# 四倍体コムギとその仮想祖先種の数量分類的関係(農学科)

# A Quantitative Study of Morphological Relationships among Natural Tetraploid Wheats and Their Putative Ancestors*

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## I INTRODUCTION

The wheat genus, *Triticum*, has diploid, tetraploid and hexaploid species with 14, 28 and 42 chromosomes. The genome constitutions of these species are AA, AABB or AAGG and AABBDD, respectively.

It is a well established fact that tetraploid wheat (AABB) and *Aegilops squarrosa* (DD) have contributed genomes to the formation of hexaploid wheat, *Triticum aestivum* (4,9) .

The tetraploid wheats are divided into two species groups, Emmer (genome constitution AABB) and Timopheevi (AAGG) group, which are isolated by the hybrid sterility through irregular meiosis. Emmer group contains wild species, *T. dicoccoides*, and many cultivated species. Timopheevi group also includes wild and semi-cultivated species, *T. araraticum* and *T. timopheevi*, respectively. These tetraploid wheats, on the one hand, have the common A genome which has derived from diploid wheat. On the other hand, the second genomes (B and G) of two groups are regarded as only partially homologous with each other because of the poor chromosome pairing in the hybrids between species of the two groups.

As to the donor of the B genome, the complete solution is not attained, though many investigations have been carried out. Tanaka (21) discussed the relationships between species of section Sitopsis of genus *Aegilops* and B genome of Emmer and Dinkel wheat. Sarkar and Stebbins (16) and Riley *et al.* (14) suggested *Ae. speltoides*, which belonged to section Sitopsis, as the B genome donor from the morphological and cytogenetical view-points, respectively. While, Sears (17) suggested *Ae. bicornis* which belongs to the same section as *Ae. speltoides* does, because of the close morphological resemblance of the amphidiploid between cultivated diploid wheat, *T. monococcum*,

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and *Ae. bicornis* to cultivated Emmer wheat, *T. dicoccum*. The cytogenetical investigations by Riley and Chapman (13), Kimber (5), and Kimber and Athwal (6) also did not favour for *Ae. speltoides* as the B genome donor.

On the other hand, the search on G genome donor was not carried out so extensively but Timopheevi group was mainly studied in connection with Emmer group (1, 7, 8, 15, 22, 23, 24, 25). The major points in dispute in this line of investigations are the causes of the poor chromosome pairing in the hybrids between two groups and the mechanisms involved in the differentiation of these two groups.

In the present study, in order to clarify the morphological relationships among these species and to examine the propriety of above-mentioned *Aegilops* species as diploid progenitor of tetraploid wheats, the morphological comparisons among these diploid and tetraploid wheats, Sitopsis species and their artificially synthesized auto- and allotetraploids have been done. For the morphological comparisons, newly developed methods of numerical taxonomy were adopted because these methods intended to group species based on quantified degree of resemblance among them (10, 11, 18, 19, 20).

## II MATERIALS AND CHARACTERS INVESTIGATED

Thirteen strains of natural species of genus *Triticum* and *Aegilops* and nine artificially synthesized auto- and allotetraploids were investigated in the present study (Table 1). Artificially synthesized allotetraploids having diploid wheats as one of the parents in common and the different species of section Sitopsis as the other parent were chosen for comparison with natural tetraploid wheats. After the analysis of results had been finished, it became questionable that the strain 1910 was *T. dicoccoides* var. *nudiglumis*, because the hybrid between 1910 and *T. timopheevi* was pollen fertile. But it is considered that this does not make the result uncertain. All the materials were obtained from the stocks maintained at the Plant Germ-plasm Institute, Kyoto University.

The 33 characters investigated (14 qualitative and 19 quantitative characters) are listed in table 2. The records for ear characters were taken from two ear samples which had been obtained from the plants grown in the field and the records for spikelet characters were taken from the middle part spikelet of each ear sample. Then the mean of two records was taken as a representative of the strain. The qualitative characters were recorded using code numbers which ranged from 1 to 3 and showed the state of character (Table 2). The measurements were taken for the quantitative characters using different units as shown in table 2. All the representative values were

**Table 1. List of species and amphidiploids used in the present study**

**I. Natural species**

| Species | Strain number | Genome symbol* | Collected from |
|---|---|---|---|
| *Triticum* | | | |
| *T. boeoticum* Boiss em. Schiem. | 101-1 | A | (unknown) |
| *T. monococcum* L. | | | |
| var. *flavescens* | 105 | A | 〃 |
| *T. dicoccoides* Körn | | | |
| var. *kotschyanum* | 108-3 | AB | Palestine |
| var. *straussianum* | 110 | AB | 〃 |
| var. *nudiglumis* | 1910 | AG | Northern Iraq |
| *T. araraticum* Jakubz. | 196-1 | AG | Armenia |
| 〃 | 196-2 | AG | 〃 |
| *T. timopheevi* Zhuk. | 107-1 | AG | Georgia |
| *Aegilops* | | | |
| *Ae. speltoides* Tausch | | | |
| var. *ligustica* | 5725B | S | Turkey |
| var. *aucheri* | 5714A | S | 〃 |
| *Ae. bicornis* (Forsk.) Jaub. et Sp. | 3-1 | $S^b$ | (unknown) |
| *Ae. longissima* Schw. et Muschl. | 4-2 | $S^l$ | Palestine |
| *Ae. sharonensis* Eig | 5-1 | $S^l$ | 〃 |

**II. Amphidiploids**

| Strain number | Genome symbol* | Derived from | Synthesized by |
|---|---|---|---|
| 201-1 | AA | *T. boeoticum* (101-1) | Cua |
| 24 | $S^bS^b$ | *Ae. bicornis* (3-1) | Kondo |
| 25 | $S^lS^l$ | *Ae. longissima* (4-1) | Cua |
| 26 | $S^lS^l$ | *Ae. sharonensis* (5-1) | Tanaka |
| 212 | $S^lA$ | *Ae. longissima* 4x (25) x *T. boeoticum* 4x (201-1) | Tanaka |
| 213 | $S^lA$ | *Ae. sharonensis* 4x (26) x *T. boeoticum* 4x (201-1) | Muramatsu |
| 214 | $S^bA$ | *Ae. bicornis* x *T. monococcum* | Sears |
| 230 | $S^bA$ | *Ae. bicornis* x *T. monococcum* (105) | Suemoto |
| T441 | SA | (*Ae. speltoides* var. *ligustica* x var. *aucheri*) $F_2$ x *T. boeoticum* | Tanaka |

* shown in haploid phase

**Table 2. List of characters investigated**

| Qualitative characters | Code number | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Articulation of ear | wedge | — | umbrella |
| Brittleness of rachis | brittle | intermediate | tough |
| Development of apical spikelet | rudimentary | 〃 | normal |
| Pubescence of rachis | hairy | 〃 | hairless |
| Bristle on face of rachis | bristle | 〃 | smooth |
| Pubescence on empty glume | hairy | 〃 | hairless |
| Prominence of keel | remarkable | 〃 | weak |
| Dehiscence of inner glume | dehiscent | 〃 | non-dehiscent |
| Development of 2nd awn of middle spikelet | remarkable | 〃 | weak |
| Development of 2nd awn of apical spikelet | 〃 | 〃 | 〃 |
| Development of lst awn of apical spikelet compared with lst awn of middle spikelet | 〃 | 〃 | 〃 |
| Solidity of straw | solid | 〃 | hollow |
| Kernel texture | flinty | 〃 | mealy |
| Adhesion of inner glume with seed | apart | 〃 | adhesive |

| Quantitative characters | Unit or formula |
|---|---|
| Length of ear | mm |
| Number of spikelets | — |
| Number of rudimentary spikelets | — |
| Length of rachis | 0.5mm |
| Shape of upper transection of rachis | width/thickness |
| Ratio | $\frac{\text{thickness of upper part of rachis}}{\text{thickness of lower part of rachis}}$ |
| Length of spikelet | 0.5mm |
| Shape of spikelet | length/width |
| Ratio | $\frac{\text{length of outer glume}}{\text{length of empty glume}}$ |
| Length of apical tooth | 0.029mm |
| Length of lateral tooth | 0.029mm |
| Length of tooth of outer glume | 0.029mm |
| Number of fertile florets | — |
| Number of veins in empty glume | — |
| Number of tooth in empty glume of apical spikelet | — |
| Length of grain | 0.71mm |
| Shape of grain | length/width |
| Shape of cross-section of grain | width/height |

standardized by dividing the deviation of each strain from the general mean for the 22 strains on each character with the standard deviation of that character, then transformed into code numbers, taking those over 0.43 to be 3, those between 0.43 and -0.43 to be 2 and those below -0.43 to be 1 (11). Records thus obtained for 33 characters in 22 strains were arranged in a 33 × 22 table.

## III METHODS OF COMPUTATION

The correlation coefficient was adopted for estimating the degree of similarity between two strains. Based on the 33 × 22 table, the correlation coefficients between strains were calculated in all possible combinations and thus the correlation matrix was constructed.

To describe the interrelations among strains, two methods, namely, Sokal's weighted variable group method of cluster analysis and centroid method of factor analysis were adopted in the present investigation.

According to Sokal's method (18, 19, 20), the grouping of strains was carried out and representation of resultant relationships among strains was made by means of the tree-like dendrogram, in which the magnitude of correlation between any two joining stems can be read on the abscissa.

The computation by the centroid method started with the same correlation matrix as used for Sokal's method. Analizing this correlation matrix by centroid method, several factors, by which the variations represented in the matrix may be adequatly accounted for, are extracted and as a result some grouping of strains may be attained. In the present study, from the original correlation matrix four centroid factor loadings were extracted according to the procedure described by Fruchter (2).

## IV RESULTS

### 1. Sokal's weighted variable group method

The result obtained by the Sokal's method is shown in Fig. 1 as a tree-like dendrogram.

From the dendrogram, three groups of strains may be recognized; the groups including strains from 101-1 to 110 (all of wheat strains), from T441 to 230 (artificially synthesized allotetraploids) and from 5714A to 24 (all of Sitopsis strains) in Fig. 1 are designated as group I, II and III, respectively.

However, it must be pointed out that the interrelations of these three groups extremely distorted in the dendrogram, because the degree of

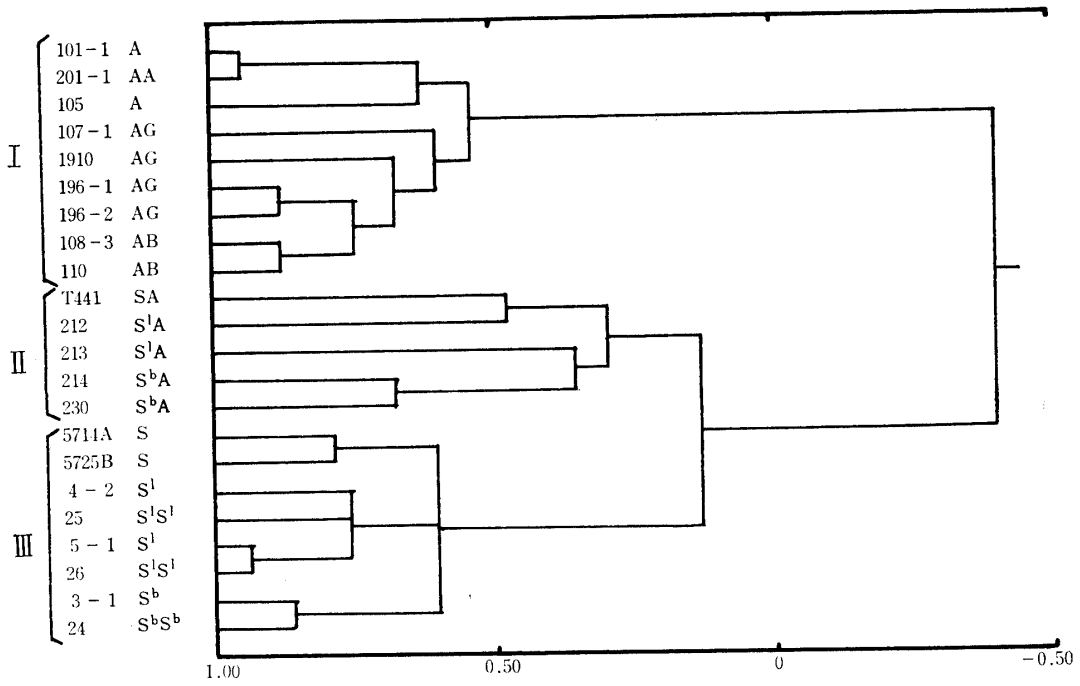


**Fig. 1 Dendrogram of 13 natural species of the genus *Triticum* and *Aegilops* and 9 amphidiploids obtained by Sokal's weighted variable group method**

similarity between group I and II ($r_{I,II}=0.105$) was comparable to that between group II and III ($r_{II,III}=0.130$), while the similarity between group I and III ($r_{II,III}=0.713$) was extremely low when compared with $r_{I,II}$ and $r_{II,III}$.

As to the inner structure of each group, such distortion as mentioned above was not observed in the dendrogram.

### 2. Centroid method

As the criteria for stopping extraction of factor loadings, both of Tucker's phi and Humphrey's rule were adopted in this study and as a result, it was found that only two factor loadings are sufficient for the purpose of this study. These two criteria may referred to Fruchter (2).

In order to recognize the groups of strains, all the strains were projected on scattering diagram with two axis which represented the first and second factors (Fig. 2). The scattering diagram showed that there were four groups of strains; two of them were identical with group II and III of the previous dendrogram obtained by Sokal's method and the third one comprised 101-1, 105, 107-1 and 201-1 (diploid and synthesized autotetraploid wheats and *T.*

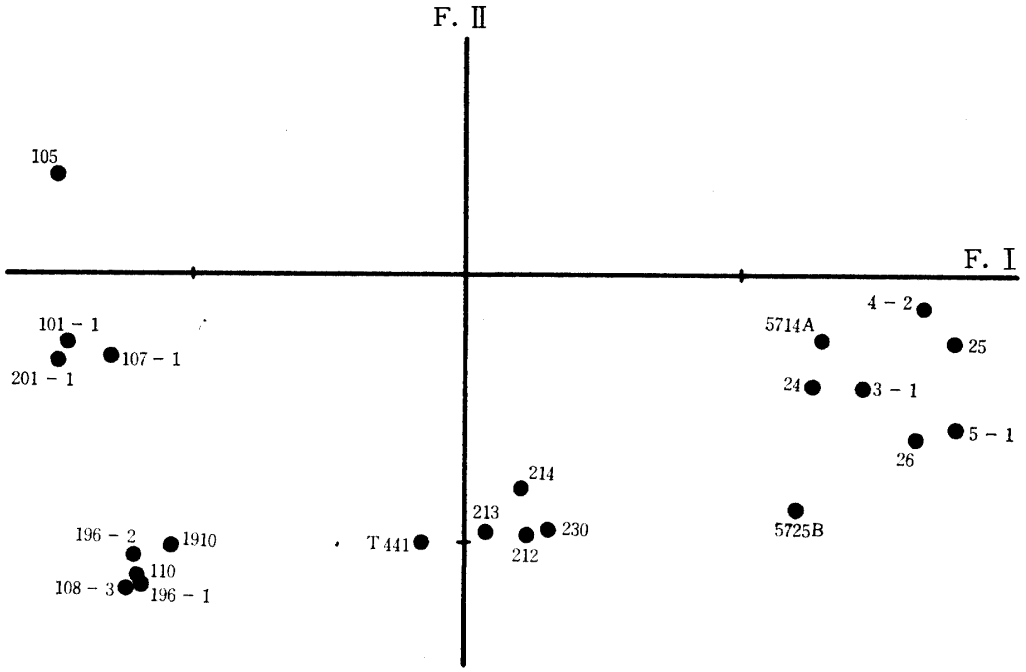


**Fig. 2 Distribution of factor loadings obtained by centroid method**

*timopheevi*) and the last one comprised 108-3, 110, 196-1, 196-2 and 1910 (natural tetraploid wheats except *T. timopheevi*) The result of factor analysis is essentially same as that of Sokal's weighted variable group method except that the group I recognized by Sokal's method was divided into two groups by the centroid method.

## V DISCUSSION

The propriety of the present methods of the classification of species and the quantification of relationships among species have been carefully discussed by some workers (10, 18) and the investigations on the different materials showed the usefulness of these methods (3, 11, 18).

In the present investigation, the fundamental concordance with classical classification was obtained. Namely, the natural strains were clearly divided into wheat and Sitopsis groups (group I and III in Sokal's method). And that, the diploid strains which had the same genome constitution showed very high correlation with each other.

On the other hand, the natural tetraploid wheats except *T. timopheevi* showed high degree of similarity with each other, in spite of their different genome constitution and the hybrid sterility found between them. This result

suggests that *T. dicoccoides* and *T. araraticum* may be closely related phylogenetically.

It is somewhat curious to know that *T. timopheevi* was not included in the group of tetraploid wheats but in the group of diploid wheats by the factor analysis.

The artificially synthesized autotetraploids had high morphological similarity with their diploid parents and so the autotetraploidization does not seem to induce the radical changes in morphology. On the other hand, the artificially synthesized allotetraploids did not link directly with those parental species but formed one group by themselves (group II in Fig. 1).
And, as a whole, group II showed the approximately same relationship with both group I and III (see RESULTS), which included their parents, diploid wheats and Sitopsis strains, respectively. Therefore, generally speaking, the allotetraploids seemed to have the intermediate morphology to their diploid parents and this agreed with the report of Riley and Bell (12).

Clustering of the all synthesized allotetraploids into one group indicates that if any species of section Sitopsis is the B or G genome donor, the natural tetraploid wheats should be included in the cluster formed by the synthesized allotetraploids. Because the present result showed that the natural tetraploid wheats did form a different group from the synthesized allotetraploids, the hypothesis, that a species of section Sitopsis is one of the parents of Emmer wheat (14, 16, 17), was not supported.

It is also noticeable that the tetraploid wheats had relatively high degree of similarity with diploid wheats.

Although any specific suggestion about the second parent of the tetraploid wheats was impossible from the present study, it can be said that, besides the survey of a possible donor of the second genome, the mechanisms of speciation through the hybridization at tetraploid level or the introgression of chromosome(s) or chromosome segments to the original amphidiploids must be considered among other possibilities.

## VI SUMMARY

In order to clarify the morphological relationships between di- and tetraploid wheats and diploid *Aegilops* species of the Sitopsis section, and to test the validity of some previous hypothesis about origin of the second genome of tetraploid wheats, thirteen strains of natural species of the genus *Triticum* and *Aegilops* and nine artificially synthesized auto- and allotetraploids were studied using two methods of numerical taxonomy.

Data were taken for 33 morphological characters in each strain. Based on these data, between-strains correlation coefficients were calculated as indices of the degree of similarity and resultant correlation matrix was analyzed by the Sokal's weighted variable group method of cluster analysis and the centroid method of factor analysis.

Both analyses gave fundamentaly similar results with the basic concordance to the classical classification. The results suggested the following points: The autotetraploidization did not induce radical changes in morphology, while allotetraploids had an intermediate morphology to their diploid parents. The natural tetraploid wheats formed a different group from the synthesized allotetraploids that were assumed as a putative ancestor of the tetraploid wheats by many workers. Therefore, it may be concluded morphologically that the tetraploid wheats do not resemble so closely the synthesized allotetraploids as to consider them as the putative type of the tetraploid wheats.

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# 四倍体コムギとその仮想祖先種の数量分類的関係

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四倍体コムギは，雑種不稔によって，お互いに隔離されている2群，すなわちエンマーコムギとチモフェビーコムギから成る。これら2群は，それぞれAABB，AAGGのゲノム構成を持ち，二倍体コムギの持つAゲノムを共有している。BゲノムとGゲノムは，部分的な染色体対合しか起さない為，部分相同ゲノムであるといわれてきたが，これらの関係は，必ずしも明きらかにされてはいない。一方，四倍体コムギの起原についても，多くの研究があり，特にBゲノム分析種として，エギロプス属シトプシス節に属する *Ae. speltoides*, *Ae. bicornis* 等があげられてきたが，いずれも確定的な根拠はない。本実験は，形態の比較研究から，これらの種が，四倍体コムギの祖先種として妥当であるか否かを，判定する為に行われた。材料は，二倍体コムギ，上記シトプシス節の4種，両者の人為合成同質及び異質四倍体，及び四倍体コムギの合計22系統を用い，主として倍数化に伴う形態の変化と，合成異質四倍体と四倍体コムギの形態的類似の程度に着目した。形態の分析には，数量分類の方法を用いた。すなわち，各系統について33の形質の調査を行い，その結果から，系統間の相関係数をすべての組み合せについて求めた。こうして得られた相関行列を Sokal's weighted variable group method と Centroid factor analysis の2方法で解析し，形態の類似度に基づく類縁関係を，明らかにした。その結果，本実験で用いた異質四倍体は，それらだけで，1つの群を構成し，それらは，全体として両親種の両方にほぼ等しい類縁度を示した。しかし，この群は，四倍体コムギの群とは，はっきりと異っていた。従って，形態の類似度に基づけば，シトプシス節の種は，四倍体コムギの直接の祖先としては，不適格であると結論される。

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