Origins of domesticated emmer and common wheat inferred from chloroplast DNA fingerprinting.

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ABSTRACT

Emmer wheat (*Triticum turgidum* subsp. *dicoccum*, genome: AABB) is one of the earliest domesticated wheat derived from the wild emmer (*T. turgidum* subsp. *dicoccoides*, genome: AABB) and that its domestication took place within the Fertile Crescent. Emmer was the main crop during the spread of Neolithic agriculture from the Fertile Crescent to Eurasia and Africa and known as the nuclear and cytoplasmic genome donor to common wheat (*T. aestivum*, genome: AABBDD). To clarify the origin of domesticated emmer as well as common wheat, we investigated the intraspecific variation of chloroplast DNA by large-scale chloroplast DNA fingerprinting. In total, 248 accessions of wild and domesticated emmer and common wheat were genotyped for 24 chloroplast microsatellite loci. All results strongly suggested the following points: 1) two distinct maternal lineages (related to two chloroplast haplotype groups) were involved in the domestication of emmer wheat and therefore the domestication occurred independently at least twice, 2) one site of the emmer wheat domestication was located in Southwest Turkey, and 3) these two maternal lineages of emmer wheat were found in common wheat, suggesting that common wheat also originated diphyletically.

INTRODUCTION

The domestication of wheat and barley was one of the most important steps toward the establishment of farming communities that later led to the civilisation in Mesopotamia.
Genetic and morphological studies revealed that common wheat (*T. aestivum*) originated through allopolyploidy between domesticated emmer wheat and a closely related wild species, *Aegilops tauschii* (2n = 14, DD) (Kihara 1944, McFadden and Sears 1944). Therefore domestication of emmer wheat was a key step in the evolution of *Triticum* species.
Genetic and morphological evidence clearly indicates that domesticated emmer was derived from a wild tetraploid wheat *T. turgidum* subsp. *dicoccoides* (2n = 28, AABB) (see Zohary and Hopf 2000 for review).
Archaeological and botanical field studies suggested that significant association between wheat and human began in southwest Asia more than 10,000 years ago (Zohary and Hopf 2000 for review). Studies on the remains in archaeological sites in the Fertile Crescent suggest that cultivation of domesticated emmer wheat was already practiced in the Levant (the east coastal region of the Mediterranean Sea including Jordan and Israel) in the early 8th

millennium BC (bc: uncalibrated dates and BC: calibrated dates). Although domesticated barley and einkorn wheat were also under cultivation in Near East, emmer dominated quantitatively in this period. Emmer wheat continued to be the main crop in this area until the Bronze Age time (by about 3000 BC), and played an important role as the grain crop in the spread of the Neolithic agricultural technology at about 6000 bc from the nuclear area of Fertile Crescent to Africa, Europe and Eurasia.

Extensive RFLP studies of chloroplast DNA show that both domesticated emmer and common wheat share the identical chloroplast genome type with wild emmer (Tsunewaki 1996). However, partly due to the highly conserved nature of the chloroplast DNA, its variation in wild emmer has not been well explored and its relation to domesticated emmer and common wheat was unclear. Recently, we have identified 24 chloroplast microsatellite loci having more than ten mononucleotide repeats in the complete sequence of the chloroplast genome of *T. aestivum* L. subsp. *aestivum* cv Chinese Spring (Ishii *et al.* 2001). The availability of highly polymorphic microsatellite markers enabled us to examine the molecular variation of the chloroplast genomes within emmer and common wheat subspecies. Here, the allelic diversity at 24 chloroplast microsatellite loci is reported. According to the geographical and frequency distribution of the chloroplast haplotypes, we discuss 'where' and 'how' the domesticated emmer and common wheat originated.

MATERIALS AND METHODS

Ninety-six accessions of domesticated emmer (subsp. *dicoccum*) and 75 accessions of wild emmer (subsp. *dicoccoides*) were used. The accessions of subsp. *dicoccum* were collected from 30 countries and / or regions covering all cultivated areas and those of subsp. *dicoccoides* from their natural distribution. Five accessions of another non free-threshing tetraploid wheat (subsp. *georgicum*) endemic to the Transcaucasus were included. In addition to these tetraploid wheat 72 accessions of hexaploid wheat (including *T. aestivum* subsp. *aestivum*, *compactum, macha, spelta* and *sphaerococcum*) were analysed. Two accessions of free-threshing tetraploid wheat (one accession each of subsp. *turgidum* conv. *turgidum* and conv. *polonicum*) and one accession of hexaploid wheat (*T. aestivum* cv Chinese Spring) were used as references.

Total DNA was extracted from fresh leaves according to the method by Liu *et al.* (1990). Twenty four chloroplast microsatellite loci (designated as *WCt1 - 24*) having more than ten mononucleotide repeats identified by Ishii *et al.* (2001) were used. PCR and following silverstaining were performed according to Ishii *et al* (2001). The allelic diversity of chloroplast microsatellites was calculated according to the gene diversity value described by Nei (1987). Based on the genotypes at all microsatellite loci, the chloroplast haplotype of each accession was determined. Genetic relationships among the chloroplast haplotypes were studied by maximum parsimony method using PAUP (Swofford 1998).

RESULTS AND DISCUSSION

Allele size at each locus was determined based on the differences in the nucleotide length from the standard variety, Chinese Spring. Among the 24 microsatellite loci examined, polymorphisms were observed at 22 loci among 176 accessions of wild emmer (subsp. *dicoccoides*) and domesticated emmer (subsp. *dicoccum* and others). While among 72 accessions of common wheat 13 loci showed polymorphisms. Within wild and domesticated emmer the number of alleles at the polymorphic microsatellite loci ranged from 2 to 5, while

In common wheat the number ranged 2 to 4. An average diversity value (H) of the wild emmer wheat (0.302, range; 0.000 – 0.610) was about two times larger than that of the domesticated emmer wheat (0.136, range: 0.000 – 0.453). Common wheat showed the lowest diversity (0.124, range: 0.000 – 0.327) among these three groups. This fact suggests that the genetic diversity of chloroplast genome in the wild progenitor is much larger than that of domesticated species and might reflect the bottle neck effect during the domestication process of emmer and following polyploidization process of common wheat.

In total, 20 haplotypes were identified in domesticated emmer and 32 haplotypes in wild emmer. Among 51 haplotypes 12 haplotypes were identical to those found in our previous study (Ishii *et al.* 2001); we therefore followed the haplotype numbering system of Ishii *et al.* (2001). We next examined genetic relationships among the haplotypes using the maximum parsimony method. The result clearly indicates that there are two well differentiated haplotype groups (group I and group II) in emmer wheat. Haplotype 10 belonging to the group I was found in 39.6 % accessions of domesticated emmer, all five accessions in subsp. *georgicum* and the two accessions in subsp. *turgidum*. Among the total of 51 haplotypes independently identified in domesticated and wild emmer, only the haplotype 10 was shared in common between them. The result clearly shows that there is a large maternal lineage associated with the haplotype 10 in domesticated emmer. In common wheat 17 haplotypes were identified. Among these about 90 % of *T. aestivum* subsp. *aestivum* and about 60 % of *T. aestivum* subsp. *spelta* shared the haplotype 10. These results strongly indicate that the large maternal lineage associated with the haplotype 10 in emmer is retained in common wheat. In contrast to the domesticated emmer, almost all haplotypes were found in relatively low frequencies in wild emmer. Only three accessions (4%) of wild emmer had this founder-haplotype. These wild emmer accessions could be a direct ancestor of domesticated emmer. The wild emmer accessions having this haplotype were found only at one location in the southwest of the Fertile Crescent. The location (hereafter refer to Kartal Dagi site) is a rocky steppe - forest of wild oak and pistachio along the slope of Kartal mountain in southern Turkey. All of our results thus attest that the Kartal Dagi region was the domestication site of emmer wheat with this major lineage. Recent archaeological studies revealed that in several archaeological sites in the northern Fertile Crescent, people were growing domesticated cereals by 7500 – 7200 bc (Nesbitt 2002). AFLP fingerprinting of the nuclear DNA has likewise suggested that domestication of einkorn and emmer wheat occurred in the northern Fertile Crescent (Karacadag in southeast Turkey (Heum *et al.* 1997, Özkan *et al.* 2002). However the Karacadag is located at about 280 km east from the Kartal Dagi.

The haplotypes in the group II have unique alleles at least nine loci compared with haplotype 10 in the group I. In domesticated emmer, we have identified two haplotypes (types 22 and 59) belonging to the group II. Interestingly, type 22 was also found in common wheat (*T. aestivum* subsp. *spelta*). Although neither haplotype 22 nor 59 were found in the wild emmer, we found closely related wild haplotypes 20, 55 and 58 in the group II. These observations clearly indicate that there is a second maternal lineage in both wild and domesticated emmer. Taken together, it is strongly suggested that the domestication of emmer wheat occurred independently at least two times. Since we found both group I and group II haplotypes in common wheat, we consider that there are at least two maternal lineages also in common wheat, which independently originated from the two groups of emmer wheat.

We could not pinpoint the domestication site of emmer with the second maternal lineage (accessions with haplotype 22 or 59). Even though we have identified closely related haplotypes 20, 21, 55 and 58 in wild emmer, they were found in four geographically distant regions. It is most probable that domestication of emmer with this second lineage occurred independently in a different location other than Kartal Dagi. However, as proposed by Zohary (1999), where multiple domestications occur, they appear to be relatively few in

number for a given crop. Our previous study of nuclear DNA polymorphism in wild and domesticated emmer supports this idea (Mori *et al.* 1997).
Further work is needed to resolve contradictory results from different techniques of DNA analysis, and to attempt to identify the likely loss of genetic diversity between the original populations of domesticated crops and current day landraces. However, it is clear that traditional models of agricultural origins, which attempt to identify a single micro-region as the key region of domestication, are likely to be inappropriate.

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