



The ancient DNA and archaeobotanical analysis suggest cultivation of *Triticum aestivum* subsp. *spelta* at Yumuktepe and Yenikapı Pottery Neolithic sites in Turkey

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Abstract Archaeobotanical materials subject to aDNA analysis were recovered from Yumuktepe and Yenikapı, two important archaeological sites in Anatolia and date back to the Pottery Neolithic Period i.e., 7th millennium BC. Many charred ancient seeds representing various cereal species including a great number of wheat grains were documented in mentioned sites. Among the cereal seeds, charred wheat samples were tentatively identified as *Triticum aestivum* subsp. *spelta* L. or *Triticum new glume* wheat (NGW) or atypical emmer or naked wheat in

Yumuktepe and Yenikapı showed similarities with the morphological characteristics of *T. aestivum* subsp. *spelta* wheat, but it was difficult to reach a firm conclusion. This study aimed to provide genetic data to enable more precise identification of charred wheat seeds using an ancient DNA (aDNA) approach. aDNAs were successfully extracted from the representative charred seeds of *T. aestivum* subsp. *spelta* or NGW or atypical emmer or naked wheat. The PCR amplification of 26SrDNA and IGS gene regions with aDNA was carried out and sequenced. The expected product sizes of IGS 158 bp for the D genome and 87 bp for the A or B genomes and DNA sequence comparisons with other wheat species revealed that *T. aestivum* subsp. *spelta* or NGW or atypical emmer or naked wheat samples included the D genome from *Aegilops tauschii* and is more likely to be *T. aestivum*

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subsp. spelta. The discovery of *T. aestivum subsp. spelta* grains in the Yenikapı and Yumuktepe suggest that the cultivation of hexaploid wheat was widespread. Further, spelta hulled wheat, which is the progenitor of the hexaploid wheat, might have been cultivated in these settlements.

Keywords Yumuktepe · Yenikapı · aDNA · *T. aestivum subsp. spelta* · *Aegilops tauschii* · IGS region

INTRODUCTION

The studies of plant residues found in archaeological excavations provide fundamental evidence to understand how the domestication and diffusion of agriculture took place. However, archaeobotanical studies have their limits due to the nature of the studied material, as they are charred and most of the time poorly preserved, causing difficulty in identifying distinct morphologies. Charring, which is a partial combustion process, affects the size and shape of seeds, causing shrinkage of the caryopsis. Based on a series of experimental studies; most charred seeds appear to shrink between 10 and 20%, with a slight bias towards greater contraction of the longer dimension, causing seeds to become more spherical (Willcox 2004; Braadbaart and van Bergen 2004; Nesbitt 2006).

Today, techniques and methods used in archaeology are improving in line with technological developments. Intensive cooperation between archaeologists and natural science researchers has made it possible to quantify previously inaccessible information to answer important archaeobotanical questions, especially through ancient DNA (aDNA) research and molecular biology studies. aDNA studies of fossil and charred forms of plant materials (wood, seeds, pollen, etc.) provide essential evidence to characterize the origin, spread and development of past agricultural systems such as cultivation time and source of cultivation. The research with aDNA also brings powerful new evidence that may complement archaeobotanical identification of excavated plant materials (Schlumbaum et al. 2008; Li et al. 2011; Ciftci et al. 2019). Once the genes which are important in the domestication processes are identified, they can be studied in ancient plant remains to assist in the understanding

of how domestication modified plant development. Consequently, questions about species identification, origin, spread and state of domestication of cultivated plants that previously could be addressed only through morphological and archaeobotanical studies, can today be explored directly (Schlumbaum et al. 2008). To understand plant domestication and recent plant evolution, previous researchers examined whether: (i) cereal crops were originally cultivated as common 'metapopulations' or as a core area for the origins of agriculture; (ii) whether they were associated with an early or recent domestication era; (iii) whether they originated from unknown domestication or subsequent genetic changes in an existing crop; (iv) whether they came from single or multiple domestication events or (v) whether they were still in the process of domestication (Willcox et al. 2008; Bogaard et al. 2021; Weide et al. 2021; Smith 2001; Fuller et al. 2011; Abbo et al. 2012; Blatter et al. 2002; Bilgiç et al. 2016; Castillo et al. 2016; Ciftci et al. 2019; Czajkowska et al. 2020; Ulaş and Fiorentino 2020).

Studies on the domestication and diffusion of wheat in recent years have focused on an interdisciplinary approach that uses disciplines such as genetics (Zohary 1996; Heun et al. 1997; Salamini et al. 2002; Özkan et al. 2002, 2005; Allaby and Brown 2003; Honne and Heun 2009; Haldorsen et al. 2011), ecology (Harlan and Zohary 1966; Ladizinsky 1985; Abbo et al. 2008a, 2008b), agronomy (Harlan 1992; Abbo et al. 2010, 2011) and emphasizes the relationship between environmental factors and human societies (Binford 1968; Flannery 1969; Cohen 1977; Rindos 1980; Cauvin 1989; Smith 2007). Especially, aDNA studies provide important contributions to discussions about the taxonomic diagnosis of some wheat species which have been problematic in archaeobotanical studies. The DNA studies carried out for the first time by Heun et al. (1997) and subsequently continued by several researchers have revealed evidence that Karacadağ Mountain, located in southeastern Turkey, is one of the first places (or cradle) of wheat domestication (Heun et al. 1997, 2008; Salamini et al. 2002; Özkan et al. 2005; Kilian et al. 2007; Haldorsen et al. 2011). In the 1990s, aDNA was extracted from some charred wheat and barley grains for the first time (Allaby et al. 1994, 1997, 1999; Brown et al. 1998; Schlumbaum et al. 1998). However, the extracted aDNA was found to be

in low amounts and short lengths (less than 60 bp.) (Allaby et al. 1997; Brown 1999). Since the publication of these early studies, numerous studies of *a*DNA from charred cereal samples have successfully obtained sufficient intact DNA fragments which can be amplified by highly specific and sensitive Polymerase Chain Reaction (PCR) (Blatter et al. 2002; Schlumbaum et al. 2008; Li et al. 2011; Oliveira et al. 2012; Fernandez et al. 2013; Bilgiç et al. 2016; Castillo et al. 2016; Ciftci et al. 2019; Czajkowska et al. 2020).

Recently, the *a*DNA study on new glume wheat (NGW), whose first use dates back to the Neolithic PPNB settlements Caferhöyük (de Moulins 1993) and Aşıklıhöyük (Ergün et al. 2018) in Anatolia and has been the subject of debate among archaeobotanists (Jones et al. 2000; Ulaş and Fiorentino 2020), has revealed that NGW wheat may belong to the *Triticum timopheevii* Zhuk. wheat group (Czajkowska et al. 2020).

The origin of hexaploid wheat cultivation is controversial indeed and it is even at the heart of this discussion. Although some morphological characters are accepted for the identification of hexaploid wheat species, accurate taxonomic identification of the caryopsis belonging to these species is often difficult (Hillman et al. 1996; Jacomet 2006). Therefore, it is often determined as a complex of *Triticum aestivum/durum* L. However, the remains of the rachis segment and glume often provide better diagnostic characters than the caryopsis (Dalnoki and Jacomet 2002; Jacomet 2006). Therefore, the earliest data on the cultivation of hexaploid wheats are based on rachis segment and glume identification.

Recent archaeobotanical and *a*DNA reports on Neolithic wheat have revealed that several species of wheat were cultivated, in addition to the three common wheat (emmer, einkorn and free-threshing wheat). Importantly, the discovery of new glume wheat (Jones et al. 2000; Schneider and Caneppele 2009; Kenéz et al. 2014; Toulemonde et al. 2014; Czajkowska et al. 2020; Ulaş and Fiorentino 2020) has reopened discussions on the types of wheat cultivated in the Neolithic period (Ulaş and Fiorentino, 2020). In addition to new glume wheat, Hillman suggested that *T. compactum* wheat was also cultivated in the PPNB Can Hasan III settlement (Hillman 1972). Another controversial wheat species cultivated in the Neolithic period is *T. aestivum* subsp. *spelta*,

which is considered to be a hexaploid, hulled and non-free-threshing primitive wheat species. It is possible to say that there is a consensus in the general archaeobotanical literature that the cultivation of spelt wheat was started in the Early Bronze period. The spelt wheat has two known races, which are the Asian and the European spelt wheats (Kuckuck and Schiemann 1957; Nesbitt and Samuel 1996). Traditionally, Asian spelt was cultivated in Transcaucasia, Iran, Tajikistan and Afghanistan, while European spelt was cultivated in Germany, Switzerland and France (Perino et al. 1996; Dedkova et al. 2004).

Ancient durum wheat (tetraploid, AABB) and bread wheat (hexaploid, AABBDD) seeds are nearly indistinguishable morphologically from each other without associated rachis fragments or entire spikes (Li et al. 2011; Hyun et al. 2020). Since the availability of seed rachis or complete spike is a rare occurrence in wheat archaeological studies, naked grains are usually labeled as “*T. aestivum/durum*”. Molecular biology tools such as specific markers and gene regions can help to differentiate morphologically indistinguishable wheat species. For instance, presence of the “D genome” was successfully demonstrated using intergenic spacer region (IGS) markers (Li et al. 2011; Oliveira et al. 2012). Amplification of an 87 bp product from the intergenic spacer region of rDNA is observed in A and B genomes, yet the D genome has a 71 bp insertion producing approximately 158 bp amplicon that makes a distinction between tetraploid (AABB) and hexaploid (AABBDD) wheat (Brown et al. 1998).

In the current study, an *a*DNA analysis was performed on some wheat grains that morphologically resemble spelt wheat or NGW or atypical emmer and/or naked wheat grains identified in the Neolithic settlements of Yumuktepe (Cilicia) and Yenikapı (Southern Thrace) dating back to the 7th millennium BC. Both settlements have key importance in understanding the spread of Neolithic agriculture to Western Anatolia and Europe due to their geographical locations (Fig. 1). The highly conserved and multiple copy numbered 26S rDNA gene extensively preferred to achieve efficient amplification of highly damaged or degraded DNA (Gugerli et al. 2005; Selkoe and Toonen 2006; Schlumbaum et al. 2008) while intergenic spacer region (IGS) was used for identification of species and determination of ploidy level in the genus *Triticum*, respectively. The results from

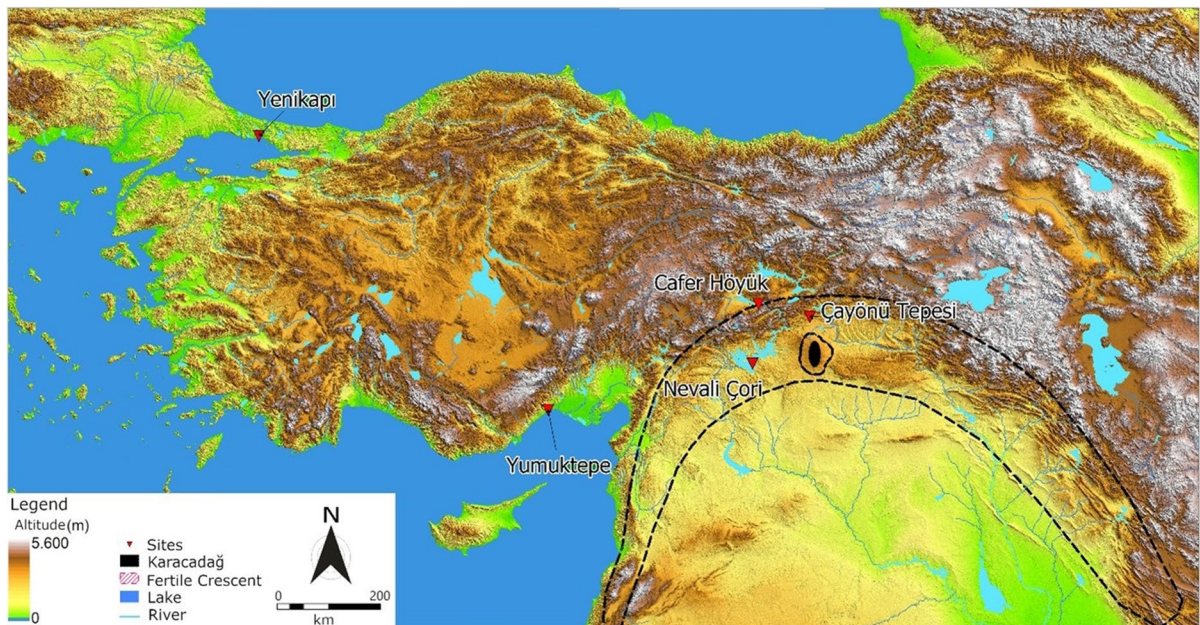


Fig. 1 The map shows the location of the Neolithic settlements of Yumuktepe and Yenikapı (Map by Savaş Saraltun)

this study would contribute to the accurate taxonomic identity of the ancient wheat species and address the domestication process of these ancient seeds. Being close to major sea routes to the south and west in the Mediterranean Sea and to Europe, findings on ancient wheat seeds from Yumuktepe (Ulaş and Fiorentino 2010, 2020) and Yenikapı (Ulaş 2020) archaeological sites would provide further insights about their center of domestication and the dispersal routes of cultivated wheats in Anatolia.

MATERIALS AND METHODS

Archaeological material

The archaeobotanical materials subject to aDNA analysis were recovered from two important archaeological sites in Anatolia, whose earliest levels date back to the Pottery Neolithic Period, i.e., 7th millennium BC.

One of these settlements is Yumuktepe (Fig. 1), which is known as one of the oldest Neolithic settlements located on the eastern Mediterranean coast of Anatolia. Yumuktepe has an uninterrupted stratigraphy from the Neolithic to the Middle Ages (Caneva

2012) and is located at the center of the modern city of Mersin, on the eastern bank of the Müftü (Efrenk) stream, 2 km north of the Mediterranean Sea. The site covers an area of about 250 × 200 m and has a height of around 23 m. About half (11 m) of this 23-m archaeological fill contains strata from the Neolithic period. It is also significant that it is located near the Gülek pass and the Belen Passes, two gates that connect Syria, the island of Cyprus, Mesopotamia and Central Anatolia. Yumuktepe had been an important hub between east and west, as well as between coasts, islands and inland regions, that allowed the site to benefit from varied and wide opportunities for communication with other cultures (Caneva 2012).

The Yenikapı site, another settlement whose archaeobotanical materials were subject to aDNA analysis in this study, was discovered during the construction of the Marmaray metro (underground rail and public transportation system) in 2004. The Yenikapı site is in the south side of Istanbul, in the south-eastern part of the Balkan peninsula and to the north side of the Marmara Sea (Fig. 1). At the Neolithic settlement Yenikapı, which can be dated between 6500 and 5800 BC (Caneva 2012), one of the richest tool and object collections of Turkey's prehistoric archaeological material were uncovered.

Archaeological rescue excavations were carried out in 40.000 square meters out of a total of nearly 58.000 square meters of land, and at a depth of -3 to -9 m below sea level. The Neolithic layers were discovered in a small part of the above-mentioned area at a depth nearly between -6.30 and -9 m. The first examinations based on cultural materials indicate that the Neolithic properties of Yenikapı are like those of Yarımburgaz 4 and Fikirtepe cultures (Kızıltan 2010; Özdoğan 2010; Kızıltan and Polat 2013).

Two exceptional situations that archaeologically characterize the İstanbul-Yenikapı settlement need to be mentioned. The first is that the Yenikapı settlement has stratification with an extended chronology from the Neolithic Period to the Ottoman era. The second is that the archaeological layers were buried under clay and mud due to the rise of the sea level and thus have been well preserved. The Neolithic farmers who were the oldest settlers at Yenikapı are considered to have settled in an area close to the banks of the Lykos/Bayrampaşa river, which is not very far from the coast, but it has dried up today. It has been suggested that the temperature increase at the end of the Pleistocene and at the beginning of the Holocene resulted in the rise in the sea level, which in turn caused the settlement to be covered with oxygen-free clay and mud. These increased oxygen-free conditions enabled organic residues to be well preserved and to be maintained until today (Algan et al. 2008; Kızıltan 2010).

Wheat samples subjected to *a*DNA analysis were obtained from the F3 trench A206 area of the Yumuktepe site and from the 1Cd3 trench of the Yenikapı site. Some information about these excavation areas will be given in detail below.

Yumuktepe—structure A206 in trench F3

The Early Neolithic remains in trench F3 of Yumuktepe consisted of irregular layers of ashes, crumbled wall plaster and burnt floors. Rough circular structures seemed to be partially dug into the ground. One such structure or area was defined as A206 and dated to 6570–6240 cal. BC. It included remains of silos with thick mud walls, which had been destroyed by a fire. In the silos carbonized seeds and pieces of plaster-bearing basket imprints were scattered all around, suggesting that the space and the structures were used to store agricultural products. Similarly built structures were found during the ethnobotanical research

carried out in the rural regions of south-eastern Anatolia (Ulaş 2021). Other furnishings of the structures were probably built with the same perishable materials, as suggested by fragments of clay boxes (Fig. 2A).

Few artifact materials were found inside these silos, but pottery fragments, lithic and bone tools were scattered in the areas outside of them. Food containers probably included both pottery and wooden vessels.

A total of 120 L of soil samples were taken from trench F3 A206 of Yumuktepe. The archaeobotanical analyses in A206 provided 1871 spikelet remains and 1459 crop plants. Among the crops, 803 of them were the seeds of different cereal species. Among the cereals, the great majority of samples were emmer wheat, but there were also 607 lentil seeds, 2 bitter vetch seeds and 49 different weed seeds. This taxa assemblage, together with the archaeological data, proves that the agricultural products were stored in this area after crop processing. In trench F3, several similar areas were found at different levels and several stamp



Fig. 2 Yumuktepe—structure A206 in trench F3 (A) and Yenikapı- trench 1cd3 (B)

seals were brought to light in association with this silo assemblage.

The dwelling structures of the same period were located at the north-east edge of the area (squares D3-E3) and consisted of square rooms with mud brick walls on stone foundations, sometimes with a red painted plastered floor and clay hearth.

Based on the combined archaeological and archaeobotanical data, it can be assumed that the F3 trench area, including A206, was probably located at the edges of the early Neolithic village, and was specifically used for several agricultural product management activities, from crop processing to storage.

Yenikapı—trench 1cd3

Trench 1cd3 is in the northeast of Yenikapı Neolithic settlement and is close to the area where the footprints of people who lived there were uncovered. On the east of this trench, there exists a stream that flooded periodically—flood traces of this stream were discovered during the excavations. Both the area which includes the footprints and the trench 1cd3 is sealed with natural sediments (sand, clay, sandstone) during these floods. Although it is close to the stream and destroyed by the floods, archaeobotanical plant remains, small-sized animal bones and snail shells were unearthed *in-situ*, together with scattered stones, in trench 1cd3. Also, in this trench, a limited number of Classical Fikirtepe pottery was found, typically red/orange/beige in color and chaff tempered. Based on these potteries, it is possible to date trench 1cd3 to 6000–6200 BC (Kızıltan and Polat 2013). In the area where trench 1cd3 is located, silos lined with stone rows in half-moon and round shapes were also found. Considering that trench 1cd3 is not in the village but to the north of it and that silos were only found there, it is thought that this area was probably the periphery of the village where daily activities such as agricultural works were carried out (Fig. 2B).

A total of 108 L of soil samples were taken from trench 1cd3 of Yenikapı. This soil sample was used both to obtain materials subject to aDNA analysis and to conduct archaeobotanical analysis. The archaeobotanical analysis which has been carried out on 30 L of the soil sample so far, shows that the grains belonging to the hexaploid wheat group are in greater number than the grains belonging to wheat species such as emmer, einkorn and NGW.

Comparing the ratios of cereal taxa obtained in the Yumuktepe F3 A206 and Yenikapı 1Cd3 areas, the settlement of Yenikapı stands out only in terms of hexaploid wheat. The two settlements have a different trend in terms of proportions in hexaploid wheat taxa during the Neolithic Period (Ulaş and Fiorentino 2020). According to the results of the archaeobotanical analysis carried out so far, while a total of 155 hexaploid wheat caryopsis have been identified in the Yenikapı settlement, this number is limited to 5 caryopses in total in the Yumuktepe settlement (Table S1). Similarly, while the number of hexaploid segments of the rachis in the settlement of Yenikapı is 26, no remains of a hexaploid segment of the rachis have yet been found in the Early Neolithic level of the settlement of Yumuktepe. On the other hand, in terms of total grain taxa rates (both grain and chaff residues), the Yumuktepe settlement has an incomparably higher amount cereal taxa value than the Yenikapı settlement (Ulaş and Fiorentino 2020). The difference in the ratio of the archaeobotanical cereal taxa can be explained by the different use functions of the two contexts in the two settlements. Area F3 A206 in the settlement of Yumuktepe is an area where the largest amounts of archaeobotanical remains are found compared to other early Neolithic contexts studied (Ulaş 2015). Out of a total of 439 emmer caryopsis identified in early Neolithic contexts, indeed, about half of these (270) came from area F3 A206 at Yumuktepe. This ratio related to emmer also applies to all archaeobotanical plant residues (Ulaş 2015; Ulaş and Fiorentino 2020). In fact, the archaeological data obtained indicate that this area was used as a sort of grain storage complex.

Description of ancient wheat grains

Charred wheat samples documented and identified as *Triticum spelta* L or NGW or atypical emmer or naked wheat in Yumuktepe and Yenikapı showed similarities with the morphological characteristics of *Triticum spelta* L wheat. Morphologically, the grain of this species has an oval back and nearly parallel sides; its upper tip is spheroid, and the lower tip is drop-shaped. When viewed from the side, its outer part is spheroid, and the inner part is flatter. The mid-section has a deep and narrow furrow, and its general view is relatively less spheroid than *Triticum aestivum/durum*, but more spheroid than *Triticum*

turgidum spp. *dicoccum* L. Although the wheats mentioned are morphologically similar to emmer and naked wheat (Figs. 3 and 4), these wheat samples need to be further examined to verify their correct taxonomical identities. The charred wheat grains from Yenikapı and Yumuktepe archaeological sites were coded as YKM09-1 to YKM09-5 and YT10-1 to YT10-5, respectively.

aDNA studies

Contamination precautions

Precautions and strategies were implemented to minimize the possible risk of modern DNA contamination. DNA extractions and Polymerase Chain Reactions (PCR) setup were conducted in a physically separated laboratory that was specially designed

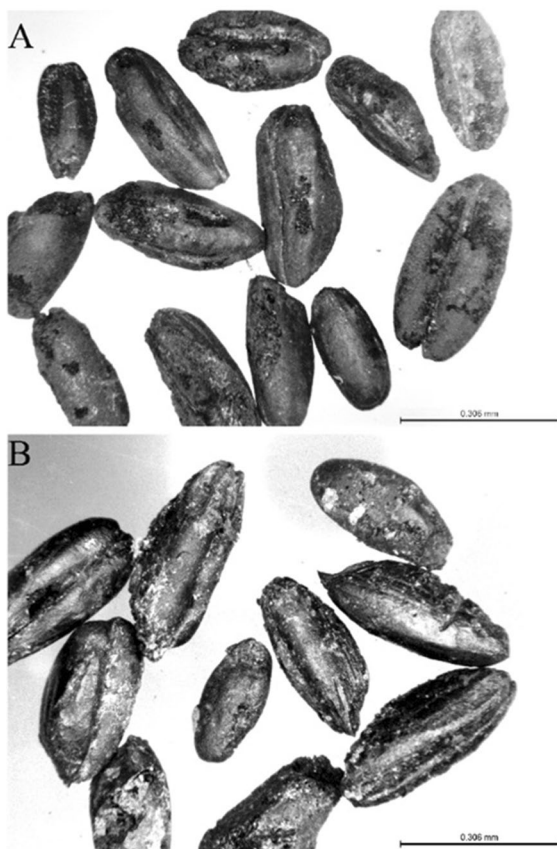


Fig. 3 Views of the spelt, NGW or atypical emmer, or naked wheat samples obtained from Yumuktepe trench A 206 (A) and Yenikapı trench 1Cd3 (B)

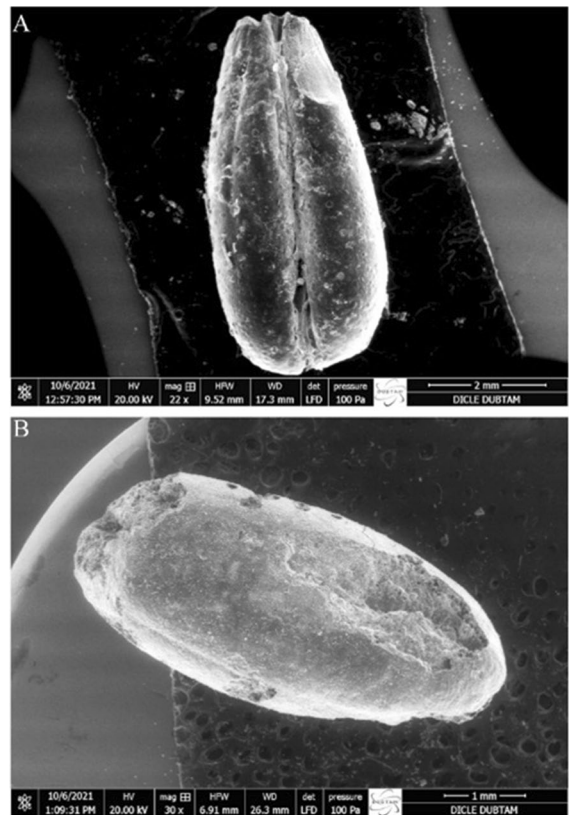


Fig. 4 Scanning electron microscopy (SEM) images for the randomly selected charred seed sample from spelt or NGW or atypical emmer wheat samples of Yumuktepe trench A 206 (A) and Yenikapı trench 1Cd3 (B) which were subjected to aDNA analysis

for ancient DNA research at Middle East Technical University, Ankara, Turkey. DNA extractions were carried out in a laminar flow cabinet that had never previously been used. The whole laboratory area was cleaned with 20% bleach, absolute ethanol and DNA AWAY™ solution (Molecular Bioproducts, Inc. San Diego, CA), providing a convenient method to decontaminate a variety of surfaces. A combination of ultraviolet (UV) irradiation, 10% bleach and 70% ethanol treatments were applied to benches, cabinet and equipment to avoid the risk of any contamination before the ancient DNA extractions were begun.

Prior to isolation of DNA and Pre-PCR, all the consumables (tubes, containers, racks and aerosol-resistant plastic tips) and reagents were autoclaved, and UV irradiated for a minimum of 15 min before each use, and pipettes were sterilized frequently.

The extraction buffer (without proteinase K) and deionized distilled water also were subjected to UV treatment. To eliminate cross-contamination, aerosol-resistant pipet tips were used. Delicate plastic materials were extensively wiped with ethanol and DNA AWAY™ solution. No other molecular experiment was performed prior to the current study to avoid the risk of contamination. Personal protective equipment covering all exposed clothing, shoes, hair, and skin was implemented during the process of the aDNA extraction.

aDNA extraction

The modified Cetyltrimethylammonium bromide (CTAB) DNA isolation method was used to extract genomic DNA of the charred wheat grains (Kistler 2012) (Supplemental Information 1). aDNA extraction was accompanied by regular use of blank (“negative control” mix plus water instead of botanical material) to detect the presence of any possible contamination by external DNA sources. Since the amount of genomic DNA extracted from ancient samples was in low concentration, a whole genome amplification strategy was applied by using the Illustra GenomiPhi HY DNA Amplification Kit (Amersham, GE Healthcare, and UK) by considering the protocol of Dagnall et al. (2018) (Supplemental Information 2). The concentration and purity of the amplified DNA were determined with a BioDrop spectrophotometer (BioDrop, Cambridge, UK). All amplified genomes were diluted 50 times for use in

PCR reactions to avoid amplification inhibition as a result of the high template DNA amount.

PCR amplification and sequence analysis

Since the quantity and the purity of the aDNA extracts do not evaluate solely the authenticity of the samples, a chloroplast DNA amplification was performed to provide evidence of endogenous DNA preservation in the ancient extracts. In order to provide evidence for sequence authenticity and assay plant DNA preservation, 3 primer pairs were designed using NCBI Primer Designing Tool Primer Blast to produce approximately 560 bp fragment of the large subunit of ribulose 1,5 biphosphate carboxylase (*rbcL*) gene (R primers in Table 1). PCR products obtained for the charred grains and one sample, namely YK09-4, were directly sequenced and aligned to each other to obtain the consensus sequence of the partial *rbcL* gene region.

PCR amplification of target sequences was carried out by using specifically designed primers for 26S rDNA and IGS gene regions in addition to the use of three negative controls including PCR mixture without DNA samples. A 25 µl total volume composed of 5 µl 5×HOT FIREPol Blend Master Mix (Solis Biodyne, Estonia), 0.5 µl each primer pair, 10 ng template DNA (5 µl diluted DNA), and 14 µl sterile PCR grade water was used for the 26SrDNA. The PCR amplification protocol was applied as follows: 95 °C for 5 min as an initial denaturation cycle, followed by 30 cycles (94 °C for 30 s, 60 °C for 30 s, then 72 °C for 1 min) with a final extension at 72 °C for 10 min.

Table 1 The studied gene regions and primers used to amplify the target sequences from charred wheat seeds

Primer name	5' to 3' sequence	Approximate product length (bp)
R1F	ATCTTGGCAGCATTCCGAGT	167
R1R	GCAACAGGCTCGATCTCGTA	
R2F	GCCTGTTGCTGGAGAAGAGA	204
R2R	TGGATTCCGTGAGGAGGACC	
R3F	TTTCCAAGGTCCTCCTCACG	190
R3R	TGGTTGGGAGTTTACATTTTCA	
26SrDNA (F) (Jovanović et al. 2011)	TTCCCAAACAACCCGACTC	150
26SrDNA (R) (Jovanović et al. 2011)	GCCGTCCGAATTGTAGTCTG	
IGSF (Li et al. 2011)	CGCCATGGAAAACCTGGGCAA	158/87
IGSR (Li et al. 2011)	ACCTCTCGTACCCGTTACGTC	

The PCR reactions for the IGS region were performed in a 20 µl total volume containing 4 µl 5×HOT FIREPol Blend Master Mix (Solis Biodyne, Estonia), 0.4 µl each primer pair, 10 ng template DNA (4 µl diluted DNA), and 10.2 µl sterile PCR grade water. The PCR protocol was adjusted as follows: 95 °C for 5 min as an initial denaturation cycle, followed by 40 cycles (94 °C for 1 min, 60 °C for 1 min, then 72 °C for 1 min) with a final extension at 72 °C for 10 min.

Details of markers and sizes of the amplified products are given in Table 1. Expected product sizes of IGS are 158 bp for the D genome and 87 bp for the A or B genome, respectively. Oliveira et al. (2012) recommended that caution must be taken in the interpretation of amplified IGS region. The absence of the D genome-specific band (158 bp) on the gel should not be taken as evidence of the absence of the D genome in a wheat sample since degradation of DNA or preferential amplification of the smaller 87 bp product. This event leads to common wheat being misidentified as a tetraploid. To ensure the presence of D genome-specific band, different PCR protocols and cycling parameters were implemented for ten charred wheat seeds (Table S3). Two modern previously extracted *T. aestivum* DNA samples (Çiftçi et al. 2019) were used to amplify IGS marker to infer the presence of the D genome in the corresponding charred wheat grains in a different laboratory.

Gel electrophoresis was carried out with 3% agarose gels at 100 V for 45 min. A low-range DNA ladder (Fermentas, Generuler, EU) was co-electrophoresed as a size marker. The gel was visualized under UV light using a gel electrophoresis visualizing system (Vilber Lourmat, France). The Gene Matrix Agarose-Out DNA Purification Kit (Eurx) was used to isolate the desired DNA fragments of the IGS rDNA gene region from agarose gel by following the product instructions in order to obtain noise-free sequence data. Sequence analysis was carried out by the BM Labosis (Ankara, Turkey) with an ABI 310 Genetic Analyzer (PE Applied Biosystem) and ABI3730XL 96 capillary automatic sequencer. The sequence data was exported to MEGA X software (Kumar et al. 2018) for sequence alignment and genetics analysis. NCBI BLAST search (Altschul et al. 1990) was used to compare DNA sequence homology. All the modern and ancient wheat accessions used for comparison in this study were retrieved from the GenBank.

RESULTS

Successful DNA extraction from the charred wheat grains and sequencing of cpDNA for authenticity

Out of 32 charred grains excavated from Yenikapı and Yumuktepe, *a*DNA was successfully extracted from ten charred wheat seeds by the modified DNA extraction method of Kistler (2012) without DNA contamination from any outer sources. The negative controls didn't include any amount of DNA (Table S2). The method yielded a moderate level of DNA concentration. Thus, the Illustra GenomiPhi HY DNA Amplification Kit (Amersham, GE Healthcare, UK) was used to implement whole genome amplification, which successfully amplified the genome of all studied charred seeds. This approach yielded a high amount of *a*DNA, which was secured to be used for further PCR reactions (Table 2).

The partial *rbcL* gene region was amplified for the charred grains successfully and the sequenced 522 bp partial fragment of sample YK09-4 showed identity to *Triticum aestivum* chloroplast genome and *Triticum aestivum* ribulose biphosphate carboxylase large chain mRNA sequence as a result of the BLAST search (Supplementary Fig. 2).

The 26S rDNA gene region

The negative controls didn't give any band on the gel. The 26S rDNA gene region was amplified for ancient wheat samples at an expected size. The ancient sequences obtained from the chromatogram data were checked with a BLAST search to determine the identity of the charred seeds which were morphologically identified as *T. aestivum* subsp. *spelta* or NGW or atypical emmer or naked wheat. According to the sequence results of the 26SrDNA gene region, YK09-3, YK09-4, YK09-5, YT10-2, and YT10-3 samples had identical DNA sequences. However, YK09-1, YK09-2, YT10-1 and YT10-4 sample sequences were differed from YK09-3, YK09-4, YK09-5, YT10-2, YT10-3 by only one base substitution at 22nd bp position. Interestingly, the YT10-5 sample differed from the other nine samples by the base changes at the 22nd and 87th bp positions (Table 3). The 151 bp ancient sequence length of 26S rDNA showed high levels of homology with the sequences from previously studied ancient *Triticum aestivum* seeds from

Table 2 aDNA concentration of the studied charred wheat seeds from Yenikapı and Yumuktepe excavation sites

Seed codes	DNA conc. (µg/ml)	230/260 260/280 absorbance ratios	DNA conc. of amplified whole genome (µg/ml)	230/260 260/280 absorbance ratio of amplified whole genome
<i>Yenikapı excavation site</i>				
YKM09- 1603–7.77–1 (YKM09-1)	75	0.85/1.56	1101	2.3/1.86
YKM09- 1603–7.77–2 (YKM09-2)	15	0.17/1.56	1229	2.35/1.85
YKM09- 1603–7.77–3 (YKM09-3)	21	0.06/1.83	1078	2.34/1.86
YKM09- 1603–7.77–4 (YKM09-4)	5	0.01/1	1172	2.24/1.86
YKM09- 1603–7.77–5 (YKM09-5)	11	0.03/2	1144	2.36/1.86
<i>Yumuktepe excavation site</i>				
YT10-F3A206-1 (YT10-1)	126	0.78/1.36	1307	2.36/1.86
YT10-F3A206-2 (YT10-2)	46	0.06/1.81	1212	2.35/1.86
YT10-F3A206-3 (YT10-3)	34	0.11/1.73	1128	2.31/1.86
YT10-F3A206-4 (YT10-4)	50	0.1/1.78	1155	2.36/1.86
YT10-F3A206-5 (YT10-5)	49	0.06/1.73	1173	2.35/1.86

Table 3 Alignment of ancient 26SrDNA sequences obtained from charred wheats of Yenikapı and Yumuktepe archaeological sites. Identical bases were marked by dots. The numbers above sequences indicate the base positions. The missing sequence IDs in table indicate the identical sequence in those positions. Reference sequences: Ancient *T. aestivum* (MK413185.1) and contemporary *T. aestivum* (MK413186.1)

Sequence ID	22	23	39	56	57	76	78	83	87	124
YKM9-1603-7.77,7.90.1	T	A	T	A	C	T	C	C	C	G
YKM9-1603-7.77,7.90.2
YKM9-1603-7.77,7.90.3	C
YKM9-1603-7.77,7.90.4	C
YKM9-1603-7.77,7.90.5	C
YT10-F3A206-1
YT10-F3A206-2	C
YT10-F3A206-3	C
YT10-F3A206-4
YT10-F3A206-5	C	T	.
<i>Triticum aestivum</i> ancient (MK413185.1)	C
<i>Triticum aestivum</i> contemporary (MK413186.1)	C	T	G	C	G	C	A	-	.	-

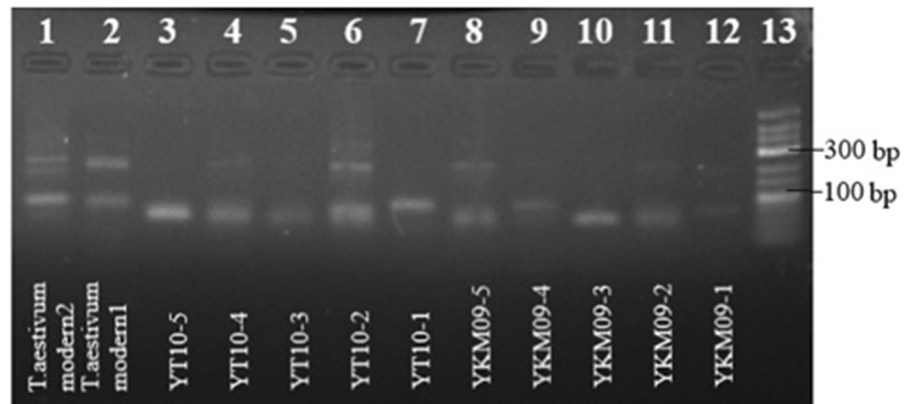
Kaymakçı archaeological site from Manisa, Turkey [Ciftci et al. 2019 (99.33% identity)]. There was also a high level of homology between the studied ancient wheat samples of the current study and the contemporary *Triticum aestivum* sequences obtained from the previous study (Çiftçi et al. 2019).

The IGS gene region

By considering the DNA sequence data from the 26S rDNA gene region, it was impossible to distinguish the ploidy levels of the charred grains, that is whether they were tetraploid or hexaploid wheats. In literature, it has been shown that the IGS gene region

could successfully identify the ploidy level of *Triticum* genus with a PCR-based method taking advantage of a 71 bp insertion in the D genome since this insertion is absent in A and B genomes (Brown and Brown 1994; Sallares et al. 1995, 1999; Li et al. 2011; Oliveira et al. 2012). The IGS region was amplified for both ancient and contemporary wheat samples (*Triticum aestivum*). All samples produced a single DNA fragment which is about 80–90 bp in length. The YKM09-3, YT10-1, YT10-3 and YT10-5 samples yielded that aforementioned fragment solely for the IGS gene region, while the remaining six charred wheat samples had an extra DNA fragment which is about 150–160 bp in length (Fig. 5), similar to the

Fig. 5 The amplified IGS gene products of charred *Triticum* sp. From Yenikapı (lanes 8–12) and Yumuktepe (lanes 3–7) archaeological sites. Lanes 1 and 2: modern *Triticum aestivum* samples. Lane 13: Thermo GeneRuler Low Range DNA Ladder



modern *Triticum aestivum* samples which were used as the reference. The presence of this band could be inferred as the presence of the D genome in the corresponding charred wheat grains. For the studied five different conditions, some of the seeds included the D genome band for a condition, while the same seeds didn't include the D genome band for other conditions (Supplementary Fig. 3). A slightly sizeable band observed in the samples numbered YT10-4 and YKM09-4 was a result of nonoptimized PCR protocols and cycling parameters implemented for obtaining D genome band.

Direct sequencing of IGS gene region for the YKM09-1, YKM09-4 and YT10-4 ancient seeds yielded 167, 131 and 188 bp sequences, respectively). The unambiguous partial sequence length for those samples were found to be 85, 98 and 81 bp length, respectively. The IGS sequences of YT10-4 and YKM09-4 samples in BLAST search yielded significant similarities with 25 and 23 NCBI accessions belonging to different *Triticum* species, respectively (Table S4). Especially, high level of homology with *Aegilops tauschii* (KF482112.1), *Triticum spelta* L. (AF147500), *Triticum aestivum* (M372699) (*Nor-D3* locus) and fossil *T. aestivum/durum* (HQ322477.1) was obtained (Table 4).

The sample YKM09-1 showed the most sequence similarity to hexaploid wheat (*T. aestivum*). The highest alignment scores were found with *T. aestivum* (AB026896.1), *T. aestivum* (X07841.1), and *Triticum* sp. (M36062.1) (Table 5). Since the direct sequencing of IGS produced partial unambiguous sequences for the YKM09-1, YKM09-4 and YT10-4 samples, to obtain the whole sequence of the D genome, IGS fragment the band of YT10-3 sample at 157 bp

was carefully cut from the agarose gel, purified and sequenced. In addition, one additional fragment that is about 250 bp in length obtained from the same sample was purified and sequenced likewise.

The sequence obtained from YT10-3 sample has very high level of homology with *Aegilops longissima* Schw. et Musch. (KF482111.1), *Aegilops tauschii* Coss (KF482112.1), *T. spelta* (AF147500), *T. aestivum* (X07841.1) and 93% *T. aestivum* (M37269.1) (Table 6). Considering sequence alignment result of the 250 bp length fragment of the same sample, about 181 bp of this sequence well aligned with the aforementioned accessions (Supplementary Fig. 1). These results confirmed the presence of the characteristic D genome insertion in IGS sequences for the charred wheat grains. The missing sequence IDs in the table indicate the identical sequences in those positions

DISCUSSION

Crop species have constantly undergone genetic changes during cultivation and breeding as a result of human interventions and selection and/or adaptation to changing environmental conditions. The cultivated plants have been spread to distinct geographical areas and have adapted to various conditions under selective pressure. Their diffusion has ensured the development of local crops possessing desired agronomic traits. The history of genetic changes in local crops of modern days could inform us on agricultural evolution and reveal their adaptive capacity to novel growth conditions. Studies of aDNA have recently become an important source of information

Table 4 Alignment results of the partial sequences of intergenic spacer region (IGS) obtained for the sample YKM09 K03-7.77,7.90-4 (YKM09-4) from Yenikapı and YT10-F3A206-4 (YT10-4) from Yumuktepe excavation sites

Sequence ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
-YKM9-1603-7.77,7.90.4		G	G	G	C	T	G	C	A	C	G	T	G	C	A	C	G	G
-YT10-F3A206-4		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
- <i>Aegilops tauschii</i> - External Transcribed Spacer (KF482112.1)	T
<i>Triticum spelta</i> L. 26S-18S Intergenic Region (AF147500.1)
- <i>Triticum aestivum</i> NOR D3 locus (M372699)	-
-Fossil <i>T. aestivum</i> IGS D Genome (HQ322477.1)

Sequence ID	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	
-YKM9-1603-7.77,7.90.4	A	C	G	T	G	A	A	C	G	G	G	T	A	C	G	A	G	A	G	G	T	A	
-YT10-F3A206-4
- <i>Aegilops tauschii</i> - External Transcribed Spacer (KF482112.1)
<i>Triticum spelta</i> L. 26S-18S Intergenic Region (AF147500.1)
- <i>Triticum aestivum</i> NOR D3 locus(M372699)
-Fossil <i>T. aestivum</i> IGS D Genome (HQ322477.1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

Identical bases were marked by dots. The numbers above sequences indicate the base positions. The missing sequence IDs in table indicate the identical sequence in those positions

on genetic changes within the cultivated species that have occurred throughout the history of agriculture.

Archaeobotanical remains can comprise recoverable DNA when excavated from frozen, desiccated environments or anoxic conditions (Palmer et al. 2012). Among all tissues, seeds and grains are particularly advantageous due to their relatively high DNA content in crop species. The nature of seeds makes them persistent for successful recovery of intrinsic DNA for plant ancient DNA studies. Charring is the most common preservation of crop seeds, yet utilisation of charred seeds for *a*DNA extraction is challenging. Many research groups have studied charred grains of cereal remains (Allaby et al. 1994, 1997; Bilgic et al. 2016; Boscato et al. 2008; Brown and Brown 1994; Ciftci et al. 2019; Fernandez et al. 2013; Kohler-Scheider and Caneppele 2009; Nasab et al. 2010; Oliveira et al. 2012; Schlumbaum et al. 1998). However, the success in *a*DNA extraction and amplification is highly dependent on the extent of charring because charred *a*DNA fragments are very short in length.

Different environmental conditions during the preservation of seeds generate eroding factors, such

as sun, rain, wind, and frost. Depending on the geographical location and the microclimate of the excavation site, the quantity and quality of DNA from charred seeds vary, although these are typically low. This makes those charred grains unsuitable for the extraction of amplifiable DNA or causes inhibition of amplification due to degradation of endogenous DNA (Fernandez et al. 2013; Oliveira et al. 2012). In the current study, extraction of relatively good quality *a*DNA was accomplished from 10 out of 32 charred seeds. Although it accounts for only one-third of the seeds, this outcome is nevertheless successful considering that the DNA in charred grains was extensively fragmented and chemically damaged. It has been reported that successful DNA recovery for charred grains is accepted to be 1 in 20 seeds (Banerjee and Brown 2002; Brown et al. 1998). The *a*DNA yield was also relatively high for charred grains, but in order to replicate polymerase chain reactions (PCRs) and amplify more than one genetic locus from a single extract, we further used whole genome amplification. It is also important to note that all 10 seeds, from which *a*DNA was extracted were fully charred and successful

Table 5 Alignment results of the partial sequences of intergenic spacer region (IGS) obtained for the sample YKM09 K03-7.77.7.90-1 (YKM09-1) from Yenikapı excavation site. Indels were designated as dashes. Dotted border lines represent the identical sequences in between positions

Sequence ID	6	16	17	18	21	22	25	27	28	32	33	41	47	56	58	59	68	70	80	81	82	83	84	85
YKM9-1603-7.77.7.90.1	T	-	T	G	-	C	G	C	G	T	T	C	A	T	T	A	-	A	A	A	A	A	A	A
<i>Triticum aestivum</i> 25S-18S Intergenic Region (ABO26896.1)	A	G	.	.	A	.	.	.	A	C	.	C	G	A	C	C	T
<i>Triticum aestivum</i> (XO7841.1)	A	G	.	.	A	G	.	.	A	C	.	C	G	A	C	C	T
Wheat Ribosomal RNA spacer DNA (M360362.1)	A	G	.	.	A	.	.	.	A	C	.	C	G	A	C	C	T
<i>Aegilops longissima</i> -External Transcribed Spacer (KF482111.1)	A	G	A	.	A	.	A	A	T	A	C	.	C	G	A	C	T
<i>Triticum spelta</i> L. 25S-18S Intergenic Region (AF147500.1)	A	G	A	C	A	.	.	A	T	A	C	G	C	G	A	C	T
<i>Aegilops tauschii</i> -External Transcribed Spacer (KF482112.1)	A	G	A	.	A	.	A	A	T	A	C	.	C	G	A	C	T	-	-	-	-	-	-	-

Table 6 Alignment of the intergenic spacer sequence (IGS) for the sample YT10-F3A206-3 (YT10-3) from Yumuktepe excavation site

Sequence ID	3	29	30	37	38	40	41	53	72	86	90	92	93	98	99	101	107	108	120	121	123	124	125	126	131	135	136
YT10-F3A206-3	T	A	G	A	-	A	C	C	G	T	T	A	A	A	C	G	G	C	G	A	C	G	T	C	-	C	
<i>Aegilops tauschii</i> (KF482112.1)	C	.	.	.	-	.	.	.	C	G	G	G	G	.	A	T	C	A	
<i>Aegilops longissima</i> (KF482111.1)	-	.	T	.	C	G	A	.	C	.	.	G	.	-	.
<i>Triticum spelta</i> L. (AF147500.1)	.	C	G	-	.	.	G	.	C	G	.	G	T	C	G	.	-	.
<i>Triticum aestivum</i> 25S-18S Intergenic Region (XO7841.1)	T	.	G	C	G	.	.	C	G	.	.	G	T	C	.	G	.	.	G	.	.	G	T	.	.	-	.
<i>Triticum aestivum</i> NOR-D3 Locus (M37269.1)	.	.	C	G	-	.	.	G	-	C	G	.	.	G	T	A	C	.	.	-	-	-	.

The 157 bp length *ad*DNA fragment was isolated from the agarose gel. The accessions with the highest alignment scores were provided along the ancient sample. Identical base positions to the sample YT10-F3A206-3 sequence were represented by dots and indels are indicated by dashes. The missing sequence IDs in the table indicate the identical sequences in those positions

amplification from those were achieved as explained in the following sections.

aDNA analysis of the rDNA regions

26S rDNA gene region yielded similar sequence results for the ancient seeds of both Yenikapı and Yumuktepe. Out of ten charred grains, four differed with one substitution and only one seed had two substitutions. This could be explained by two possible scenarios; (i) The seeds belong to different species, although they were identified morphologically as one, (ii) A more consistent scenario is that there had been seed exchanges between the two communities. In addition, the sequence similarities between seeds of these excavation sites and ancient *Triticum aestivum* seeds from Kaymakçı, Manisa are prominent. Although there was also high homology between the studied ancient wheat samples of the current study and the contemporary *Triticum aestivum* sequences, it is not possible to infer the genome composition of the ancient seeds in question based on solely 26S rDNA sequences.

In order to shed light on the ploidy level of the charred grains, another rDNA region, IGS was amplified. The primers designed for amplification of this IGS region produced clear amplification products with the aDNA from charred grains in this study. The 71 bp insertion in the D genome was useful to identify the genome composition of ancient wheat samples in question as proved to be in literature (Brown and Brown 1994; Li et al. 2011; Oliveira et al. 2012; Sallares et al. 1995, 1999). All charred grains produced the fragment specific to A/B genomes at about 80–90 bp. Seven charred wheat grains had an extra fragment that is about 150–200 bp in length, like that of the modern *Triticum aestivum* samples used as a reference. The presence of this band confirms the possession of the D genome in the corresponding charred wheat grains. Direct sequencing of IGS fragments for three ancient seeds from both Yenikapı and Yumuktepe yielded 167, 131 and 188 bp sequences, respectively. However, the unambiguous partial sequence lengths for those samples were found to be 85, 98 and 81 bp in length. The sequences of grains showed high homology with *Aegilops tauschii* (KF482112.1), *Triticum spelta* L. (AF147500) and *Triticum aestivum* *Nor-D3* locus (M372699). Since

the direct sequencing of the IGS fragment was problematic and produced a shorter unambiguous sequence due to premature termination of sequencing reaction, D genome-specific bands of sample YT10-3 from Yumuktepe (157 bp) were purified from the gel and sequenced. This 157 bp fragment was fully sequenced and global alignment of this sequence with accessions in the GenBank database again confirmed the presence of the D genome in the YT10-3 sample. On the other hand, there was another fragment at approximately 250 bp for the same sample and was also sequenced by gel purification to determine whether that specific grain belongs to a different species and produced a different amplification product. The result was not promising such that only 181 bp sequence of this 250 bp fragment aligned with D genome bearing accessions. This could stem from the process called template switching during PCRs of degraded aDNA samples. The remnants of fragmented aDNA are comprised of segments of aDNA molecules concatenated so that the resulting sequence of an amplification becomes a combination of different parts of a genome/genomes by PCR jumping (Pääbo et al. 1990). This results in chimeric products including also some part of the desired gene region (Allaby et al. 1999; Pääbo et al. 1990; Brown et al. 2015). Although three of the samples did not produce the D-genome-specific fragment, this result should be discussed carefully. The lack of this band in some of those grains could have arisen from the preferential amplification of smaller length fragments in PCRs because aDNA is extremely fragmented and degraded. This could lead to inaccurate identification of the wheat species under discussion as tetraploid. Thus, in the interpretation of the IGS results, though the presence of 150–200 bp fragment indicates D genome possession, the absence of that does not always mean the species has only A or B genome (This study; Oliveira et al. 2012).

The results of this study illustrate the crucial need to use a combination of morphological and molecular methods in the differentiation of wheat grains in archaeobotanical assemblages. Nucleotide substitutions between ancient and modern wheat seeds corroborate intensive human interventions, notably the process of domestication and breeding activities over time. Despite being morphologically indiscernible, it was concluded that the charred grains from

Yumuktepe and Yenikapı possess the D genome, as the presence of a D genome characteristic fragment of the IGS region confirms.

Archaeobotanical background of *Triticum aestivum* subspp. *spelta* prehistoric cultivation and possibly cultivation of spelta in Yumuktepe and Yenikapı Neolithic sites

While the settlements in Europe where free-threshing hexaploid wheat has been dated as early as the Neolithic Period, its use became widespread in the Bronze Age (in 3000–2000s B.C.) (Marinova and Valamoti, 2014). Ancient DNA analysis of some wheats at Çatalhöyük, a Neolithic site of PN, suggests that *T. aestivum* subspp. *spelta* wheat may have been cultivated at this site (Bilgiç et al. 2016). *Triticum* cfr. *spelta* was identified previously in the Chalcolithic and Neolithic Period settlements in Bulgaria (Marinova 2003). However, the same researchers have stated that this finding needs to be checked since these materials might be *Triticum* “new” glume wheat type (Marinova and Valamoti 2014). *T. spelta*, hulled hexaploid wheat species, had been more precisely identified in Archondiko in Greece (Valamoti et al. 2008; Kroll 1983; Jones et al. 1986), and the Bronze Age settlements (dating back 3000 B.C.) in Thrace (Popova 1995), which are the regions close to Yenikapı site in Istanbul.

The presence of two different spelta wheat subspecies (‘European Spelta’ and ‘Asian Spelta’) has been discussed by various authors (Nesbitt and Samuel 1996; Dvorak and Luo 2001; Blatter et al. 2004). Although opinions on the presence of both species are diverse, it has been asserted that spelta of Asia formed as a hybridization of a tetraploid species and wild diploid *Aegilops squarossa* L. (Zohary and Hopf 2000) while the spelta of Europe was formed through autohybridization (Andrews 1964; Akeret 2005). Genetically, it has been accepted that hexaploid wheat species with AABBDD genome (such as *T. aestivum*) could be created through artificial hybridization between *T. turgidum* spp. *durum* (AABB genome) and *Ae. tauschii* (DD genome) (van Zeist 1976; Zohary and Hopf 2000; Nesbitt 2001). Therefore, it would not be unexpected to obtain a hybrid species similar to *T. spelta*, which is morphologically close to *T. dicoccum* and *T. aestivum*.

The range of *Aegilops tauschii* covers a very large area from Northeastern Anatolia to Armenia, Azerbaijan, the coast of the Caspian Sea, Pakistan, Afghanistan, and the northeastern part of Kyrgyzstan, not only the Fertile Crescent (Zohary and Hopf 2000). This wild diploid species with a D genome has adapted to various climatic and environmental conditions ranging from the desert-like environment on the mountain foothills in Afghanistan to the mild coast of the Caspian Sea. The identification of this species in Northeastern Anatolia, where continental climatic conditions and steppe plant cover prevail, but not around the Karacadağ region with continental climatic conditions, where the wild progenitors of *T. dicoccum* and *T. monococcum* L. are present seems like a remarkable contradiction (Heun et al. 1997; Salamini et al. 2002; Özkan et al. 2005; Kilian et al. 2007; Heun et al. 2008; Haldorsen et al. 2011). The adaptation of certain hexaploid species such as spelta to mild humid or harsh continental climate conditions is reported to be a result of *A. tauschii* with the D genome (Zohary and Hopf 2000; Nesbitt 2001; Ulaş and Fiorentino 2020). This genetic makeup is mentioned as the reason why certain species such as spelta were preferred in agriculture in the cold and mild regions such as Europe, not in the Near East with continental climatic conditions (Nesbitt 2001).

The identification of *A. tauschii* around Karacadağ- Urfa, where Çayönü, Nevalı Çori and Göbekli Tepe settlements are located (Karagöz et al. 2006) indicated that the natural geographical range of *A. tauschii* is extended further. Based on the identification of *T. compactum* in Can Hasan III (Hillman 1972), *T. aestivum/durum* in Çayönü (van Zeist 1972), the hexaploid rachis remains in Caferhöyük III and IV levels (de Moulins 1993), *T. spelta* grains in the Yenikapı and Yumuktepe and suggested that cultivation of free-threshing hexaploid wheat was widespread. This could potentially indicate that spelta hulled wheat, which is the progenitor of the free-threshing hexaploid wheat, might have been cultivated in those settlements. Additionally, discovering *T. aestivum* subspp. *spelta* and *Triticum* “new” glume wheat type in the Yenikapı and Yumuktepe Neolithic settlement is an indicator that the spelta might have arrived in Southern Thracia and Cilicia with other species (such as *T. dicoccum*, *Triticum* “new” glume wheat type and *T. monococcum*) from these

regions of Anatolia. The identification of the range of *Aegilops tauschii*, the “Achilles Heel” of this hypothesis, around the primary agricultural economy settlements such as Çayönü, Nevali Çori in the Fertile Crescent also indicates that the origin of spelta wheat needs to be traced to the settlements on the foothills of Karacadağ in southeastern Turkey.

CONCLUSIONS

There are various views and theories about the origins and the diffusion pathways and dynamics of *T. aestivum* subsp. *spelta*. The identification of probable spelta wheat in Yenikapı and Yumuktepe settlements has created the necessity for revising these views and theories.

Contrary to the prior assertions, the identification of the range of *Aegilops tauschii*, the wild diploid species, around the primary agricultural settlements of the agricultural economy in the Fertile Crescent such as Çayönü, Nevali Çori encourages further search for the origin of spelt wheat found in the settlements on the foothills of Karacadağ since wild progenitors of *T. dicoccum* and *T. monococcum* still grow in the area.

Identification of *T. aestivum* subsp. *spelta* in the Yenikapı and Yumuktepe settlements implies that the origin of this species needs to be traced to Anatolia and the Near East.

The views and theories regarding the origin of the Neolithic culture date back to the 1960s and 1970s. Considering new information provided by genetic and archaeobotanical studies as well as the settlements that have recently been found in the Southeastern Anatolia Region of Turkey such as Göbeklitepe, Kortiktepe, Gusirhöyük, the region cannot be regarded as a “periphery” in the transition process to the agricultural economy (Özdoğan et al. 2011; Abbo et al. 2012). Hence, by means of these new findings, the role of the settlements in the Southeastern Anatolia Region in the emergence of domesticated plant species is again brought up for discussion (Ulaş and Fiorentino 2020).

The results of the Yenikapı and Yumuktepe archaeobotanical research point at the diversity of the wheat species. The research we have carried out has shown that the farmers of Yenikapı and Yumuktepe in terms of new agricultural methods and products (such as *T. aestivum* subsp. *spelta*, *Triticum* “new” glume wheat

type), had a more advanced agricultural economy, compared to other regions of Anatolia.

However, the results obtained in this study should be considered preliminary, as the examined samples represent a limited collection from only two Neolithic sites. For this reason, more reliable information can be obtained from authentic aDNA analyses in addition to archaeobotanical analyses on hexaploid wheat groups that are difficult to classify taxonomically. This study also highlights the need for such further studies.

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Declarations

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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