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ORIGIN OF POLYPLOID WHEATS REVEALED BY RFLP ANALYSES[1]

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Summary. This paper reviews the results of our works carried out during the past few years on the RFLP analyses on nuclear DNAs of common wheat and its ancestors, which are summarized as follows: (1) The analyses of seven accessions belonging to three einkorn species revealed that *Triticum monococcum* was derived from *T. boeoticum*, whereas *T. urartu* provided the A genome to emmer and common wheats, (2) about 30 accessions each of *T. dicoccoides* and *T. araraticum* revealed that the interspecific differentiation between them was greater in magnitude than their intraspecific variations, which is in favor of the diphyletic origin of the emmer and timopheevi groups, (3) the analyses of five *Aegilops squarrosa* accessions collected from different localities revealed a great geographical differentiation in this species, and some accessions from the south coastal area of the Caspian Sea in Iran are closely related to common wheat, and (4) eight common wheat accessions investigated also revealed some geographical differentiation; European *T. spelta* was assumed to have originated from a cross between common and emmer wheats, while Tibetan semi-wild wheat was of a recent derivative from Chinese common wheat.

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1. RFLP analyses of einkorn wheat (Takumi et al. 1991)

The Einkorn group of wheat that is diploid (2n=2x=14), having the A genome, includes two wild species, *T. boeoticum* and *T. urartu*, and a single cultivated species *T. monococcum*, and is known to be the A genome donor to polyploid wheats (Kihara 1924). To determine the immediate ancestor of *T. monococcum* and the precise A genome donor to polyploid wheats, RFLP analyses of einkorn wheat were carried out.

Two accessions each of *T. boeoticum* (code B1 and B2) and *T. urartu* (U1 and U2), and three accessions of *T. monococcum* (M1, M2 and M3) were studied together with a single accession of each *T. durum* (D) and *T. aestivum* (A) as the referants.

Total DNA was extracted from young seedlings of each accessions, digested with two 6-base cutters (BamHI and HindIII), electrophoresed, hybridized to 46 genomic clones of CS as probes after Southern blotting, and autoradiographed. The procedures of these treatments were the same as those described by Liu et al. (1990).

Fig. 1 shows the autoradiograms of Southern blots of two enzyme-probe combinations, i. e., BamHI-Tag94 and HindIII-Tag94. Of the 92 possible enzyme-probe combinations, eight combinations did not give readable autoradiogram. Based on the autoradiograms of the remaining 88 enzyme-probe combinations, total and common hybrid fragments were counted between every pair of the nine accessions. Applying these data to Nei's formula (Nei 1987), genetic distances between all accessions were calculated. Based on these distances, a dendrogram showing the genetic relatedness among the nine accessions was constructed by the UPGMA method of Sokal and Sneath (1963) as shown in Fig. 2.

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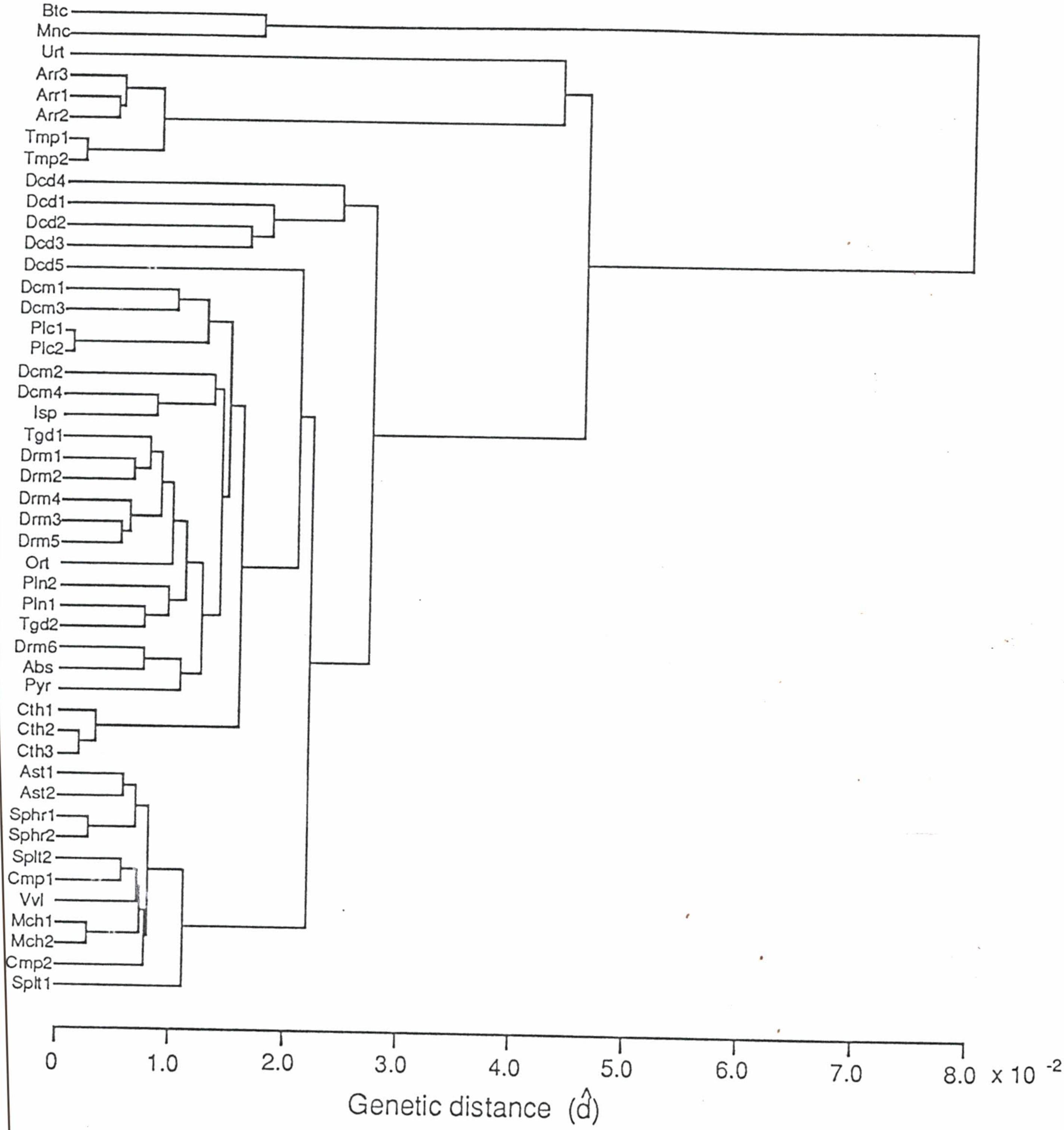

Figure 4. A dendrogram showing genetic relationships among 47 accessions of the genus *Triticum* constructed by the cluster analysis using genetic distances (Nei 1987) which were caluculated from the RFLP data based on 60 probe-enzyme combinations

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From the above results the following conclusions can be drawn; (1) Intraspecific variation seems to be larger in *T. boeoticum* than in the two other species, (2) interspecific variation among the three einkorn species is much larger than the intraspecific variation in all species, (3) *T. monococcum* is the cultivated form of *T. boeoticum*, and (4) *T. urartu* provided its nuclear genome to emmer and common wheats. The third and fourth conclusions are in complete agreement with those of Dvorak (1988), which were obtained by RFLP analyses using a highly repeated sequence as probe.

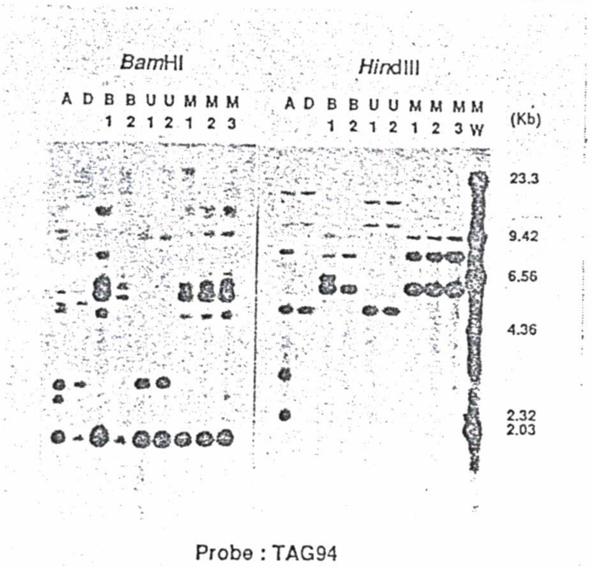

Fig. 1. Southern hybridization patterns of the total DNAs of seven accessions of einkorn wheat. The total DNAs were digested with BamHI (A) and HindIII (B) and proved with a genomic DNA clone Tag94. B1 & B2: *T. boeoticum* accessions, U1 & U2: *T. urartu* accessions, M1-M3: *T. monococcum* accessions, D: *T. durum* var. *reichenbachii*, A: *T. aestivum* cv. Chinese Spring, MW:molecular marker.

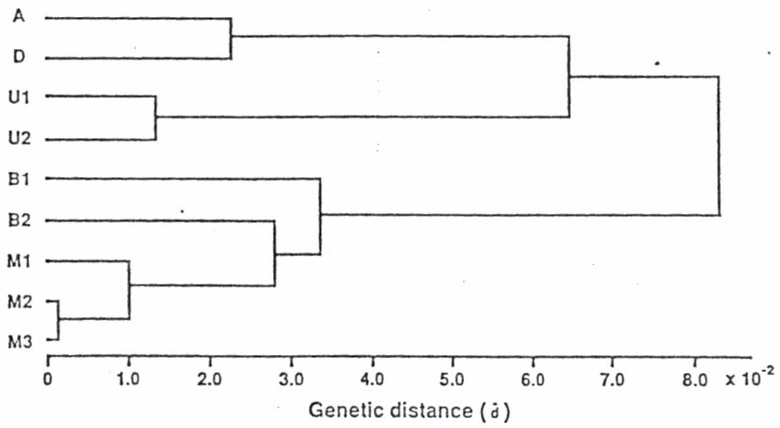

Fig. 2. A dendrogram showing genetic relationships among seven accessions of einkorn wheat and two accessions of polyploid wheats, that was constructed by the UPGMA method based on genetic distances. Abbreviations are the same as those in Fig. 1.

2. RFLP analyses of wild tetraploid wheats (Mori et al. 1991)

Two groups are known in tetraploid wheat, i.e., Emmer and Timopheevi groups having a haploid genome constitution of AB and AG, respectively (Lilienfeld and Kihara 1934). The origin of these two tetraploid wheat groups is the largest unsolved problem in wheat evolution. The differentiation of the two groups occurred undoubtedly before their cultivation, because each group includes a wild species, i.e., *T. dicoccoides* in Emmer group and *T. araraticum* in Timopheevi group. The present investigation aimed to contribute to the problem of their origin.

Thirty-two accessions of *T. dicoccoides* and 24 accessions of *T. araraticum* were selected to cover their entire distribution areas. Their total DNA was handled in the same ways as described above for einkorn wheat, together with the total DNA of a single accession of each *T. monococcum, Ae. speltoides, T. durum, T. timopheevi* and *T. aestivum* as the referants, and their Southern hybridization patterns were compared. In this case, two 6-base cutters, BamHI and HindⅢ, were used for DNA restriction, and 25 genomic DNA and 10 cDNA clones prepared from CS were employed as probes, which were prepared by Liu et al. (1990) and Mori et al. (1991), respectively. Fig. 3 shows an example of the Southern hybridization patterns.

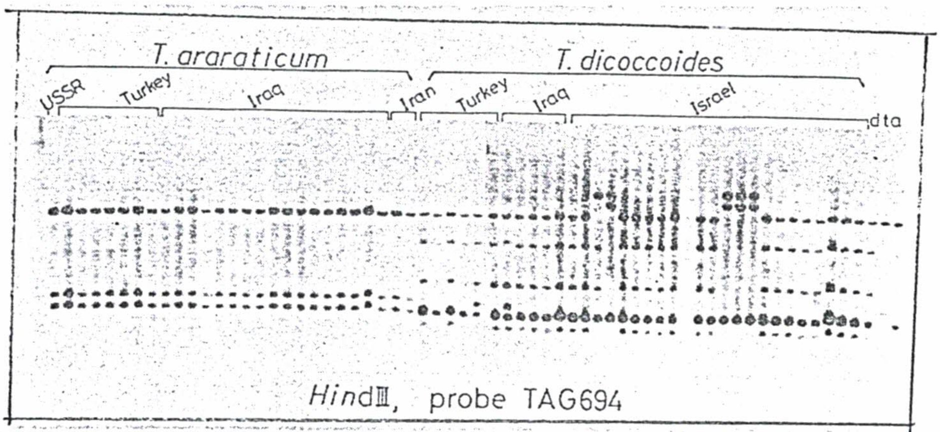

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Fig. 3. Southern hybridization patterns of ~~some~~ accessions of *T. dicoccoides* and *T. araraticum*.

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By comparing the Southern hybridization patterns obtained from 70 enzyme-probe combinations, we scored the numbers of total and common hybrid fragments for all pairs of the accessions, calculated the genetic distances and constructed a dendrogram showing their genetic relationship in the same way as described for einkorn wheat. Fig. 4 shows the dendrogram obtained.

The intraspecific genetic distances obtained between 32 *T. dicoccoides* accessions ranged from 0.0001 to 0.0205 with the average of 0.0135, whereas those between 24 *T. araraticum* accessions ranged from 0.0010 to 0.0100 with the average of 0.0036. On the contrary, the interspecific genetic distances between *T. dicoccoides* and *T. araraticum* accessions ranged from 0.0435 to 0.0507 with the average of 0.0482.

In the dendrogram, the single *T. timopheevi* accession was affiliated to the cluster of *T. araraticum* accessions, whereas *T. durum* was included in the cluster of *T. dicoccoides* accessions, to which *T. aestivum* accession was joined. *T. monococcum* and *Ae. speltoides* accessions formed a loose cluster that was distantly related to the cluster containing all the accessions of polyploid wheats.

From the above results, the following conclusions can be drawn: (1) Intraspecific variation of *T: dicoccoides* is about three times larger than that of *T. araraticum*, (2) the two wild tetraploid species show clear differentiation, having no intermediate types, and (3) *T. timopheevi* was originated from *T. araraticum*, and *T. durum* from *T. dicoccoides.* The first conclusion suggests a rather recent origin of *T. araraticum*, compared to that of *T. dicoccoides.* This and the second conclusion lead us to support the diphyletic origin of the two tetraploid wheat groups.

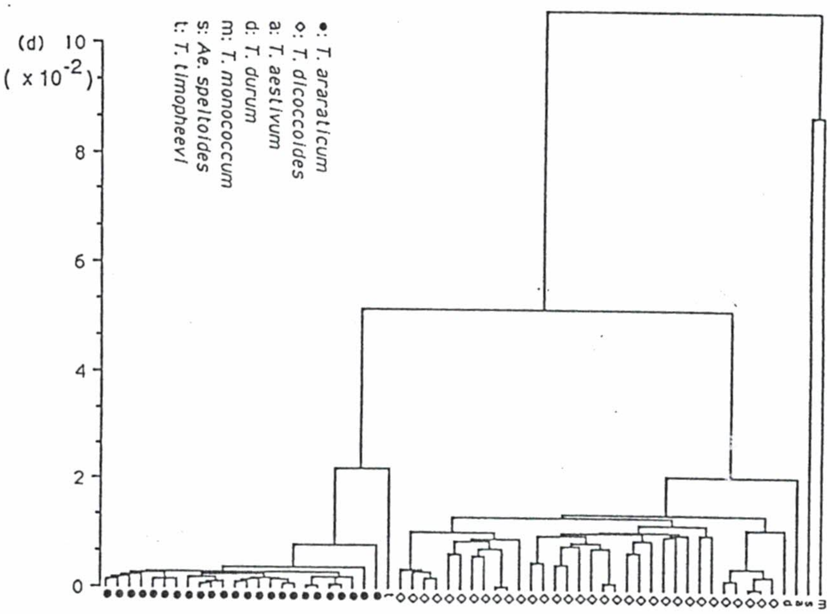

Fig. 4. A dendrogram showing genetic relationships among the accessions of *T. dicoccoides* and *T. araraticum*, together with some referant accessions.

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3. RFLP analyses of *Ae. squarrosa* (Achiwa 1990)

Ae. squarrosa is the D genome donor to common wheat that is hexaploid (2n=6x=42), having A, B and D genomes (Kihara 1944; McFadden and Sears 1944). This investigation was undertaken to clarify the genetic diversity of *Ae. squarrosa* and to speculate the birthplace of common wheat by comparing their RFLP characteristics.

Five accessions of *Ae. squarrosa* were studied. Table 1 shows their source. Their total DNA, together with that of CS as the referant, was extracted and treated in the same way as described for einkorn wheat. The Southern hybridization patterns were obtained from the combinations of three 6-base cutters (BamHI, EcoRI and HindIII)

Table 1. Accessions of common wheat and *Ae. squarrosa* used in RFLP analyses (a durum wheat was used as a referant)

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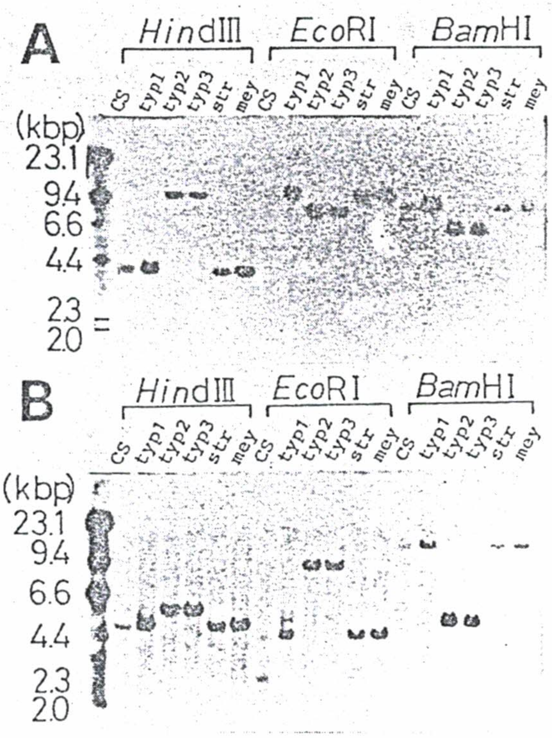
Accession	Abbreviation	Ploidy	Nuclear genome
T. aestivum cv. Chinse Spring	CS	6x	AABBDD
〃 ssp. *tibetanum*	Tbt	〃	〃
〃 cv. S-615	S615	〃	〃
〃 var. *erythrospermum*	Tve	〃	〃
T. compactum cv. No. 44	Cmp	〃	〃
T. sphaerococcum var. *rotundatum*	Sphr	〃	〃
T. macha var. *subletschchumicum*	Mch	〃	〃
T. spelta var. *duhamelianum*	Splt	〃	〃
T. durum var. *reichenbachii*	Drm	4x	AABB
Ae. squarrosa var. *typica* No.1 (KU20-1)	Typ1	2x	DD
〃 var. 〃 No.2 (KU20-2)	Typ2	〃	〃
〃 var. 〃 No.3 (KU20-3)	Typ3	〃	〃
〃 var. *meyeri* (KU20-10)	Myr	〃	〃
〃 ssp ~~var.~~ *strangulata* (KU20-9)	Str	〃	〃

Note: KU number is the accession number at the Plant Germplasm Institute, Faculty of Agriculture, Kyoto University, Japan

and 27 DNA clones as probes. The DNA clones used included 22 genomic clones prepared from *Ae. squarrosa* var. *typica* accession KU20-3, and five cDNA clones of CS. Fig. 5 shows an example of the Southern hybridization patterns.

By analyzing the Southern hybridization patterns obtained from 81 enzyme-probe combinations, we scored the numbers of total and common hybrid fragments between all pairs of the accessions, calculated the genetic distances, and constructed a dendrogram showing their genetic relatedness in the same way as described previously.



Probe: (A) ASG2, (B) ASG104

Fig. 5. Southern hybridization patterns of five accessions of *Ae. squarrosa* with a common wheat as a referant.

As seen in Fig. 6, Typ2 and Typ3 formed a cluster, whereas Typ1 was more closely related to Myr and Str than to other *typica* accessions. Typ1, Myr and Str were collected from west to south coastal area of Caspian Sea, while Typ3 from Tashkent, USSR, far east from the Caspian Sea. (Origin of Typ2 is unknown.)

The genetic distance from a common wheat CS was the shortest to Str and Myr (both d=0.0332), followed by Typ1 (d=0.0404). Two other *typica* accessions gave a longer distance to CS (d=0.0516-0.0533).

From the above results, the following conclusions are tentatively drawn; (1) *Ae. squarrosa* shows geographical differentiation of its nuclear genome as detected by RFLP analyses, and (2) *Ae. squarrosa* var. *meyeri* and subsp. *strangulata* have the D genome most closely related to common wheat genome with respect to RFLPs. The second conclusion is in agreement with the results of comparative gene analyses of common wheat and its ancestors which indicated that the probable birthplace of common wheat is in the south-west coastal area of Caspian Sea (Tsunewaki 1968; Nakai 1979; Nishikawa et al. 1980).

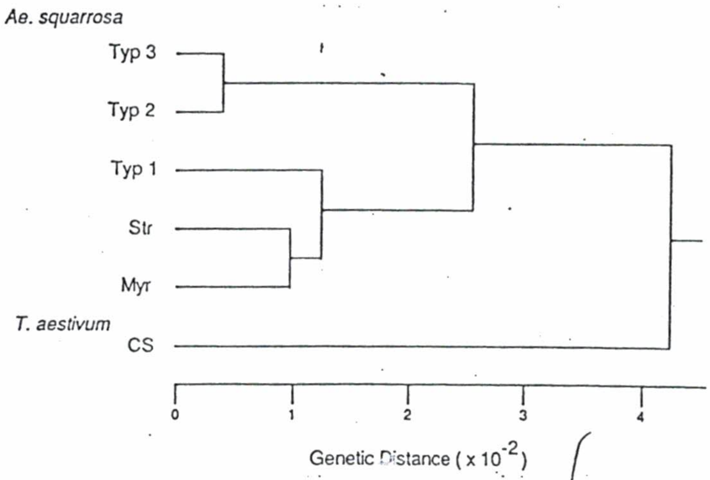


Fig. 6. A dendrogram showing genetic relationships among five *Ae. squarrosa* accessions and a common wheat.

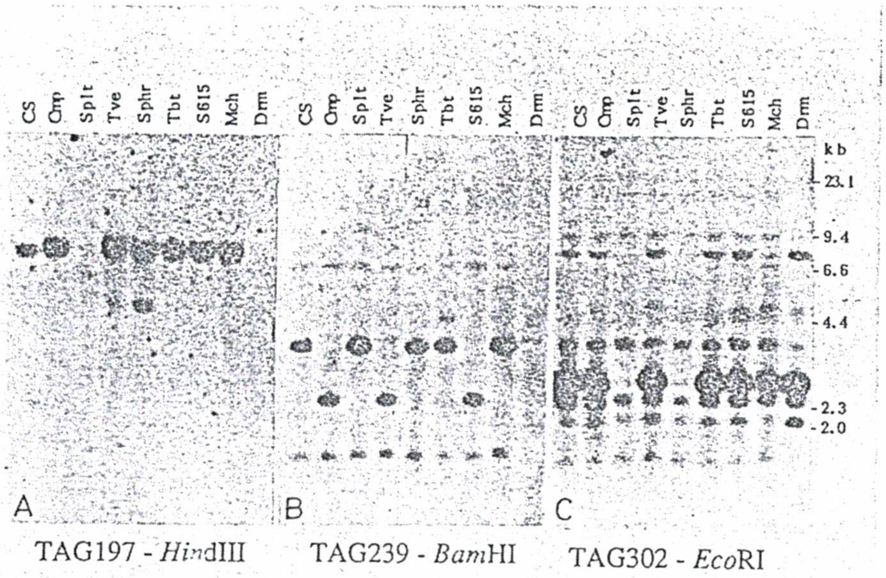
4. RFLP analyses of common wheat (Liu et al. 1990)

Common wheat that is a cultivated hexaploid with a single haploid genome constitution ABD, includes six species (or subspecies, depending upon the taxonomic system used), five of which were employed in the present investigation as shown in Table 1. The one excluded was *T. vavilovii*, which is considered as a mutant form of *T. spelta*. In total, four accessions of *T. aestivum* and single accession each of other species were studied, together with a *T. durum* accession as the referant.

The procedures employed to obtain their Southern hybridization patterns were the same as described previously, in which two endonucleases (EcoRI and HindⅢ) and 50 genomic clones of CS as probes were used in combination. Fig. 7 shows an example of the Southern hybridization patterns.

Fig. 8 shows a dendrogram showing the genetic relationship among eight common wheats and a single emmer wheat. It was constructed in the same way as were the other dendrograms. The eight common wheat accessions were classified into three groups, one consisting of CS, Tbt, Mch and Sphr, the second one with Cmp, S615 and Tve, and the last one with only Splt. Tbt that is a spelt type hexaploid with brittle spikes and was found as a weed in Tibet (Shao et al. 1983), was closely related to a Chinese cultivar (CS). Mch is an endemic species of Transcaucasus that is also a spelt type. Sphr is endemic in west India and Pakistan, that is free-threshing. This means that all four accessions comprised the first cluster were of Asian origin. Cmp and Tve were maintained in Hokkaido University at the time when Sakamura (1918) studied their chromosome number. Although no records on their origin are available at present, their morphology and growth habit, such as high photoperiod-sensitivity and weak winter growth habit, suggest their origin from central or northern Europe. S-615 was a spring cultivar grown in Canada. Thus we may say that the second cluster consists of the accessions from Western countries. These findings suggest that common wheat had differentiated geographically into the eastern and western populations, which is in agreement with the results on the distribution of hybrid necrosis genes, i.e., the frequency of *Ne1* gene is high (ca. 37%) in the former and low in the latter (ca. 10%), whereas that of the *Ne2* gene is low (ca. 5%) in the former and high (ca. 26%) in the latter (Tsunewaki 1970).

Splt is an accession of European *T. spelta* belonging to the old collection of Hok-



TAG197 - *Hin*dIII TAG239 - *Bam*HI TAG302 - *Eco*RI

Fig. 7. Southern hybridization patterns of eight common wheat accessions and a *T. durum* accession as the referant. Abbreviations are given in Table 1.

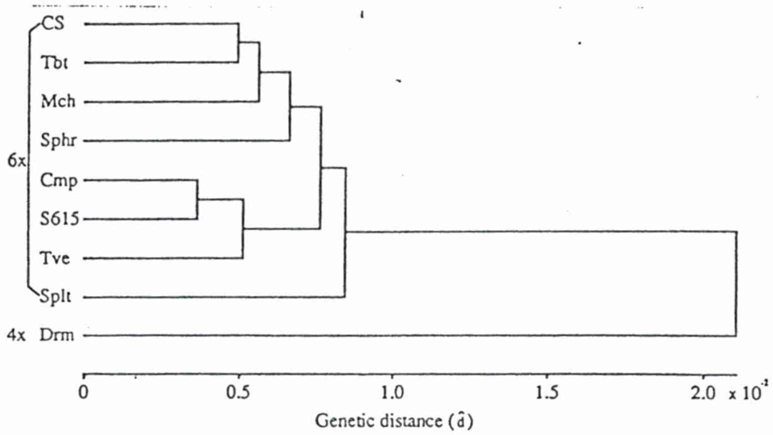


Fig. 8. A dendrogram showing the genetic relationships among eight common wheats and a durum wheat.

kaido University. Various works suggested that this species is a hexaploid derivative of the hybrid between common and emmer wheats (Mac Key 1963; Tsunewaki 1968; Gerlach et al. 1978). The chromosome mapping of the RFLP sites observed between CS and Splt also indicated the possible origin of Splt as a hybrid between common and emmer wheats (Liu and Tsunewaki 1991). The observed, separated genetic relationship of Splt to all other common wheats is well explained by the proposed origin of *T. spelta*.

In conclusion, the present results of RFLP analyses of common wheat support its geographical differentiation into the eastern and western populations, and the previously proposed origin of *T. spelta*.

5. General discussion on the origin of polyploid wheats

RFLP analyses of einkorn wheat indicated that *T. urartu* provided the A genome to both emmer and common wheats. We have not studied yet the origin of the A genome in the timopheevi group. RFLP analyses of chloroplast and mitochondrial genomes (Ogihara and Tsunewaki 1988; Terachi et al. in press) indicated that the cytoplasm donor to the timopheevi group was *Ae. speltoides*, and that no diploid *Aegilops* species other than *Ae. speltoides* can be considered as the cytoplasm donor to the emmer and common wheat groups. In addition, the present RFLP analyses of *T. dicoccoides* and *T. araraticum* demonstrated a discrete genomic difference between the two species and suggested an older origin of the former than the latter. These results strongly support the diphyletic origin of these species. Common wheat was suggested to have originated in the south to west coastal area of Caspian Sea from the RFLP data of *Ae. squarrosa*, and its geographical differentiation into the eastern and western populations was supported by the RFLP data of different accessions of common wheat.

References

√ ACHIWA, T. (1990) RFLP analysis of nuclear DNA in *Aegilops squarrosa* L. Master Thesis Fac Agr Kyoto Univ. pp.77

DVORAK, J. (1988) Cytological and molecular inferences about the evolution of wheat. In: Miller, T. E. and Koebner, R. M. D. (ed) Proc Ⅶ Int Wheat Genet Symp Cambridge. p.187-192.

GERLACH, W. L., APPELS, R., DENNIS, E. S. and PEACOCK, W. J. (1978) Evolution and analyses of wheat genomes using highly repeated DNA sequence. In: Ramanujam, S. (ed) Proc V Int Wheat Genet Symp New Delhi. p.81-91.

KIHARA, H. (1924) Cytologische und genetische Studien bei wichtigen Getreidearten mit besonderer Rücksicht auf das Verhalten der Chromosomen und die Sterilität in den Bastarden. Mem Coll Sci Kyoto Imp Univ. Bl:1-200.

KIHARA, H. (1944) Die Entdeckung der DD-Analysatoren beim Weizen. Agr Hort Tokyo 19:889-890.

LILIENFELD, F. A. and KIHARA, H. (1934) Genomanalyse bei *Triticum* und *Aegilops*. V. *Triticum timopheevi* Zhuk. Cytologia 6:87-122.

LIU, Y. G., MORI, N. and TSUNEWAKI, K. (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.

LIU, Y. G. and TSUNEWAKI, K. (1991) Restriction fragment length polymorphism (RFLP) analysis in wheat. II. Linkage maps of the RFLP sites in common wheat. Jpn J Genet 66:617-633.

3 MAC KEY, J. (1966) Species relationship in *Triticum*. In: MAC KEY, J. (ed) Proc Ⅱ Int Wheat Genet Symp Lund. p.237-276.

MCFADDEN, E. S. and SEARS, E. R. (1944) The artificial synthesis of *Triticum spelta*. Rec Genet Soc Amer 13:26-27.

MORI, N., LIU, Y. G., NAKAMURA, C. and TSUNEWAKI, K. (1991) Genetic differentiation between two wild tetraploid wheats, *Triticum dicoccoides* and *T. araraticum* as revealed by RFLP analysis of organellar and nuclear DNA. Seiken Ziho 72:76-78.

NAKAI, Y. (1979) Isozyme variations in *Aegilops* and *Triticum*. IV. The origin of the common wheats revealed from the study on esterase isozymes in synthesized hexaploid wheats. Jpn J Genet 54:175-189.

NEI, M. (1987) Molecular evolutionary genetics. Columbia Univ Press, New York. pp. 106-107.

NISHIKAWA, K., FURUTA, Y. and WADA, T. (1980) Genetic studies on alpha-amylase isozymes in wheat. Ⅲ. Intraspecific variation in *Aegilops squarrosa* and birthplace of hexaploid wheat. Jpn J Genet 55:325-336.

OGIHARA, Y. and TSUNEWAKI, K. (1988) Diversity and evolution of chloroplast DNA in *Triticum* and *Aegilops* as revealed by restriction fragment analysis. Theor Appl Genet 76:321-332

SAKAMURA, T. (1918) Kurze Mitteilung über die Chromosomenzahlen und die Verwandschaftsverhaltnisse der *Triticum*-Arten. Bot Mag Tokyo 32:151-154.

SHAO, Q., LI, C. and BASANG, C. (1983) Semi-wild wheat from Xizang (Tibet). In: SAKAMOTO, S. (ed) Proc Ⅵ Int Wheat Genet Symp Kyoto. p.111-114.

SOKAL, R. R. and SNEATH, P. H. A. (1963) Principles of numerical taxonomy. Freeman & Co., San Francisco. pp.359.

TAKUMI, S., NASUDA, S., LIU, Y. G. and TSUNEWAKI, K. (1991) Nuclear genome differentiation between three species of einkorn wheat as revealed by RFLP analysis. Japan J Breed 41 (Suppl 1):464-465.

TERACHI, T., OGIHARA, Y. and TSUNEWAKI, K. ~~(1991)~~ The molecular basis of genetic diversity among cytoplasms of *Triticum* and *Aegilops*. Ⅷ. Mitochondrial RFLP analysis using cloned genes as probes. Mol Biol Evo (in press). トル

TSUNEWAKI, K. (1968) Origin and phylogenetic differentiation of common wheat revealed by comparative gene analysis. In: Finlay, K. N. (ed) Proc Ⅲ Int Wheat Genet Symp Canberra. p.71-85.

TSUNEWAKI, K. (1970) Necrosis and chlorosis genes in common wheat and its ~~and its~~ ancestral species. Seiken Ziho 22:67-75. トル