Multiple origins of U genome in two UM genome tetraploid *Aegilops* species, *Ae. columnaris* and *Ae. triaristata*, revealed based on the polymorphism of a genome-specific PCR fragment

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To elucidate the evolutionary mode of the formation of species via polyploidization, we conducted phylogenetic analysis of the U genome of the UM genome tetraploid *Aegilops* species, *Ae. columnaris* and *Ae. triaristata*. Using the genome-specific PCR primer set U31, we investigated the variation of the U genome of 48 accessions each of *Ae. columnaris* and *Ae. triaristata* and 72 accessions of their diploid ancestor *Ae. umbellulata*. As a result, three alleles were distinguishable by amplified length and CAPS polymorphisms, namely, allele I = normal size with an *Msp*I site, allele II = normal size without an *Msp*I site, and allele III = shorter size caused by a 123bp deletion. All three alleles were detected both in diploid and tetraploid accessions. Sequence comparison indicated the inheritance of alleles I and III from the diploid to the tetraploids, suggesting multiple origins of the U genome of the tetraploids. Regarding allele II, however, the sequence comparison indicated that parallel mutations at the *Msp*I site produced allele II several times. The phylogenetic tree based on the sequences of the U31 region demonstrated the presence of a third lineage of the U genome from *Ae. umbellulata* to *Ae. columnaris*. Consequently, we concluded that the U genome had at least three origins in *Ae. columnaris*, and at least two, probably more, in *Ae. triaristata*.

Key words: *Ae. columnaris*, *Ae. triaristata*, multiple origins, polyploidy, U genome-specific primer

INTRODUCTION

Polyploidization, the stable amplification or coexistence of more than two genomes in a nucleus, has played an important role in plant evolution. Masterson (1994) reported that 70% of angiosperms have experienced genome duplication at least once. Researchers have long pointed out the evolutionary potential of polyploidization, that is, it may produce more genetic diversity in nuclear genomes and consequently allow polyploids to be distributed in a wider area and to adapt to more varied niches than diploids (Kihara, 1954; Levin, 1980; Osborn et al., 2003).

The taxonomical complex of the genera *Aegilops* and *Triticum* (goatgrass and wheat, respectively) is one of the

most extensively investigated groups with respect to allopolyploidy. Extensive genome analysis with cytological techniques revealed the diploid ancestors of most of the polyploids in these genera (Lilienfeld, 1951). Subsequently, plasmon analysis with molecular techniques determined the cytoplasm type for each species (Wang et al., 1997). In a study of the plasmon analysis, Murai and Tsunewaki (1986) found that *Ae. triuncialis* L., a UC genome tetraploid *Aegilops* species, has two reciprocal maternal lineage within a species. This was the first molecular evidence for multiple origins of polyploid species in these genera, although the possibility had been discussed because a high level of morphological variation exists in hexaploid wheat (Dekaprelevisch, 1961; Kuckock, 1964; MacKey, 1966). The development of DNA technology and accumulation of information have made it possible to generate genome-specific primers, which facilitate the tracing of the nuclear lineage in polyploids. To date,

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several studies using such genome-specific primers have proved the existence of multiple origins of polyploids in these genera (Chee et al., 1995; Talbert et al., 1998; Vanichanon et al., 2003; Caldwell et al., 2004).

Ae. columnaris Zhuk. and *Ae. triaristata* Willd., which belong to the section Polyeides in the genus *Aegilops*, are tetraploids possessing the UM genome [nomenclature follows Kihara and Tanaka (1970)]. Their diploid ancestors are *Ae. umbellulata* Zhuk. for the U genome and *Ae. comosa* Sibth. et Sm. for the M genome, and the cyto-

plasm donor for both species is *Ae. umbellulata* (Lilienfeld, 1951; Wang et al., 1997). These two UM genome species are morphologically similar and genetically close to each other (Kimber and Feldman, 1987; Resta et al., 1996; Badaeva, 2002). Among the section Polyeides, multiple origins of the U genome have been demonstrated for two tetraploid species, *Ae. triuncialis* and *Ae. biuncialis* Vis. (Chee et al., 1995; Vanichanon et al., 2003). This fact raised the question whether the other Polyeides polyploids also have multiple origins of the U genome. In

Fig. 1. Three alleles of locus U31. (A) Amplified length polymorphism of locus U31. Lanes 1–6 are *Ae. umbellulata* (accession code umb03, umb21, umb04, umb28, umb13, and umb14, from left to right), 7–12 *Ae. columnaris* (clm01, clm19, clm07, clm47, clm17 and clm22), and 13–18 *Ae. triaristata* (trr03, trr12, trr01, trr02, trr05 and trr48). M is size marker ϕ X174 digested with *Hae*III. (B) CAPS profile of locus U31 digested with *Msp*I. Sample order is the same as in (A). Lanes 1, 2, 7, 8, 13 and 14 represent allele I, 3, 4, 9, 10, 15 and 16 allele II, 5, 6, 11, 12, 17 and 18 allele III. (C) Schematic gene structure of three alleles of U31. The broken line indicates deletion.

Table 1. Number of accessions for each allele of U31 in three *Aegilops* species

U31 Allele	Sequence at <i>Msp</i> I site ^a	<i>Ae. umbellulata</i>	<i>Ae. columnaris</i>	<i>Ae. triaristata</i>
allele I	CCGG	35 (0.49)	35 (0.73)	42 (0.88)
allele II		27 (0.38)	2 (0.04)	4 (0.08)
	CCAG	<i>21</i>	<i>1</i>	<i>0</i>
	CTGG	<i>3</i>	<i>0</i>	<i>4</i>
	CTGA	<i>3</i>	<i>0</i>	<i>0</i>
	CCG-	<i>0</i>	<i>1</i>	<i>0</i>
allele III		8 (0.11)	11 (0.23)	2 (0.04)
allele IV (null)		2 (0.03)	0 (0.00)	0 (0.00)
Total		72 (1.00)	48 (1.00)	48 (1.00)

Frequency of each allele in the species is shown in the parenthesis. The number of accessions for each sequence type of allele II is shown in italics.

^a: - indicates a deletion.

Table 2. List of *Aegilops* accessions used for sequence analysis of entire U31 region

Lab. code ^a	Accession No. ^b	Origin	U31 allele ^c
<i>Ae. umbellulata</i>			
umb03	KU2731	Turkey	I
umb21	KU4109	Iran	I
umb32	IG119380	Syria	I
umb04	KU2760	Turkey	II (CTGA)
umb28	IG49022	Iraq	II (CCAG)
umb31	IG107074	Iran	II (CCAG)
umb13	KU12189	Greece	III
umb14	KU12199b	Greece	III
umb25	IG47866	Turkey	III
<i>Ae. columnaris</i>			
clm01	KU11-1	Turkey	I
clm19	KU2953A	Armenia	I
clm24	KU5011	Iraq	I
clm07	KU2706	Iran	II (CCAG)
clm47	KU12779	Syria	II (CCG-)
clm17	KU2719	Iran	III
clm22	KU4194	Turkey	III
clm43	KU6584	Turkey	III
<i>Ae. triaristata</i>			
trr03	KU2930	Azerbaijan	I
trr12	KU4135	Iraq	I
trr29	KU6244	Turkey	I
trr01	KU10-4	Turkey	II (CTGG)
trr02	KU10-3	Turkey	II (CTGG)
trr37	KU6379	Turkey	II (CTGG)
trr05	KU4122	Iraq	III
trr48	KU4125	Iraq	III
<i>Ae. bicornis</i>			
bcr01	KU3-2	Israel	II

^a: Laboratory designated accession code used in this study.

^b: Accession number given by the original genebanks. KU and IG are the code indicating Kyoto University and ICARDA Genebank, respectively.

^c: The polymorphic sequences at the *Msp*I site are shown in the parentheses for allele II. - indicates a deletion.

this study, we generated U genome-specific primers and applied them to large numbers of *Ae. columnaris* and *Ae. triaristata*, and the U-genome diploid *Ae. umbellulata*. The observed polymorphisms of the U genome-specific fragment revealed the multiplicity of the origin of the U genome in these two species.

MATERIALS AND METHODS

Plant materials and DNA extraction A total of 72 accessions of *Ae. umbellulata* (accession code umb01 – umb72) and 48 accessions each of *Ae. columnaris* (clm01 – clm48) and *Ae. triaristata* (trr01 – trr48) were used in this study. Thirteen accessions of *Ae. umbellulata* were provided by the genebank of the International Center for Agricultural Research in the Dry Areas (ICARDA) and all the rest were from the collection of the Plant Germ-plasm Institute, Graduate School of Agriculture, Kyoto University. Since *Ae. triaristata* consists of tetraploid and hexaploid accessions, only tetraploid accessions were used

in this study. The materials were chosen to cover most of the geographical distribution for each species. Total DNA was extracted from seedlings using a modified CTAB method (Murray and Thompson, 1980).

Construction of the U genome-specific primers

Several U genome-specific AFLP fragments detected previously (Sasanuma et al. 2004) were recovered from acrylamide gels and sequenced. Specific primers for each fragment were designed. The genome-specificity of the primers was verified by diagnostic PCR using all 12 diploid species of the genera *Aegilops* and *Triticum*. From the 39 AFLP fragments sequenced, one U genome-specific primer set, U31, was obtained. This primer set was used for the following analyses. This primer set amplified the S^b genome of *Ae. bicornis* in addition to the U genome. The U31 region of the S^b genome was included in the evolutionary analysis as an outgroup.

PCR amplification and CAPS analysis PCR was con-

Accession code	U31 allele	Nucleotide position																											
		42	45	50	52	54	60	61	98	112	115	133	139	140	196	203	228	350	228	230	263	290	291	292	313	329	332	336	339
Consensus		G	C	G	A	A	A	C	C	T	G	C	G	A	G	G	123bp	A	G	A	C	G	G	G	A	A	C	T	
umb03	(I)	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.
umb21	(I)	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	C
umb32	(I)	.	T	.	.	.	.	.	T	.	.	.	.	.	A	A	.	.	.	C	A	.	.	.	.	.	.	.	.
clm01	(I)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
clm19	(I)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
clm24	(I)	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
trr03	(I)	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
trr12	(I)	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
trr29	(I)	.	.	A	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
umb04	(II)	C	.	.	G	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.	A	.	.	.	.	.
umb28	(II)	.	T	.	G	.	.	.	.	.	.	.	.	.	A	A	.	.	.	C	A	.	A	.	A	G	.	.	.
umb31	(II)	.	T	.	G	.	.	.	.	.	.	.	.	.	A	A	.	.	.	C	A	.	A	.	A	.	.	.	.
clm07	(II)	.	T	.	G	.	.	.	.	.	.	.	.	.	A	A	.	.	.	C	A	.	A	.	A	.	.	.	.
clm47	(II)	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
trr01	(II)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	T	.
trr02	(II)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.
trr37	(II)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.
umb13	(III)	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-
umb14	(III)	.	T	.	G	.	.	.	.	A	.	-	-	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-
umb25	(III)	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-
clm17	(III)	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-
clm22	(III)	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-
clm43	(III)	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-
trr05	(III)	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-
trr48	(III)	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-
bcr01	(II)	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	C

Fig. 2. Nucleotide substitutions detected in the U31 region among the 26 sequences. The nucleotides identical to the consensus sequence are represented by dots. Dashes mean deletions. The substitutions at the *Msp*I site are highlighted by a bold-line square. The allele II sequences are shown by gray background. The 20 single substitutions detected only in bcr01 are not shown.

ducted with the primer set U31 (U31a 5'-TATGATAAA-CAAGGCGAAAA-3' and U31b 5'-GGCTGTGGTGTAT-GATGACT-3'). The reaction conditions were 1 cycle of 94°C, 5min; 30 cycles of 94°C, 1min; 55°C, 1min, 72°C, 2min; and finally 1 cycle of 72°C, 7min. PCR amplification of polymorphisms was investigated by electrophoresis in agarose gels. The U31 product of one accession each of the three species was sequenced to find restriction sites suitable for the cleaved amplified polymorphic sequence (CAPS) analysis. *MspI* restriction variation was thereby detected among the sequences. The amplified products of all accessions were accordingly digested with *MspI* and electrophoresed in agarose gels to examine the restriction polymorphism.

DNA sequencing The PCR products were sequenced for phylogenetic analysis. The infrared fluorescent IRD41-labeled U31a and U31b primers were used for sequencing with a DNA sequencer model 4000L (Aloka). To avoid errors of the polymerase reaction, the PCR products were directly used as the sequencing template. For construction of a phylogenetic tree, PAUP 3.1.1 (Swofford, 1993) was used.

RESULTS

PCR amplification and CAPS analysis of the U31 region Amplification length polymorphism was detected with PCR primer set U31 (Fig. 1A). Most of the accessions produced fragments with the expected size (approximately 390bp), but several accessions showed a product with shorter size (approx. 270bp). As shown in Fig. 1A, the shorter products were detected in all three species. In addition to these two types, we detected a non-amplified type in two accessions of *Ae. umbellulata*.

CAPS analysis with *MspI* revealed restriction polymorphism within a species (Fig. 1B). The products with normal size segregated into cleaved and non-cleaved types, while all the shorter-size products were non-cleaved. Consequently, based on both the amplification length and the restriction polymorphisms, the following three alleles were distinguishable; allele I = normal size with an *MspI* site, allele II = normal size without an *MspI* site, and allele III = shorter size. The structures of the three alleles are schematized in Fig. 1C. The non-amplified type was defined as allele IV. The number and frequency of the alleles in each species are summarized in Table 1. The three alleles, I, II and III, were present in all three species, although the frequency differed with the ploidy level. For all three species, the frequency of allele III was low.

Sequence analysis of the U31 region To verify the evolutionary orthology of each allele, we sequenced the *MspI* site and the deletion region of U31 for several

accessions. In allele I, all 18 sequences determined from both the diploid (12 sequences) and the tetraploid accessions (three sequences for each) had an *MspI* site (CCGG) at the same position. Similarly, eight sequences of allele III from both the diploid (three sequences) and the tetraploids (three from *Ae. columnaris* and two from *Ae. triaristata*) contained a 123bp large deletion at the same position.

In contrast to these two alleles, allele II had variable nucleotide substitutions at the *MspI* site. Among all of the 33 accessions with allele II, four sequence types, CCAG, CTGG, CTGA and CCG-, were found at the *MspI* site. CCAG, CTGG and CTGA were detected in *Ae. umbellulata*, CCAG and CCG- in *Ae. columnaris*, and CTGG in *Ae. triaristata*. Table 1 shows the number of accessions for each sequence type. The outgroup species, *Ae. bicornis*, had allele II whose *MspI* site was CTGG.

Phylogenetic tree To reveal the evolutionary relationships among the alleles and species, we determined the

Fig. 3. Strict consensus tree of 39 minimal length trees for the U31 region. The length of the shortest trees was 180. Numbers on the branches indicate the consensus indices for each cluster. Gaps were treated as a fifth base. The tree was rooted by defining the sequence of bcr01 as an outgroup.

entire sequence of the U31 region for nine accessions of *Ae. umbellulata* and eight accessions each of *Ae. columnaris* and *Ae. triaristata*. The accessions used for sequencing are listed in Table 2. Excluding the primer sites and their flanking sequences, the alignment length was 339bp. Fig. 2 summarizes the nucleotide substitutions and Fig. 3 shows a strict consensus tree. In the tree, all eight sequences of allele III formed a distinct cluster. On the other hand, the sequences of alleles I and II were mixed with each other and dispersed in the tree showing a star-like topology. In detail, however, the three sequences of allele II with CCAG from *Ae. umbellulata* and *Ae. columnaris* (umb28, umb31 and clm07) formed a cluster. We could not find any other clusters including both diploid and tetraploid, except for a cluster of clm24 and umb21, which was not supported by a high statistical value.

DISCUSSION

The primary purpose of this study was to determine the lineage of the U genome in two UM genome tetraploid species, *Ae. columnaris* and *Ae. triaristata*. The PCR products of the U31 region demonstrated amplification length polymorphism for these two species and the U-genome diploid *Ae. umbellulata*. The size difference was caused by a 123bp large deletion for both diploid and tetraploid. This result indicated that at least two distinct genetic sources of *Ae. umbellulata* contributed to the U genome of the two tetraploids. CAPS analysis with *MspI* also revealed two restriction patterns common to the three species. Sequence analysis, however, indicated that the non-cleaved type (allele II) was generated several times by parallel mutations at the *MspI* site. Therefore, the CAPS variation does not represent an evolutionary process of the U genome. Although the outgroup species *Ae. bicornis* possessed CTGG at the *MspI* site, the entire sequence of the U31 region was much different from that of any U genome species. The plasticity of the *MspI* site implies the possibility of a parallel mutation in *Ae. bicornis*, so that we could not determine if the CTGG at the *MspI* site represents an ancestral sequence of the U genome.

In the phylogenetic tree, a distinct cluster comprising two *Ae. umbellulata* accessions and one *Ae. columnaris* accession (umb28, umb31 and clm07) was formed, which indicates the presence of a third origin of the U genome of *Ae. columnaris* (Fig. 3). A distinct cluster containing three accessions of *Ae. triaristata* (trr01, trr02 and trr37) was also found, but this cluster did not include diploid accessions. Although the three accessions of *Ae. triaristata* had CCTG at the *MspI* site, three *Ae. umbellulata* accessions possessing the same nucleotides at the *MspI* site did not show a high level of sequence similarity with them (data not shown). Those distinct sequences of *Ae.*

triaristata may have been derived from another *Ae. umbellulata* not used in this study. The expected maximum frequency of a rare allele to escape in a sample size of 72 at a 95% confidence interval is 0.041. Consequently, estimating conservatively, we conclude that the number of origins of the U genome is at least three for *Ae. columnaris*, and at least two, possibly more, for *Ae. triaristata*.

The frequency and level of variation indicated that the longer type of U31 (alleles I and II) was ancestral and was introduced into the tetraploids first via polyploidization, and that the shorter type (allele III) originated later. A question therefore arises; how was the second type introduced into each tetraploid? Three events could have been involved in the process, namely, (i) independent second polyploidization, (ii) introgression from diploid to tetraploid by interploidal hybridization, and (iii) gene flow between tetraploids. It seems possible that any of these might have occurred because evidence for each event has been reported in the genus *Aegilops* (Murai and Tsunewaki, 1986; van Slageren, 1994; Guadagnuolo et al., 2001). Furthermore, the high level of sequence similarity of allele III among the three species obscures the process responsible for the second origin. The geographical distribution, however, gives a hint. Allele III of *Ae. umbellulata* was restricted to western Turkey and Greece, whereas that of the two tetraploids was found in eastern Turkey and eastward. The lack of overlap of the distribution rules out recent and frequent introgression from diploid to tetraploid. On the other hand, the distributions of allele III for the two tetraploids overlap with each other in northern Iraq. Moreover, our unpublished AFLP data indicated that the accessions of *Ae. triaristata* possessing allele III are genetically closer to *Ae. columnaris* than the other *Ae. triaristata*. Hence, we deduced that allele III was introduced from diploid to a tetraploid at a considerably early time by a second polyploidization or interploidal introgression, and the other tetraploid gained the allele by gene flow between the tetraploids.

Recent molecular analyses have suggested that the evolutionary features of the formation of polyploids are more complex than previously thought. Regarding the *Aegilops-Triticum* group, multiple origins of polyploids have been clarified in several cases; *Ae. triuncialis* (Murai and Tsunewaki, 1986; Chee et al., 1995; Vanichanon et al., 2003), *Ae. biuncialis* (Chee et al., 1995), *T. aestivum* L. (Talbert et al., 1998; Caldwell et al., 2004), *Ae. cylindrica* Host (Caldwell et al., 2004), and *Ae. columnaris* and *Ae. triaristata* in this study. These results indicate that multiple origins are the general rule for polyploid species in these genera. Another question is how the multiple species sharing the common UM genome formed via polyploidization. Regarding this question, two proposed hypotheses have remained controversial for a long time. One hypothesis postulates the independent origin,

namely, each tetraploid species was derived through independent polyploidization (Kihara, 1954; 1963). The other is called the pivotal-differential theory, that is, the speciation of tetraploids was caused by rearrangements of the differential genomes at a tetraploid level (Zohary and Feldman, 1962). In both theories, it is considered that the speciation of tetraploids is caused by variation of the M genome while the U genome is stable and less variable. Contrary to these previous hypotheses, our present study indicated that the U genome is also variable and was introduced to each tetraploid multiple times. Further investigation of the speciation among the polyploid species will be required to elucidate the "origin of species" via polyploidization.

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REFERENCES

- Badaeva, E. D. (2002) Evaluation of phylogenetic relationships between five polyploid *Aegilops* L. species of the U-genome cluster by means of chromosome analysis. *Genetika* **38**, 799–811 (in Russian with English summary).
- Caldwell, K. S., Dvorak, J., Lagudah E. S., Akhunov, E., Luo, M. C., Wolters, P., and Powell, W. (2004) Sequence polymorphism in polyploid wheat and their D-genome diploid ancestor. *Genetics* **167**, 941–947.
- Chee, R. W., Lavin, M., and Talbert, L. E. (1995) Molecular analysis of evolutionary patterns in U genome wild wheats. *Genome* **38**, 290–297.
- Dekaprevich, L. L. (1961) The species *Triticum macha* Dek. et Men. In the light of the most recent investigations on the origin of hexaploid wheat. *Zeitschr. Pfl. Züchtung* **45**, 17–30.
- Guadagnuolo, R., Savova-Bianchi, D., and Felber, F. (2001) Gene flow from wheat (*Triticum aestivum* L.) to jointed goatgrass (*Aegilops cylindrica* Host.), as revealed by RAPD and microsatellite markers. *Theor. Appl. Genet.* **103**, 1–8.
- Kihara, H. (1954) Consideration on the evolution and distribution of *Aegilops* species based on the analyser-method. *Cytologia* **19**, 336–357.
- Kihara, H. (1963) Interspecific relationship in *Triticum* and *Aegilops*. *Seiken Zihō* **15**, 1–12.
- Kihara, H., and Tanaka, M. (1970) Addendum to the classification of the genus *Aegilops* by means of genome-analysis. *Wheat Inform. Serv.* **70**, 1–2.
- Kimber, G., and Feldman, M. (1987) Wild wheats: An introduction. Special Report No. 353, University of Missouri-Columbia, Columbia.
- Kuckock, V. (1964) Experimental investigation on the origin of wheat. *Zeitschr. Pfl. Züchtung* **51**, 97–140.
- Levin, D. A. (1980) Polyploidy and distribution. In: *Polyploidy: Biological relevance* (ed.: W. H. Lewis), pp. 45–60. Plenum, New York.
- Lilienfeld, F. A. (1951) H. Kihara: Genome-analysis in *Triticum* and *Aegilops*. X. Concluding review. *Cytologia* **16**, 101–123.
- MacKey, J. (1966) Species relationships in *Triticum*. In: *Proc. 2nd Intl. Wheat Symp.*, Lund, Sweden (ed.: J. MacKey), pp. 237–276. Berlingska Boktryckeriet Lund, Lund.
- Masterson, J. (1994) Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. *Science* **264**, 421–424.
- Murai, K., and Tsunewaki, K. (1986) Molecular basis of genetic diversity among cytoplasm of *Triticum* and *Aegilops* species. IV. CtDNA variation in *Ae. triuncialis*. *Heredity* **57**, 335–339.
- Murray, M. G., and Thompson, W. F. (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* **8**, 4321–4325.
- Osborn, T. C., Pires, J. C., Birchler, J. A., Auger, D. L., Chen, Z. J., Lee, H. S., Comai, L., Madlung, A., Doerge, R. W., Colot, V., and Martienssen, R. A. (2003) Understanding mechanisms of novel gene expression in polyploids. *Trends Genet.* **19**, 141–147.
- Resta, P., Zhang, H. B., Dubcovsky, J., and Dvorak, J. (1996) The origins of the genomes of *Triticum biunciale*, *T. ovatum*, *T. neglectum*, *T. columnare*, and *T. rectum* (Poaceae) based on variation in repeated nucleotide sequences. *Am. J. Bot.* **83**, 1556–1565.
- Sasanuma, T., Chabane, K., Endo, T. R., and Valkoun, J. (2004) Characterization of genetic variation in and phylogenetic relationships among diploid *Aegilops* species by AFLP: incongruity of chloroplast and nuclear data. *Theor. Appl. Genet.* **108**, 612–618.
- Swofford, D. L. (1993) PAUP (Phylogenetic Analysis Using Parsimony) version 3.1.1. Computer program distributed by the Illinois Natural History Survey, Champaign, Ill.
- Talbert, L. E., Smith, L. Y., and Blake, N. K. (1998) More than one origin of hexaploid wheat is indicated by sequence comparison of low-copy DNA. *Genome* **41**, 402–407.
- Vanichanon, A., Blake, N. K., Sherman, J. D., and Talbert, L. E. (2003) Multiple origins of allopolyploid *Aegilops triuncialis*. *Theor. Appl. Genet.* **106**, 804–810.
- van Slageren, M. W. (1994) Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig (Poaceae). Joint publication of ICARDA, Aleppo, Syria and Wageningen Agricultural University, The Netherlands.
- Wang, G. Z., Miyashita, N. T., and Tsunewaki, K. (1997) Plasmid analyses of *Triticum* (Wheat) and *Aegilops*: RCR-single-strand conformational polymorphism (PCR-SSCP) analyses of organellar DNAs. *Proc. Natl. Acad. Sci. USA* **94**, 14570–14577.
- Zohary, D., and Feldman, M. (1962) Hybridization between amphidiploids and the evolution of polyploids in the wheat (*Aegilops-Triticum*) group. *Evolution* **16**, 44–61.