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The Molecular Structure of Einkorn Wheat
(*Triticum monococcum* ssp. *monococcum*)

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Abstract

Triticum and Aegilops, which form wheat, belong to the Triticeae tribe in the Gramineae family. Morphological markers, biochemical markers, and molecular markers are extensively applied in the identification of plant genetic resources including wheat. Morphological markers, for their limited number and being influenced by environmental conditions and biochemical markers for their lower level of polymorphism are restricted in diversity studies. One of the first domesticated plants is einkorn wheat (*Triticum monococcum* ssp. *monococcum*). It will guarantee a consistent supply of nutritious food items suited to various stressful conditions of the climate. Unfortunately, it was only used since the Bronze Age for cultivation in the breeding of wheat. This review aims to the molecular structure of einkorn wheat based on the previous studies.

Keywords: Genetic diversity, Distribution, Einkorn, Genotype, *Triticum monococcum* ssp. *monococcum.*

1. Introduction

Wheat supplies human with calorie and protein which are required for body. It is the basic food material 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 and consumer demands have diversified. The most common consumption forms of wheat are flour, bread, pasta, semolina, biscuits, bulgur, and vermicelli. The products outside of traditional products in the world and Turkey are sweets, starches, and so on (Zencirci and Karagöz, 2005). Intensive studies have been carried out on *Triticum monococcum* ssp. *monococcum*, in particular, for its diploid feature and for the possibility, the information to be obtained from it can be easily applied to the breeding of durum and bread wheat. That has made einkorn very attractive for the researchers. To answer the question of where einkorn was first cultivated, comparative DNA analyzes were conducted by Heun et al. (1997) among approximately 1400 wild einkorn (*T. monococcum* ssp. *boeoticum*) wheat and einkorn (*T. monococcum* ssp. m*onococcum*). As a result of the analyses, it was concluded that the population closest to the cultured form was from Karacadag / Diyarbakir region. In the morphological analyses performed by different researchers, it was stated that einkorn wheat is resistant to biotic and abiotic stress conditions, therefore, it can be used in wheat breeding. It carries A genome which exist in the structure of both durum and bread wheat (Yaman and Zencirci, 2015).

Triticum is divided into three groups according to its chromosome numbers: diploid (2n = 14), tetraploid (2n = 28), and hexaploid (2n = 42). Diploid cultured wheat is *T. monococcum* ssp. *monococcum* (2n = 14, AA), two tetraploid ones are *T.turgidum* ssp. *dicoccoides* (2n = 28, AABB) and *T. timopheevii* (2n = 28, AAGG), and a hexaploid one is *T. aestivum* L. (2n = 42; AABBDD) (Feldman et al., 1988). These species can also be classified differently according to their utilization. Hexaploid wheat is extensively for bread, baklava, pie, and biscuit making while tetraploid one for pasta and bulgur. Diploid wheat, on the other hand, is mostly used for bulgur and rarely for bread and some pasta production today (Zencirci et al., 2018).

Selection of DNA marker system in the characterization of plant gene resources, depends on the research purpose, population structure, plant variety, marker system availability, and time and cost required for the analyses. Each system has its advantages and shortcomings. When Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Simple-Sequence Repeat (SSRs), and Inter Simple Sequence Repeats (ISSR) DNA marker techniques were compared, SSR and AFLP for polymorphism, RAPD and ISSR techniques for cost, RFLP for reproducibility were found superior. While laboratory facilities considered RAPD, SSR, and ISSR are found easily applied in laboratories where radioactive material use and conditions are limited.

Southeastern Anatolia is a very important region for wheat genetic resources. Especially, with the studies carried out in recent years, it has been shown that einkorn (*T. monococcum ssp. monoccocum*) and durum wheat (*T. durum*) have been taken into the first culture in Karacadag / Diyarbakır (Ünlü et al. 2018). It is necessary to carry out necessary studies on this valuable material collected in the region in order to determine genetic resouces against abiotic and biotic stresses, especially in prospective wheat breeding studies.

2.The Origin Einkorn Wheat

The einkorn wheat, *T. monococcum* ssp. *monococcum*, was among the first crops domesticated in the Fertile Crescent. As indicated by grain remnants in archaeological sites, it was domesticated from its wild forebear, *T. monococcum* ssp. *aegilopoides*, around 7,500 BC, during the Pre-Pottery Neolithic A (PPNA) or B (PPNB) periods (McCorriston and Hole, 1991). First postulated that Jordan Valley was the territory of the domestication of einkorn (Jones et al., 1998). However, an investigation on 288 einkorn and its wild precursor by AFLP markers, regardless, revealed that wild populace from the Karacadag Mountains of southeast Turkey. That suggested that restraining occurred in this locale. The most reliable grain remains of einkorn were also found at settlements near Karacadag (Heun et al., 1997).

By researching haplotype variety among in more than twelve million nucleotides sequenced at eighteen loci across three hundred twenty-one wild and ninety-two domesticated einkorn accessions, revealed that *T.monococcum* ssp. *aegilopoides* experienced a procedure of natural genetic differentiation before domestication, prompting the emergence of three genetically unmistakable wild einkorn races assigned as a, b, and c (Kilian et al., 2007). Similarly, some authors discovered higher nucleotide and haplotype diversity in cultured einkorn than in the wild einkorn bunch b, demonstrating that einkorn did not endure a reduction of its diversity during training, and suggested multiple autonomous domestication events. Kilian et al. (2009) proposed a "dispersed-specific" training scenario. A sedentary Natufian society (Bar-Yosef, 2002) first harvested and afterward cultivated *T. monococcum* spp. *aegilopoides*. In a later phase of agricultural extension, the society was moved to different locations, potentially for a new start (Willcox, 2005). This hypothesis concurs well with the results of archaeological excavations. Apparatuses for grinding seeds were found in the most of Fertile Crescent destinations a long time before the huge seed remains of einkorn (Bar-Yosef, 2002), supporting the view that humans in the area were familiar with the harvest of wild seeds (Weiss et al., 2006).

The domestication of wild einkorn and further selection by farmers prompted important physiological and morphological changes. Some spring structures present in the predominantly winter wild ancestor were most likely chosen by a man for culture (Golovnina et al., 2010). The completely brittle rachis became semi-fragile, permitting the harvest of nearly 100% of the grain, contrasted with 50–80 % on wild forms (Davies and Hillman, 1990). The size of the kernels expanded (Zohary and Hopf, 2000) and they turned out to be marginally simpler to select, decreasing the endeavors required for de-husking. Stays of two-grained structures were found in archaeological destinations in Syria and in Greece and encompassing territories (Kroll, 1992). It is not clear, be that as it may, regardless of whether these structures spoke to unmistakable genotypes or if the presence of an extra flower by spikelet was expected to particular to ecological conditions (Kreuz and Boenke, 2002).

3.Genetic Diversity

Evaluation the decent einkorn increases gathered in various nations and stored in distinct gene banks would extensively encourage the assessment of helpful traits and practice in wheat improvement. Knüpffer (2009) detailed various 5,067 einkorn accesions preserved in 54 gene-banks. Just a little proportion of these accessions has anyway been broken down. Diversity investigations of einkorn dependent on morphological attributes, isozymes, and molecular markers have generally concerned a diminished diversity (Zaharieva and Monneveux, 2014).

Guzy et al. (1989) revealed a high inconstancy for yield inside einkorn. Sharma et al. (1981) portrayed abroad variety for plant stature, seed weight, flour protein, and lysine content, spike weight, and earliness in 93 einkorn populations and identified two which were shorter and sooner than durum wheat 'Modoc' and bread wheat 'Anza'. Wide intra-specific morphological diversity has been additionally announced for vegetative, ear, and phytoliths. Castagna et al. (1995) observed, in various areas, significant differences for yield-related traits including earliness in 21 accesions. Empilli et al. (2000) analyzed 16 morpho-agronomic characteristics in 1039 accesions *T. monococcum* subsp. *monococcum* ssp. *aegilopoides* and convar. A high diversity was observed for earliness and plant height. A lower diversity existed in seed attributes, which 40 genotypes were identified with heavier thousand weight - < 49 g) in 13 populations. Most accessions demonstrated low SDS (sodium dodecyl sulfate) sedimentation, indicating a bread-production quality. Regardless, eight accessions had SDS more prominent than eighty ml, equal to excellent bread wheat 'Salmone' . Moreover, a larger grain number per spike and heavier grain weight in a thirty-seven einkorn samples from Romania and Hungary. Guzmán et al. (2009) studied twenty-nine Spanish selections for morphological characters. He named just four botanical varieties, i.e., *tauricum* Drosd, *monococcum*, *macedonicum and nigricultum Flaksb*. Brandolini et al. (2013) has also revealed a larger phenotypical variation in 169 einkorn populations for 24 plant, spike, and other part characteristics. They discovered significant contrasts among samples from various origins for heading date, number of spikelets per spike, seed length, protein substance, and Sodium Dodecyl Sulfate (SDS) sedimentation volume.

The investigation of storage proteins by Payne and Waines (1987) in wild einkorn (*T. monococcum* subsp. *Aegilopoides*) for HMW (High Molecular Weight) glutenin subunits. In excess of 80 diverse gliadin electrophoretic designs, Metakovsky and Baboev (1992) found 109 einkorn.

4.Genetic Transformation

Domesticated einkorn (*Triticum monococcum* ssp. monococcum) is one of the world's oldest cereal crop 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), and low genome size (~ 5.7GB). But einkorn, for the application of modern biotechnology, like transgenesis is still a recalcitrant monocotylatedone plant (Örgeç et al., 2021; Feldman et al., 1988).

The efficient genetic transformation of the seed using bio-mediated DNA delivery is recorded (Miroshnichenko et al., 2018). For effective transformation, it was important to adjust to 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. Tissues of the bombarded einkorn regeneration plants are recalcitrant, but certain improvements in the culture medium have shown that transgenic events are more efficient. Independent transgenic plants at frequencies range from 0.0 to 0.6 percent were produced in different experiments.

5.The Previous Studies of 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 may give close to 100% results in determining the diversity in plant populations and the relationships among these individuals. Molecular markers are used to identify individuals in gene resources and to prevent duplications, determination of kinship relationships, mapping, and selection.

Empilli et al. (2000) examined the morphological variation in 1,344 genotypes of *T. monococcum* ssp *monococcum, T. monococcum* ssp *boeoticum, and T. monococcum* ssp *sinskajae* subspecies, for a total of 17 morphological and quality traits. They determined some *T. monococcum* ssp *monococcum* genotypes with good agronomic characters.

Chabane et al. (1999), with AFLP marker, investigated the genetic variation between 6 *T. urartu* genotypes collected from the northern (Aleppo) and southern (Sweida) Syria and 12 Turkish genotypes and found that AFLP primers produced a total of 176 bands, with band lengths of 50. They reported that the six genotypes collected from the northern and southern Syria formed a separate group from Turkish genotypes in the cluster analysis. *T. Urartu* also differed by the region.

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

Alvarez et al. (2006), in their study using SDS-PAGE and A-PAGE methods to investigate different alleles of seed storage proteins in Spanish origin monococcum genotypes, they found 3 different alleles for Glu-A1m and 6 alleles for Glu-A3m, Gli-A1m and Gli-A2m loci. They reported that 7 and 14 alleles were detected for these alleles, respectively, and that there were variations in genotypes for these alleles, and that these alleles could be used to improve the quality of wheat breeding.

Gülbitti-Onarici et al. (2007), Turkey's use of the different regions of the toplanmş *Triticum aestivum, T. dicoccoides, T. urartu* and *T. monococcum* ssp. *boeoticum* boeotic types of AFLP marker genotypic identification, polymorphism and Umeda WOR they examined the phylogenetic relationships, 33 AFLP primer combinations of the form 875 polymorphic bands, 133 of the polymorphic band *T. monococcum* ssp *boeoticum* to, 66 of *T. urartu* and 141 of *T. dicoccoides* are specific bands, *T. monococcum* ssp *boeoticum*, *T. urartu* and *T. dicoccoides* polymorphism rates respectively; They reported that it was found as 42.63%, 32.34% and 27.71%, and that *T. urartu* was the progenitor of *T. dicoccoides* and *T. aestivum*.

In Demirel (2020) study, genetic relationships of 14 einkorn wheat (*Triticum monococcum L.*) were determined using 7 ISSR markers. As a result of the research, 68 polymorphic bands were obtained and the rate of polymorphism (P%) was calculated as 97.5% on average. Genetic diversity (H) value was between 0.38 and 0.50, and the average was determined to be 0.45. Polymorphism information content (PIC) was between 0.30 and 0.37, and the average was found to be 0.34. The average Jaccard similarity value was determined as 0.4554. According to the result of the dendogram, while genotypes are grouped in two clusters, it was determined that they are divided into four sub-clusters in the PCoA graph. It was concluded that ISSR markers can be used to determine genetic variation and characterize phylogenetic relationships.

In Karahan et al. (2019) study, it was determined that 64 genotypes of einkorn wheat obtained from different countries were grown under Çukurova conditions and some of them were suitable for Çukurova conditions, while others were not suitable because the required vernalization requirement was not met, and there was also a wide variation in terms of agro-morphological characteristics. The obtained data can be used in the development of einkorn types suitable for the purpose.

Cao et al. (1999), in their study investigating the usability of RAPD technique in reclassifying incorrectly classified wheat samples analyzed control genotypes by RAPD markers for 12 genotypes. They reported that five 5 out of the 12 genotypes examined were *T. turgidum* ssp *dicoccum*, one was *T. timophevii* ssp timophevii and six were *T. monococcum* ssp *monococcum.* These results were also supported by cytological analyzes.

6.Conclusion

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.

Other than its enthusiasm as healthy food, einkorn has likewise been considered for quite a while as a potential source of genetic variation for wheat breeding. Its powerful usage has been to a great extent postponed by the difficulty to create interspecific hybrids with satisfactory fertility. This problem has been to some degree defeated by using the strategy of extension crossing of tetraploid wheat / einkorn *amphiploids*. By contributing novel genes and permitting direct recombination exchanges einkorn is presently progressively utilized for the transfer of valuable characteristics into cultivated wheat and *triticale*. Einkorn is as of now to a great extent utilized in several breeding programs, for instance at ICARDA (International Center for Agricultural Research in Dry Areas) to improve rust resistance, earliness, and early energy in durum wheat and at the Agricultural research organization of the Hungarian Academy of Sciences, Martonvasar, to transfer biotic stresses resistance and ice tolerance into bread wheat (Van Slageren, 1994).

In any case, there is reestablished enthusiasm for this crop because of the dietary qualities of its grain, its adjustment to low - embrace agriculture, and the elevated level of resistance to insects and diseases, which makes einkorn suitable for organic farming.

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