernard, 1995), and in 36 diploid wild einkorn (*Triticum boeoticum*) from West Iran by RAPD, AFLP and SSR markers (Naghavi et al., 2007). A larger collection of miRNAs and small RNA molecules were used for analysis of *Triticum monococcum* 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 (*T. monococcum*s sp. *Monococcum*) landrace populations originating from Turkey were investigated to determine the genetic diversity for high-molecular-weight (HMW) glutenin subunits, and the gliadins. Turkish cultivated einkorn populations displayed

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 Ö., 2021).

6. CONCLUSIONS AND RECOMMENDATIONS

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 early Bronze Age. Then, they started to be replaced by high yielding and free threshing wheat varieties (*Triticumaestivum* 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 Turkey (Fritsch, R., Hammer, K., & Szabo, 1996; Ozbek Ö., 2021).

When reviewing the agro-morphological characters averages, we found that one of the best characters was in population 27 of IZA - Bolu / Göynük / ÇaylakKöyüfor germination strength, root count, and root length. Germination power provides a better yield at harvest because the population has a better germination strength, the highest number of roots, and the best root length compared to other populations.

Population 21, IZA - Bolu / Seben / GüneyceKöyü had the lowest in coleoptile length, root count, root length, fresh root weight, and dry root weight (Table 8.1 in appendix).

Dendograms revealed that some agro-morphological characteristics in certain populations have no correlation with the 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 have no correlations. Population 15, on the other hand, did not 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 was not correlated for germinating strength with the other and Population 35 did not 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 for the average Tukey HSD test also revelead that (Table 8.1 in appendix) Population 27 had the best characters, Population 21 had the worst characters.

Molecular markers have been used efficiently to reveal phylogenetic relationships in plant species. 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 Aegilops species (MIZUMOTO et al., 2002).

The wheat landraces have accumulated an enormous amount of genetic variation for 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 of their uses and, for the improvement and the efficient genetic diversity maintenance and utilization of plant species (DESHEVA et al., 2020).

Recently people's interest in healthy foods also increased the interest in the cultivation of wheat landraces in Europe, and North African countries Morocco, Egypt and Ethiopia as well as in Turkey. Nevertheless, it seems that einkorn wheat landraces are, today, growing only in Bolu, Çankırı, Çorum, Kayseri, Sinop, and Kastamonu provinces in Turkey (Özberk, İ., Atay, S., Altay, F., Cabi, E., Özkan, H., & Atlı, 2016).

The study found apparent differences between the population in seven characters, and as can be noticed that there are differences between the populations in different cities in Turkey. Accordingly, it is recommended that more extensive study should be carried out among the population in the most apparent characteristics through the germination of the population for a longer period until the crop ripens. And study the difference between the population in terms of leaf length, plant length, spike length, spike weight, number of seeds per spike, seed weight, and population study in 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 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 Turkey and in other countries for agro-morphological and molecular characteristics.

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8. APPENDIX

8.1 The Agro-Morphological Characters Materials

The following items are the materials used in the agro-morphological characters experiments:

- Autoclave sterilization device to sterilize the soil at 121 ° C for 15 minutes (Figure 8.1). The NÜVE Autoclave device capacity is 40 liters. Sterilization temperatures were 105.0 ° C / 135.0 ° C, five ready sterilization programs, and Programmable microprocessor N-sMArt control unit.
- Domestos bleach containing sodium hypochlorite 20 dh to 30% to sterilize the seeds before planting (Figure 8.2).
- Tween 20 is used to sterilize (Number 8.22184.1000 - Merck KGaA) (Figure 8.3).
- MtopsMs300hs Magnetic Stirrer device for mixing seeds with sterilizers for 10 minutes (Figure 8.4).
- Climate room is a seed room with a temperature of 23 ° C, 16 hours during the day and 8 hours at night (apic of climate room).
- The sterile cabin for cultivation on the petri dish.
- AS 220.C2 PLUS sensitive scale (Figure 8.5).
- Filter paper
- Petri dishes
- Distilled water
- The INCU-SHAKER™ drying device (incubator) (Figure 8.6). The INCU-SHAKER™ Mini is the compactest shaker of its kind. A wide 11.5" x 9.5" workspace embraces many platforms for use: various flasks, pipes and other ordinary vessels.
- Greenmore Agricultural soil: Greenmore Wonderful Plant Soil Flower Soil Pot Soil with Humus Additive. The most important component of the soil is organic substances that make up the structure of the soil and are the most important growth stimulating substance. With a pH level (6-7) and Organic Matter: 76.4%. (Figure 8.7).

Figure 8.1. Autoclave sterilization device.

Figure 8.2. Domestosbleach (sodium hypochlorite)

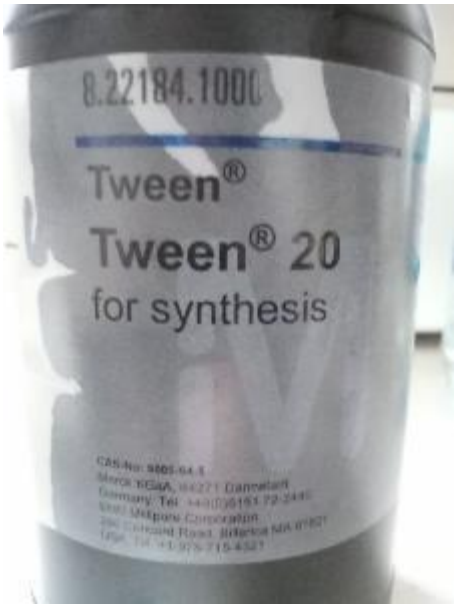

Figure 8.3. Tween 20

Figure 8.4. MtopsMs300hs Magnetic Stirrer device

Figure 8.5. AS 220.C2 PLUS Sensitive scale

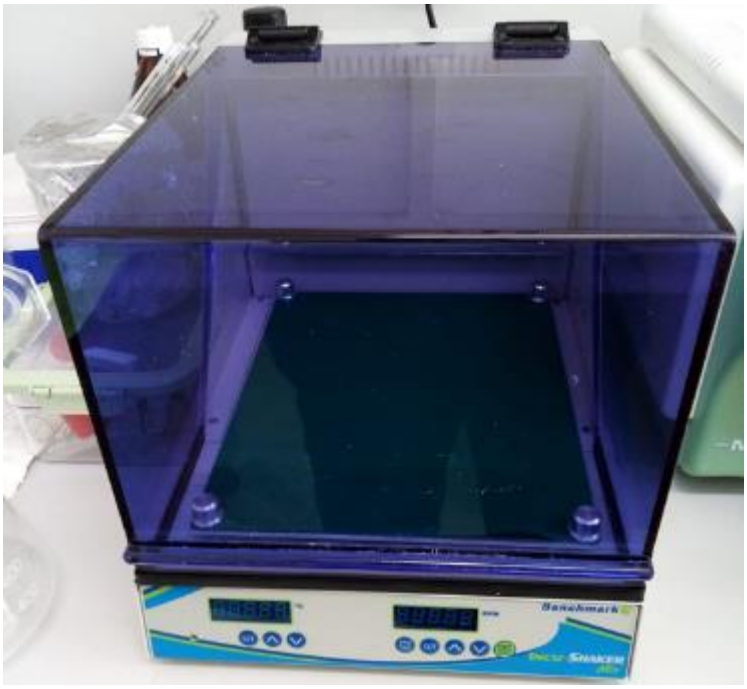

Figure 8.6. The INCU-SHAKER drying device (incubator)

Figure 8.7. The Greenmore agricultural soil

8.2 The Molecular Characters' Materials

The following items are used in the molecular character experiments:

1. Glacial acetic acid 25 Litre Cat No: 1.00056-2,5LT. (Merck)
2. Ethanol 25 Liter Cat No: 12221-2.SLT. (Sigma)
3. DNA Ladder 1 Kb Lambda DNA Marker Cat No: N3232S. (NEB)

4. DNA Ladder 20 bp Lambda DNA Marker Cat No: PI 598. (NEB)
5. PCR Super Mix High Fidelity 100u Cat No: 1725310. (Biorad)
6. Plant Genomic DNA Purification Kit. (MN)
7. Gel and PCR Clean Up Kit 250 preps. (MN)
8. ElektroforezJelBoyası Cat No: ABM01. (ABM)
9. Gel Loading Dye Blae 4 ml Cat No: B702 IS. (NEB)
10. Ultrapure ddH2O500ML. (Serva)
11. Primer Forward 50 mer HPLC Cat No: PRZHPLC50. (PRZ)
12. Primer Reverse 55 mer HPLC Cat No: PRZHPLC50. (PRZ)
13. Liquid Nitrogen
14. FAPG1 Buffer
15. FAPG2 Buffer
16. FAPG3 Buffer (concentrate)
17. W1 = Wash Buffer (concentrate)
18. W2 = Wash Buffer (concentrate)
19. Elution Buffer
20. RNase A (lyophilized)
21. Filter Column
22. FAPG Column
23. TAE Buffer
24. EtBr
25. 6X Loading dye
26. Collection Tube
27. Filter Column
28. Ethanol (96-100%)
29. dH2O
30. Ice
31. Centrifuge (Microfuge 20R – BECKMAN COULTER) (Figure 3.8).
32. Vortexing (BenchMixer™ and Mortexer™ Vortex Mixers by Benchmark Scientific) (Figure 3.9).
33. myFuge™ 8 Mini Centrifuge by Benchmark Scientific (Figure 3.10).
34. AS 220.C2 PLUS sensitive scale (Figure 3.11).
35. Benchmark Scientific BSH1002 Digital Dry Bath Heater (Figure 3.12).
36. PCR (T professional – Thermocycler) (Figure 3.13).

37. Electrophoresis is an electrokinetic mechanism that divides loaded particles by an electrical load field into fluid. In life sciences, it's most commonly used for protein molecules or DNA separation and can be done by various procedures, based on the molecules' form and scale. (Figure 3.14).
38. The Microwave (Arçelik) (Figure 3.15).
39. The D.N.R. Bio-Imaging Systems which is gel documentation and analysis equipment for the molecular biology research community (Figure 3.16).

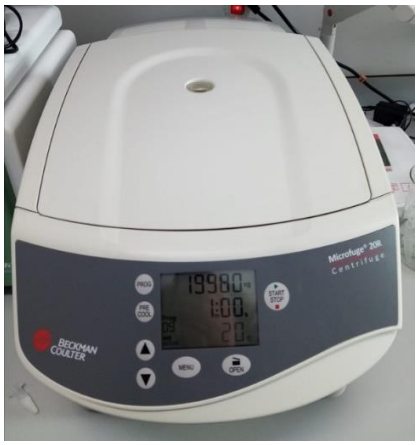

Figure 8.8. The Centrifuge (Microfuge 20R – BECKMAN COULTER)

Figure 8.9. The Vortexing (BenchMixer™ and Mortexer™ Vortex Mixers by Benchmark Scientific)

Figure 8.10. myFuge™ 8 Mini Centrifuge by Benchmark Scientific

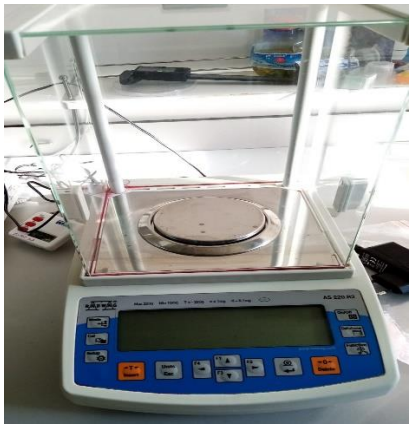

Figure 8.11. AS 220.C2 PLUS sensitive scale

Figure 8.12. Benchmark Scientific BSH1002 Digital Dry Bath Heater

Figure 8.13. The PCR (T professional – Thermocycler)

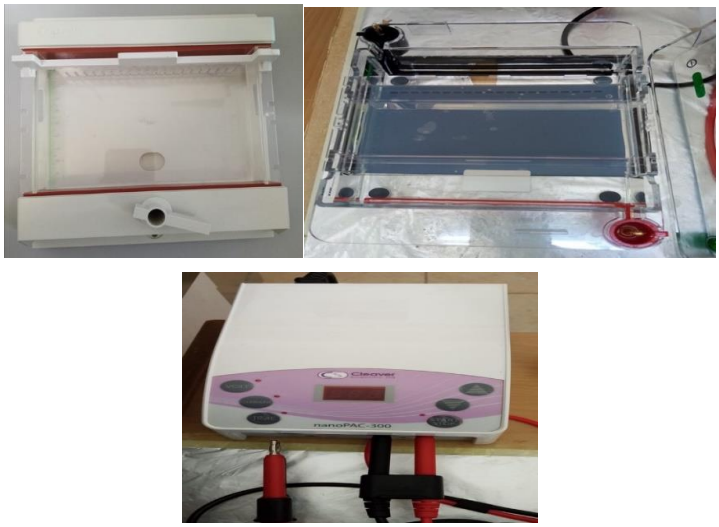

Figure 8.14. The Electrophoresis

Figure 8.15. The Microwave (Arçelik)

Figure 8.16. The D.N.R. Bio-Imaging Systems

8.3 Differences Among 48 Einkorn Discriminated by Tukey HSD

Table 8.1. Differences among 48 einkorn discriminated by Tukey HSD

Cultivars	LW*	CL	RC	RL	FRW	DRW	GP
1	353.25$^{e-f-g-h}$	1.01$^{b-c-d-e-f-g-h}$	3.77$^{d-e-f-g-h-I-j}$	1.88$^{a-b-c-d-e-f-g-h}$	43.20^{a}	19.90^{a-b}	7.33^{a-b}
2	361.35^{f-g-h}	1.20$^{e-f-g-h-i-j}$	3.97$^{f-g-h-i-j-k}$	2.30$^{c-d-e-f-g-h-i-j-k}$	64.07^{a}	22.93^{a-b}	9.00^{b}
3	361.58^{f-g-h}	1.32$^{g-h-i-j-k-l-m}$	4.47^{i-j-k}	2.34$^{c-d-e-f-g-h-i-j-k}$	101.87^{a}	29.57^{a-b}	10.00^{b}
4	244.20$^{a-b-c-d-e-f-g}$	1.23$^{e-f-g-h-i-j-k-l}$	4.47^{i-j-k}	2.24$^{b-c-d-e-f-g-h-i-j-k}$	90.33^{a}	28.37^{a-b}	10.00^{b}
5	271.76$^{a-b-c-d-e-f-g}$	1.37$^{h-i-j-k-l-m-n-o}$	4.70^{j-k}	3.54^{k}	100.57^{a}	35.17^{b}	9.67^{b}
6	193.58$^{a-b-c-d}$	1.23$^{e-f-g-h-i-j-k-l}$	4.07$^{f-g-h-i-j-k}$	2.04$^{b-c-d-e-f-g-h-i-j}$	81.97^{a}	24.13^{a-b}	9.67^{b}
7	426.90^{h}	1.45$^{i-j-k-l-m-n-o}$	4.27$^{g-h-i-j-k}$	3.22^{i-j-k}	108.67^{a}	34.53^{b}	10.00^{b}
8	376.52^{g-h}	0.99$^{b-c-d-e-f-g-h}$	3.73$^{c-d-e-f-g-h-i-j}$	3.00$^{f-g-h-i-j-k}$	125.87^{a}	25.90^{a-b}	8.33^{a-b}
9	183.16$^{a-b-c-d-}$	1.01$^{b-c-d-e-f-g-h}$	4.00$^{f-g-h-i-j-k}$	2.80$^{e-f-g-h-i-j-k}$	77.80^{a}	18.43^{a-b}	9.33^{b}

Cultivars	LW*	CL	RC	RL	FRW	DRW	GP
10	190.50 a-b-c-d-	0.75 b-c-d	3.20 b-c-d-e-f-g	0.98 a-b	23.37 a	5.20 a-b	8.00 a-b
11	194.18 a-b-c-d-	0.72 a-b-c	2.67 a-b-c-d	1.17 a-b-c	37.60 a	5.83 a-b	8.33 a-b
12	201.43 a-b-c-d-	0.88 a-b-c-d-e-f	3.37 b-c-d-e-f-g-h-I	1.51 a-b-c-d-e	70.23 a	13.13 a-b	9.33b
13	277.94 a-b-c-d-e-f-g-h	0.88 b-c-d-e-f	3.10 b-c-d-e-f	1.12 a-b-c	27.00 a	7.13 a-b	9.33b
14	299.04 c-d-e-f-g-h	0.82 a-b-c-d-e	2.77a-b-c-d-e	1.48 a-b-c-d-e	51.90 a	8.23 a-b	9.67b
15	235.30 a-b-c-d-e-f-g	1.24 e-f-g-h-i-j-k-l	3.87 e-f-g-h-i-j	3.07 g-h-i-j-k	141.60 a	21.73 a-b	9.00b
16	186.72 a-b-c-d-	0.74 a-b-c	2.60 a-b-c	0.92a-b	27.10 a	5.93 a-b	8.33 a-b
17	245.12 a-b-c-d-e-f-g	0.85 a-b-c-d-e-f	2.47a-b	1.48 a-b-c-d-e	23.00 a	10.63 a-b	8.00 a-b
18	196.58 a-b-c-d	0.78 a-b-c-d	2.47 a-b	1.39 a-b-c-d	25.10 a	5.17 a-b	8.67b
19	165.40 a-b-c	0.89 a-b-c-d-e-f	3.63 c-d-e-f-g-h-i-j-k	1.69 a-b-c-d-e-f	36.77 a	8.87 a-b	8.67b
20	183.42 a-b-c-d-	0.77 a-b-c-d	2.77 a-b-c-d-e	1.14 a-b-c	23.40 a	5.73 a-b	9.00b
21	187.32 a-b-c-d-	0.49a	1.80a	0.54a	9.37a	2.23a	8.00 a-b
22	200.17 a-b-c-d-	0.87 a-b-c-d-e-f	3.20 b-c-d-e-f-g	1.76 a-b-c-d-e-f-g	59.10 a	12.40 a-b	9.33b
23	229.04 a-b-c-d-e-f-g	1.02 b-c-d-e-f-g-h	3.50 b-c-d-e-f-g-h-i-j	1.43 a-b-c-d-e	29.23 a	10.10 a-b	9.00b
24	238.20 a-b-c-d-e-f-g	1.21 e-f-g-h-i-j-k	4.07 f-g-h-i-j-k	2.50 d-e-f-g-h-i-j-k	102.73 a	23.10 a-b	8.67b
25	194.08 a-b-c-d-	1.23 e-f-g-h-i-j-k-l	4.13 f-g-h-i-j-k	1.84 a-b-c-d-e-f-g-h	66.47 a	15.30 a-b	9.33b
26	205.86 a-b-c-d-e	1.36 h-i-j-k-l-m-n-o	4.23 f-g-h-i-j-k	2.38 c-d-e-f-g-h-i-j-k	80.53 a	17.73 a-b	8.33 a-b
27	141.50a	1.68 m-n-o	4.60 j-k	5.27l	127.70 a	23.57 a-b	5.33a
28	217.92 a-b-c-d-e-f	1.62 k-l-m-n-o	3.80 d-e-f-g-h-i-j	1.84 a-b-c-d-e-f-g-h	36.10 a	14.77 a-b	9.00b
29	171.08 a-b-c	1.64 l-m-n-o	3.73 c-d-e-f-g-h-i-j	1.75 a-b-c-d-e-f-g	32.90 a	14.00 a-b	8.67b
30	265.86 a-b-c-d-e-f-g	1.77o	3.90 e-f-g-h-i-j	3.16 h-i-j-k	82.77 a	23.03 a-b	8.33 a-b
31	226.26 a-b-c-d-e-f	1.74 n-o	4.27 g-h-i-j-k	3.26 j-k	85.10 a	26.83 a-b	9.00b
32	188.20 a-b-c-d-	1.69 m-n-o	4.47i-j-k	3.07g-h-i-j-k	86.37 a	22.47 a-b	9.00b
33	211.05 a-b-c-d-e	1.70 m-n-o	4.20 f-g-h-i-j-k	1.96 b-c-d-e-f-g-h-i-j	43.50 a	17.30 a-b	9.00b
34	199.50 a-b-c-d-	1.76o	3.93 f-g-h-i-j-k	1.88 b-c-d-e-f-g-h	48.23 a	14.77 a-b	9.67b
35	169.48 a-b-c	1.56 j-k-l-m-n-o	4.50i-j-k	1.96 b-c-d-e-f-g-h-i-j	75.60 a	18.03 a-b	10.00b

Cultivars	LW*	CL	RC	RL	FRW	DRW	GP
36	147.43 a-b	1.26 f-g-h-i-j-k-l	4.13 f-g-h-i-j-k	1.92 b-c-d-e-f-g-h-i	52.90 a	13.63 a-b	9.33 b
37	266.12 a-b-c-d-e-f-g	1.04 b-c-d-e-f-g-h-i	4.13 f-g-h-i-j-k	2.69 d-e-f-g-h-i-j-k	102.53 a	17.93 a-b	8.67 b
38	256.84 a-b-c-d-e-f-g	1.34 h-i-j-k-l-m-n	3.97 f-g-h-i-j-k	1.42 a-b-c-d	29.43 a	8.33 a-b	8.67 b
39	203.24 a-b-c-d-e	1.16 c-d-e-f-g-h-i-j	3.93 f-g-h-i-j-k	1.72 a-b-c-d-e-f	62.20 a	13.17 a-b	9.67 b
40	263.00 a-b-c-d-e-f-g	1.11 b-c-d-e-f-g-h-i	4.37 h-i-j-k	1.79 a-b-c-d-e-f-g	62.20 a	14.47 a-b	9.33 b
41	227.48 a-b-c-d-e-f-g	1.16 d-e-f-g-h-i-j	4.27 g-h-i-j-k	1.72 a-b-c-d-e-f	50.67 a	13.73 a-b	9.33 b
42	172.73 a-b-c	1.03 b-c-d-e-f-g-h-i	3.57 b-c-d-e-f-g-h-i-j-k	1.85 a-b-c-d-e-f-g-h	47.63 a	9.30 a-b	9.33 b
43	173.44 a-b-c	0.98 b-c-d-e-f-g-h	3.47 b-c-d-e-f-g-h-i-j	1.55 a-b-c-d-e	39.80 a	8.87 a-b	9.33 b
44	247.27 a-b-c-d-e-f-g	1.34 g-h-i-j-k-l-m-n	3.27 b-c-d-e-f-g-h	2.09 b-c-d-e-f-g-h-i-j	66.87 a	11.53 a-b	10.00 b
45	296.20 b-c-d-e-f-g-h	1.20 e-f-g-h-i-j-k	3.27 b-c-d-e-f-g-h	1.38 a-b-c-d	29.97 a	8.80 a-b	9.00 b
46	290.40 a-b-c-d-e-f-g-h	0.93 b-c-d-e-f-g	3.30 b-c-d-e-f-g-h	1.52 a-b-c-d-e	35.27 a	8.33 a-b	9.00 b
47	325.93 d-e-f-g-h	1.09 b-c-d-e-f-g-h-i	3.40 b-c-d-e-f-g-h-I	1.75 a-b-c-d-e-f	47.17 a	11.00 a-b	9.67 b
48	186.20 a-b-c-d-	1.32 g-h-i-j-k-l-m	3.83 e-f-g-h-i-j	1.96 b-c-d-e-f-g-h-i-j	30.23 a	10.40 a-b	9.33 b

* LW= Leaf Weight (mg); CL= Coleoptile Length (cm); RC= Root Count (n); RL= Root Length (cm); FRW= Fresh Root Weight (mg); DRW= Dry Root Weight (mg); GP= Germination Power (%).