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Maternal lineages in polyploid wheat species inferred from organellar DNA fingerprinting

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ABSTRACT

To understand the origin of polyploid wheat species from the view point of maternal lineage, we have been studying the molecular variation of organellar genomes using DNA fingerprinting techniques. Chloroplast and mitochondrial microsatellites were used to study intraspecific variation of *Triticum* and related *Aegilops* species. Surveys in free-threshing emmer and common wheat indicated that only one of the two maternal lineages in non-free-threshing emmer wheat was transferred to free-threshing common wheat. Chloroplast DNA variation in wild and domesticated timopheevi (*T. timopheevi*) wheat suggested the monophyletic origin of domesticated timopheevi wheat. Molecular variation in mitochondrial DNA in *Ae. speltoides* revealed the contrasting variability between chloroplast and mitochondrial genome.

INTRODUCTION

The genus *Triticum* consists of a polyploid series and is classified into four groups, einkorn (genome constitution: AA, 2n=14), emmer (AABB, 2n=28), timopheevi (AAGG, 2n=28), and common wheat (AABBDD, 2n=42). Polyploid species in *Triticum* and its closely related genus *Aegilops* originated through the allopolyploidization process, i.e., interspecific hybridization and subsequent chromosome doubling[1]. The A genomes of both tetraploid and hexaploid wheat originated from einkorn wheat. The origin of the B and G genomes has been debated; however recent studies of nuclear and organellar DNAs provided molecular clues supporting the hypothesis that *Ae. speltoides* was the donor for both B and G genomes[2,3,4]. Common wheat (*T. aestivum*) originated through allopolyploidization between domesticated emmer wheat and *Ae. tauschii* (DD, 2n=14). Therefore, in addition to the polyploidization, domestication of wild emmer wheat was a key step in the evolution of agriculturally important tetra- and hexaploid species in *Triticum*.

Extensive RFLP studies of chloroplast DNA provided a clear picture of phylogeny and evolution of polyploid species in *Triticum* and related wild *Aegilops* speices[2,5]. These studies show that both domesticated emmer and common wheat share the identical chloroplast genome type with wild emmer, while timopheevi wheat showed a distinctive chloroplast DNA (ctDNA) type[6]. However within species variation and its relation to the maternal lineage of polyploid wheat have not been well explored. Since the chloroplast genome of wheat is highly conserved, almost no RFLPs could be identified within the subspecies[6]. Ishii *et al.* (2001)[7] have identified 24 chloroplast microsatellite loci having more than ten mononucleotide repeats in the complete sequence of the chloroplast genome of *T. aestivum* cv. Chinese Spring. The development of highly polymorphic microsatellite markers enabled us to examine the molecular variation of the chloroplast genomes within wheat species.[7, 8] These studies suggest that there are two well-differentiated plastogroups in domesticated emmer wheat (*T. turgidum* ssp. *dicoccum*), and one of these maternal lineages originated in south Turkey. However, recent analyses on the molecular variation in nuclear DNA[9,10] suggested that emmer wheat might be domesticated in southeast Turkey. Therefore, as to the site of emmer wheat domestication, our results, on the basis of chloroplast DNA, did not agree with that by the nuclear DNA analyses.

Here, we summarise the following results: 1) variation of ctDNA in free-threshing emmer, and common wheat, 2) ctDNA variation in wild and domesticated timopheevi wheat, and 3) molecular variation in mitochondrial DNA (mtDNA) in *Aegilops speltoides*. Based on these results we discuss the evolution and domestication of polyploid wheat from the maternal lineage viewpoint.

MATERIALS AND METHODS

Seventy four accessions of free-threshing common wheat (*T. aestivum* ssp. *aestivum*), 39 accessions of domesticated emmer wheat (*T. turgidum* ssp. *dicoccum*), 136 accessions of free-threshing emmer wheat (ssp. *turgidum*), 59 accessions of wild timopheevi wheat (*T. timopheevi* ssp. *armeniacum*), seven accessions of domesticated timopheevi wheat (ssp. *timopheevi*) and 80 accessions of *Ae. speltoides* were used.

Total DNA was extracted from fresh leaves according to the method by Liu *et al.* (1990)[11]. Twenty four chloroplast microsatellite loci (designated as *WCt1 - 24*) having more than ten mononucleotide repeats identified by Ishii *et al.* (2001)[7] were examined. For mtDNA analysis, 21 mitochondorial micorosatellite loci (*WCt1 – 21*)[12] were examined. The allelic diversity of chloroplast microsatellites was calculated according to the gene diversity value (H) described by Nei (1987)[13]. Based on the genotypes at all microsatellite loci, the ctDNA haplotype (plastotype) or mtDNA haplotype was

determined for individual accessions. Genetic relationships among the plastotype or mtDNA haplotype were studied by the maximum parsimony method and neighbour joining (NJ) method[14].

RESULTS AND DISCUSSION

Variation of ctDNA in free-threshing emmer wheat

We have been studying the chloroplast microsatellite variation in wild and domesticated emmer wheat. These results revealed that there are two well differentiated maternal lineages associated with two distinctive plastogroups (Group I and II, respectively) in domesticated emmer wheat (*T. turgidum* ssp. *dicoccum,* non-free-threshing) (Mori et al. 2003[8], Mori *et al*. in prep.). In order to determine if both of the two plastogroups exist in evolutionary advanced free-threshing emmer wheat, we surveyed ctDNA variation in 134 accessions of *T. turgidum* ssp. *turgidum* (free-threshing). These plant materials were collected mainly in Southwest Asia and North Africa. Polymorphic banding patterns were observed at 11 out of 24 chloroplast microsatellite loci. The number of alleles per polymorphic locus ranged from 2 to 3 with an average number of 1.78, and the diversity index (H) ranged from 0.01 to 0.47 with an average of 0.08. These values are clearly lower than that of non-free-threshing emmer wheat (average allele no. = 2.13, and average H = 1.78). Twenty-four plastotypes were found among 134 free-threshing emmer wheat. Interestingly all 24 plastotypes belonged to the plastogroup I. This result suggested that the second maternal lineage associated with plastogroup II has not passed down to the free-threshing emmer wheat. However it should be noted that most of the accessions we studied were collected in Southwest Asia. Therefore, it is necessary to study the free-threshing emmer wheat collected in other regions of the world, and these surveys are now underway.

Maternal lineage of free-threshing common wheat

Our previous study regarding the chloroplast and nuclear DNA variation in common wheat indicated the existence of at least two maternal lineages in common wheat, especially in ssp. *spelta* in Europe[15]. However analysis of nuclear DNA variation suggested nuclear introgressions in ssp. *spelta*, and accordingly indicated that the origin of European *spelta* was a secondary event[15]. Therefore all data indicated that only one maternal lineage associated with plastogroup I in emmer wheat was introduced directly in the polyploidy evolution of common wheat (Figure 1).

In order to confirm the results shown above, we studied the ctDNA variation in 74 accessions, in total, of free-threshing common wheat (*T. aestivum* ssp. *aestivum*) collected in Europe, Mediterranean, Southeast Asia, North Africa and North America using 24 chloroplast microsatellites. The average number of alleles and average gene diversity (H) in the free-threshing common wheat in Europe was 1.30 and 0.02, respectively. These values are much smaller than that of emmer wheat (shown above). Only eight plastotypes were found and all these plastotypes belonged to plastogroup I, supporting the hypothesis shown inFig.1. These results also suggested the lower ctDNA polymorphism in free-threshing common wheat compared with non-free-threshing common wheat[15] or emmer wheat. Interestingly, these values were slightly lower than that of ssp. *aestivum* in Southwest Asia (average allele no.=1.5, average H=0.040)[15]. This result might indicate the gradual loss of ctDNA variation in the marginal region of cultivation of the free-threshing common wheat.

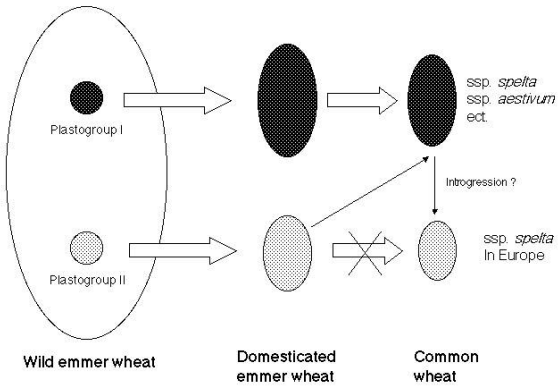

Figure 1. Schematic drawing of two hypothetical maternal lineages in emmer and common wheat.

Variation of ctDNA in timopheevi wheat

Domesticated species of timopheevi wheat (*T. timopheevi* ssp. *timopheevi*) are known to be endemic to the Transcaucasia region. It is generally accepted that the wild progenitor of this wheat is *T. timopheevi* ssp. *armeniacum*. However the origin of domesticated timopheevi wheat is still not clear. To study the domestication of timopheevi wheat, we conducted ctDNA fingerprinting using wild and domesticated timopheevi wheat. Allelic variation was evaluated at 23 chloroplast microsatellite loci for 66 accessions, in total, of wild and domesticated timopheevi wheat. In wild timopheevi, an average number of alleles and an average gene diversity (H) were 1.86 and 0.195, respectively, while domesticated timopheevi wheat showed monomorphic gel-banding pattern and no variation was found (average no. allele=1, average H=0). These results indicated that ctDNA variation in both wild and domesticated timopheevi wheat was much smaller than that in emmer wheat. Twelve plastotypes were found in 66 accessions of wild timopheevi, while all seven accessions of domesticated timopheevi shared single plastotype (Type 7). These results suggested the monophyletic origin of domesticated timopheevi wheat. The accessions collected in north Iraq and west Iran showed a smaller gene diversity (H) than that estimated for the accessions collected in south Turkey and north Syria. Although we could not detect plastotype 7 of domesticated timopheevi in wild timopheevi wheat, accessions collected in Transcaucasia did not show close relationship with domesticated timopheevi wheat. Further analysis is in underway.

Mitochondrial DNA diversity in Aegilops speltoides

We have been studying intraspecific diversity of mitochondrial DNA in *Aegilops speltoides* and its relation to the polyploidy evolution in *Triticum*. Mitochondrial microsatellite variability at 21 loci having more than ten mononucleotide repeats[12] was examined in 80 accessions of *Aegilops speltoides*. Polymorphic banding patterns were obtained at 19 out of 21 mitochondrial microsatellite loci. The number of alleles per polymorphic microsatellite ranged from 2 to 8 with an average of 3.43, and the diversity values (H) ranged from 0.03 to 0.68 with an average of 0.26. These values are about two thirds of chloroplast microsatellite values in the same species (Table 1). These results support the previous report[12] that variability of mitochondrial microsatellite is much less than that of chloroplast microsatellite. Based on the allele variation at all loci, a total of 42 mitochondrial haplotypes were identified in *Ae. speltoides*. A phylogenetic tree, constructed using the NJ method, showed four clearly differentiated subgroups in *Ae. speltoides*. All accessions of emmer and common wheat were grouped into a single clade that was separated clearly from the clades in *Ae. speltoides* accessions.

In contrast to the smaller number of mitochondrial haplotypes observed in polyploid wheat species (number of haplotype ranged from 1 to 3)[12], *Ae. speltoides* showed a much larger number of haplotypes (42 haplotypes). These results indicated that *Ae. speltoides* has wider intraspecific variation. In order to specify the possible candidates for the cytoplasm donor of tetraploid wheat, a larger scale survey on mitochondrial microsatellite would be necessary.

Table 1. Average number of alleles, diversity index (H) an number of haplotype for mitochondrial and chloroplast microsatellites observed in *Ae. speltoides*

	No. acc. examined	No. allele	Diversity index (H)	No. haplotype
Chloroplast	92	4.000	0.374	80
Mitochondria	80	3.430	0.258	42

CONCLUSION

Analyses on the molecular variation using highly variable microsatellites in chloroplast and mitochondrial genomes enabled us to explore the within subspecies diversity in *Triticum* and *Aegilops*. Present results here suggested that at least two maternal lineages were involved in the domestication of emmer wheat, and free-threshing hexaploid wheat was originated from one of these lineages. In addition, a contrasting mode of evolution between chloroplast and mitochondrial genomes was revealed. More information on the organellar genomes is required to understand whole maternal lineage and evolutionary history of *Triticum*.

REFERENCES

1. Lilienfeld, F. A., 1951 H. Kihara: genome-analysis in *Triticum* and *Aegilops*. X. Concluding review. Cytologia 16: 101 – 123.
2. Tsunewaki, K., 1996 Plasmon analysis as the counterpart of genome analysis, pp. 271 – 299 in *Method of genome analysis in plants*, edited by P. P. Jauhar. CRC press, New York.
3. Miyashita, N. T., N. Mori, and K. Tsunewaki 1994 Molecular variation in chloroplast DNA regions in ancestral species of wheat. Genetics 137: 883-889.
4. Kilian, B., H. Ozkan, O. Deusch, S. Effgen, A. Brandolini, J. Kohl, W. Martin and F. Salamini 2007 Independent wheat B and G genome origins in outcrossing *Aegilops* progenitor haplotypes. Mol. Biol. Evol. 24: 217-227.
5. Ogihara, Y. and K. Tsunewaki 1988 Diversity and evolution of chloroplast DNA in *Triticum* and *Aegilops* as revealed by restriction fragment analysis. Theor. Appl. Genet. 76: 321-332.
6. Mori, N., T. Terachi and K. Tsunewaki 1988 Organellar genome differentiation in wild tetraploid wheats, *T. dicoccoides* and *T. araraticum*. In Proc. VII Int. Wheat. Genet. Symp., Cambridge, Vol 1 pp 109-114.
7. Ishii, T., N. Mori, and Y. Ogihara 2001 Evaluation of allelic diversity at chloroplast microsatellite loci among common wheat and its ancestral species. Theor. Appl. Genet. 103**:** 896 - 904.
8. Mori, N., T. Ishido, S. Hirosawa *et al*. 2003 Origin of domesticated emmer and common wheat inferred from chloroplast DNA fingerprinting. Proc. X Int. Wheat. Genet. Symp., Paestum, pp 25-28.
9. Ozkan, H., A. Brandolini, C. Pozzi, S. Effgen, J. Wunder, F. Salamini 2005 A reconsideration of the domestication geography of tetraploid wheat. Theor. Appl. Genet. 110: 1052-1060.
10. Luo, M.-C., Z.-L. Yang, F. M. You, T. Kawahara, J. G. Waines and J. Dvorak 2007 The structure of wild and domesticated emmer wheat populations, gene flow between them, and the site of emmer domestication. Theor. Appl. Genet. 114: 947-959.
11. Liu, Y.-G., Mori, N. and K. Tsunewaki 1990 Restriction fragment length polymorphism (RFLP) analysis in wheat. I. Genomic DNA library construction and RFLP analysis in common wheat. Jpn. J. Genet. 65: 367-380.
12. Ishii, T., C. Takahashi, N. Ikeda, O. Kamijima and N. Mori 2006 Mitochondrial microsatellite variability in common whaet and its ancestral species. Genes Genet. Syst. 81: 211-214.
13. Nei, M. 1987 *Molecular evolutionary genetics*. Columbia University Press, New York.
14. Saitou, N. and M. Nei 1987 The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Boil. Evol. 4: 406-425.
15. Hirosawa, S., S. Takumi, T. Ishii, T. Kawahara, C. Nakamura and N. Mori 2004 Chloroplast and nuclear DNA variation in common wheat: insight into the origin and evolution of common wheat. Genes Genet. Syst. 79: 271-282.