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Wheat phylogeny determined by RFLP analysis of nuclear DNA. 3. Intra- and interspecific variations of five *Aegilops* Sitopsis species

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Abstract The level of intra- and interspecific variations on nuclear DNA in five *Aegilops* species of the Sitopsis section were investigated using restriction fragment length polymorphism (RFLP) analysis. A total of 18 accessions, i.e. 7 of *Ae. speltoides*, 3 of *Ae. longissima*, 2 of *Ae. searsii*, 3 of *Ae. sharonensis* and 3 of *Ae. bicornis*, were used. One accession each of *Triticum aestivum*, *T. durum*, *T. urartu* and *Ae. squarrosa* was included as reference material. Five enzymes and 20 probes were used. Among the five Sitopsis species studied, *Ae. speltoides* had the largest intraspecific variation ($\pi = 0.061$), which was as high as the interspecific variation observed among the other four species. The section Sitopsis was divided into two distinct groups: one containing only *Ae. speltoides* and the other, *Ae. longissima*, *Ae. searsii*, *Ae. sharonensis* and *Ae. bicornis*. This grouping by RFLP analysis is in agreement with the taxonomical classification of the subsections.

Key words Intraspecific variation · Interspecific variation · *Aegilops* Sitopsis species · RFLP analysis · Nuclear DNA

Introduction

Because of the agronomical importance of common and durum wheat (*Triticum aestivum* and *T. durum*, respectively), the phylogenetic relationship among the species of two related genera, *Triticum* and *Aegilops*, has been studied extensively (e.g. Lilienfeld 1951; Sarkar and Stebbins 1956). The genus *Triticum* is composed of four groups (Einkorn, Emmer, Timopheevi and Common wheat), and the genus *Aegilops* consists of six sections (Polyeides, Cylindropyrum, Comopyrum, Amblyopyrum, Sitopsis and Vertebrata). Many species of *Triticum* and *Aegilops* have evolved by polyploidy. For example, common wheat *T. aestivum* is the amphiploid between an Emmer wheat and *Ae. squarrosa* (Vertebrata section) (Kihara 1944; McFadden and Sears 1946), and Emmer wheat is the amphiploid between *T. urartu* (Einkorn wheat) and an *Aegilops* Sitopsis species (Sarkar and Stebbins 1956; Dvořák et al. 1990).

Molecular techniques provide new means for determining quantitative expression of phylogenetic relationships and the variation detected can be calibrated over time (Nei 1987). Their recent advent has enabled us to take this kind of approach in a phylogenetic investigation of *Triticum* and *Aegilops* species. In fact, restriction fragment length polymorphism (RFLP) analyses on nuclear (Liu et al. 1990; Takumi et al. 1993; Mori et al. 1995), chloroplast (Ogihara and Tsunewaki 1988) and mitochondrial DNA (Breiman 1987; Graur et al. 1989; Terachi et al. 1990) have revealed essentially the same phylogenetic relationships among *Triticum* and *Aegilops* species, thereby establishing their usefulness in the determination of interspecific relationships. Furthermore, intraspecific variation has been investigated for Einkorn wheat (Takumi et al 1993; Castagna et al. 1994), wild tetraploid wheats (Miyashita et al. 1994; Mori et al. 1995), common wheat (Liu et al. 1990; Siedler et al. 1994) and Sitopsis section of the genus *Aegilops* (Miyashita et al. 1994). These studies have shown that *Triticum* and *Aegilops* species have as much intraspecific variation in their nuclear and mitochondrial DNAs as other plant species, but very little in their chloroplast DNA.

The section Sitopsis has been attracting a lot of attention because this section has been suspected of containing the B genome donor to polyploidy wheats. In the last decade, molecular studies using RFLPs of chloroplast DNA (Ogihara and Tsunewaki 1988; Miyashita et al. 1994) and mitochondrial DNA (Breiman 1987; Graur et al. 1989; Terachi et al. 1990) and

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Southern blot analyses of nuclear repeated sequences (Dvořák and Zhang 1990) were conducted for this section. From these studies, three general conclusions were obtained. First, *Ae. speltoides* is distinctly separate from other Sitopsis species in the phylogenetic relationship. Second, *Ae. speltoides* shares the most similar band pattern with Common, Emmer and Timopheevi wheats among the five Sitopsis species. Third, *Ae. speltoides* has a higher intraspecific variation than other four Sitopsis species so far as organellar DNAs are concerned. However, no extensive study has been done on intraspecific variation of nuclear DNA in the section Sitopsis. In the study presented here, the intra- and interspecific variations of the nuclear DNA in five *Aegilops* Sitopsis species were investigated by RFLP analysis. The purpose of this study was to clarify the phylogenetic relationship of these *Aegilops* species based on the nuclear DNA variations.

Materials and methods

Plant materials

Seven accessions of *Aegilops speltoides*, 3 accessions each of *Ae. longissima*, *Ae. sharonensis* and *Ae. bicornis* and 2 accessions of *Ae. searsii* were used. As references, a single accession each of *Triticum aestivum* (AABBDD genome), *T. durum* (AABB), *T. urartu* (AA) and *Ae. squarossa* (DD) was also used. All of the seeds used in this study were supplied from the Plant Germplasm Institute, Faculty of Agriculture, Kyoto University, Japan. These accessions have been maintained by continuous selfing. Passport data of these species are shown in Table 1.

Total DNA extraction

Total DNA was extracted from each accession using the CTAB method (Ramser 1992). After the extracted total DNA was dissolved completely, its concentration was measured on an agar gel by comparing it with a standard DNA of known concentration.

RFLP analysis

Total DNA was digested with the following five restriction endonucleases: *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV and *Hin*dIII. The digested DNA was electrophoresed in 1% agarose gels for 20–22 h at about 20 V and then transferred to the nylon membrane Hybond-N^+ (Amersham) by alkaline blotting. After prehybridization at 68 °C in a mixture of 5 × SSC, 0.1% sodium N-lauroyl sarcosinate, 0.02% SDS and 0.5% Blocking reagent (Boehringer Mannheim), the solution was replaced with the same solution containing 5% dextran sulfate and [^{32}P]-labeled probe. A total of 20 DNA clones derived from common wheat (Liu and Tsunewaki 1991) were used as the probes (Table 2). The sizes of the probes are 1.0–4.1 kb. For DNA labeling, Random Primed DNA Labeling Kit (Boehringer Mannheim) was used. After hybridization at 67 °C for 20 h, the membrane was washed at 65 °–70 °C, first with 1 × SSC, containing 0.1% SDS, for about 20 min, and then if necessary, with 0.2 × SSC, containing 0.1% SDS, for 20–50 min. The membrane was then exposed to Fuji X-ray film, using one or two intensifying screens at −70 °C for 1–10 days.

Cluster analysis

The autoradiograms of all the informative probe-enzyme combinations were scored. For each pair of the 22 accessions, the total and shared numbers of bands were determined. From the proportion of shared bands, we calculated the genetic distance between a pair of accessions according to Nei (1987). This distance is expressed as the number of nucleotide substitutions per nucleotide site, and this was used as π_{ij}, defined as the rate of different nucleotides between two DNA arrays (Nei 1987). The distance matrix of 231 combinations of 22 accessions was used to reconstruct the phylogenetic tree. Trees were constructed using the unweighted pair-group method with arithmetic mean (UPGMA) method (Sokal and Sneath 1963) and the neighbor joining (NJ) method (Saitou and Nei 1987).

Table 1 Accessions of *Aegilops* and *Triticum* species used

Accession	Species and lower taxon		Ploidy	Genome	KU No.[a] and origin
Spe 1	*Ae. speltoides*	var *typica*	2x	SS	2207, Turkey
Spe 2	*Ae. speltoides*	var *typica*	2x	SS	5725B, Turkey
Spe 3	*Ae. speltoides*	var *typica*	2x	SS	7712, Iraq
Spe 4	*Ae. speltoides*	var *typica*	2x	SS	7776, Iraq
Spe 5	*Ae. speltoides*	var *typica*	2x	SS	7802, Iraq
Spe 6	*Ae. speltoides*	var *typica*	2x	SS	7883, Iraq
Spe 7	*Ae. aucheri* (a form of *Ae. speltoides*)	var *typica*	2x	SS	7701, Iraq
Lon 1	*Ae. longissima*	var *typica*	2x	S^lS^l	4-1, Israel
Lon 2	*Ae. longissima*	var *typica*	2x	S^lS^l	4-4, unknown
Lon 3	*Ae. longissima*	var *typica*	2x	S^lS^l	4-5, unknown
Sha 1	*Ae. sharonensis*	var *typica*	2x	S^lS^l	5-1, unknown
Sha 2	*Ae. sharonensis*	var *typica*	2x	S^lS^l	5-2, Israel
Sha 3	*Ae. sharonensis*	var *typica*	2x	S^lS^l	5-3, Israel
Sea 1	*Ae. searsii*	var *nova*	2x	S^sS^s	4-7, Syria
Sea 2	*Ae. searsii*	var *nova*	2x	S^sS^s	4-8 Israel
Bic 1	*Ae. bicornis*	var *typica*	2x	S^bS^b	3-1, unknown
Bic2	*Ae. bicornis*	var *mutica*	2x	S^bS^b	3-3, Egypt
Bic3	*Ae. bicornis*	var *typica*	2x	S^bS^b	5782, Egypt
CS	*T. aestivum*	cv Chinese Spring	6x	AABBDD	184-1, China
Dur	*T. durum*	var *reichenbachii*	4x	AABB	125, unknown
Ura	*T. urartu*	var *albonigrum*	2x	AA	199-15, Lebanon
Squ	*Ae. squarrosa*	var *typica*	2x	DD	20-3, Tashkent

[a] KU No. is the accession number of the Plant Germplasm Institute, Kyoto University (Tanaka 1983)

Table 2 Wheat genomic DNA clones used as probes. Locus and carrier chromosome are after Liu and Tsunewaki (1991). Restriction enzyme used in excision of the insert is also shown

Clone no.	Insert size (kb)	Restriction enzyme	Locus	Carrier chromosome
Tag 334	1.5	*Hin*dIII	334	6A
Tag 356	1.0	*Bam*HI, *Dra*I, *Hin*dIII	356	7B
Tag 370	1.5	*Bam*HI	370 a	2B
Tag 398	2.5	*Bam*HI	370 a	2B
Tag 431	--	*Bam*HI	431 a	2D
"		*Bam*HI	431 b	4A
Tag 483	1.3	*Bam*HI	483	1A
Tag 460	2.6	*Eco*RI	460	2A
Tag 479	1.5	*Hin*dIII	479	6A
Tag 529	3.6	*Hin*dIII	529 a	2B
"		*Eco*RV	529 b	2D
Tag 536	1.7	*Dra*I	536	7B
Tag 547	2.5	*Eco*RI	547 a	6A
"		*Eco*RI	547 b	6A
"		*Eco*RI	547 c	6B
"		*Eco*RI	547 d	6A
Tag 549	--	*Eco*RI	549 a	7B
"		*Eco*RV	549 b	1A
Tag 554	2.1	*Eco*RV	554 a	2A
"		*Eco*RV	554 b	5B
"		*Eco*RV	554 c	2B
Tag 558	2.3	*Hin*dIII	558	1D
Tag 652	3.8	*Eco*RV	652 a	1D
"		*Eco*RV	652 b	6A
Tag 687	2.4	*Bam*HI	687	2B
Tag 750	3.7	*Dra*I	750	7B
Tag 754	3.6	*Bam*HI	754	7B
Tag 762	4.1	*Bam*HI	762	6A
Tag 764	2.6	*Hin*dIII	764	1B

Results

Restriction fragment length polymorphism (RFLP)

Of 100 probe-enzyme combinations tested, 90 produced readable autoradiograms, all of which showed RFLPs. Examples of those autoradiogram are shown in Fig. 1. Generally, as in these two autoradiograms, the band patterns of *Ae. speltoides* accessions are quite different from each other, while those of the accessions of other four Sitopsis species are similar to each other.

In total, 1408 different fragments were found. The total number of bands scored in each accession and the proportion of shared bands between each pair of the accessions are summarized in Table 3. *T. aestivum* and *T. durum* have larger numbers of bands than any other species because of their higher ploidy. *Ae. speltoides* has a high level of intraspecific variation, relative to the other four Sitopsis species, as indicated by the lower proportions of shared bands. On average, *Ae. speltoides* shared only 36% of its bands between a pair of accessions. On the other hand, *Ae. longissima*, *Ae. searsii*, *Ae. sharonensis* and *Ae. bicornis* showed a 64%, 89%, 62% and 78% similarity, respectively, between the accessions in each species.

The genetic distance calculated between each pair of accessions is also shown in Table 3. The average of pairwise π_{ij} between accessions for each Sitopsis species is shown in Table 4. This value is a measure of intraspecific variation, defined as the nucleotide diversity (π) (Nei 1987). *Ae. speltoides* showed the highest level of intraspecific variation (0.061); it is two- to nine-fold larger than those of the other four species.

The net distances between all pairs of the five Sitopsis species as estimated by the formula, $\text{net}(A\text{–}B) = d_{AB} - (d_A + d_B)/2$, are shown in Table 5. Here, d_{AB} is the average distance between all pairs of the accessions of species A and species B, and d_A and d_B are the average distances between the accessions of species A and between those of species B, respectively (Nei 1987). The longest distance was between *Ae. speltoides* and *Ae. searsii* (0.100), and the shortest was between *Ae. longissima* and *Ae. sharonensis* (0.007).

The distances between each of the five Sitopsis species and *T. aestivum* (CS) or *T. durum* (Dur) are shown in Table 6. These distances were calculated by the formula $d_{(A-B)} = d_{(A-B)} - d_A/2$, where A is the Sitopsis species, B is CS or Dur, $d_{(A-B)}$ is the average distance between species A and B and d_A is the average distance within species A. While this estimate does not correspond to the exact net distance because d_B, representing the intraspecific variation in CS or Dur, is neglected, it can be

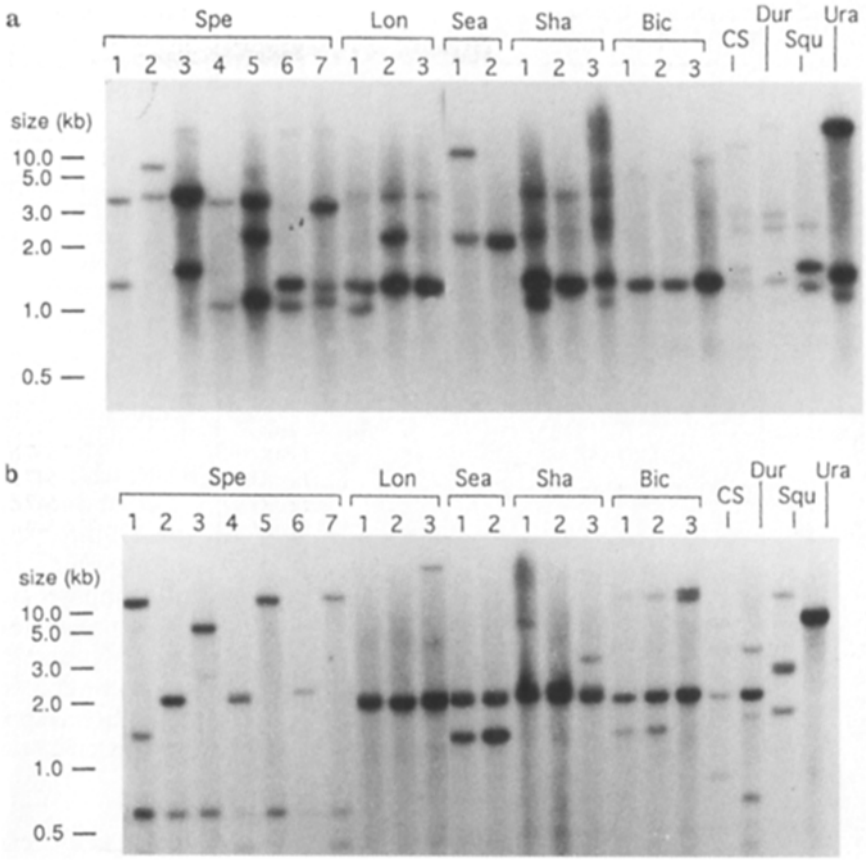

Fig. 1a, b Autoradiograms showing RFLPs among the 22 accessions of the *Aegilops* and *Triticum* species studied. The probe-enzyme combinations shown were **a** Tag 334-*Hin*dIII and **b** Tag 554-*Dra*I

regarded as a reasonable parameter of the net distance if the fact that cultivated species generally have a very small amount of intraspecific variation compared to their wild relatives is taken into consideration. The species which had the smallest distance to both *T. aestivum* and *T. durum* was *Ae. speltoides*.

Cluster analysis

On the basis of genetic distances, two trees were constructed using the UPGMA method (Sokal and Sneath 1963) and neighbor joining method (Saitou and Nei 1987); these two trees are very similar to each other (Figs. 2 and 3). The 22 accessions analyzed were divided into three main clusters. One cluster was comprised of the 7 *Ae. speltoides* accessions (group 1 in Figs. 2 and 3), the second one, of the accessions of other Sitopsis species (group 2) and the third one, of the 3 *Triticum* accessions and *Ae. squarrosa* accession (group 3).

In both trees, each Sitopsis species in group 2 formed a different second-order cluster from the others. The branch length between *Ae. longissima* and *Ae. sharonensis* was the shortest of all those between different species. The branches between the 7 accessions of *Ae. speltoides* were much longer than those between the accessions of each of the other four Sitopsis species.

Discussion

Intraspecific variation

In this study *Ae. speltoides* was revealed to have a much higher level of intraspecific variation than the other Sitopsis species. The level of the former was as high as those of interspecific variation among the other four species (see Figs. 2 and 3 and Tables 4 and 5). Nucleotide diversity (π) for *Ae. speltoides* (0.061) was 2–9 times larger than those of the other species (range was 0.007–0.028) (Table 4) and was almost the same as the genetic distances between *Triticum* species of different ploidies. In Einkorn wheats (Takumi et al. 1993) and wild tetraploid wheats (Mori et al. 1995), the value of π were estimated using essentially the same method to the one used here. According to these two studies, the average genetic distance (π) within species was 0.026 in *T. boeoticum* (2 accessions), 0.013 in *T. urartu* (2 accessions), 0.006 in *T. monococcum* (3 accessions), 0.014 in *T. dicoccoides* (32 accessions) and 0.004 in *T. araraticum* (24 accessions). The level of polymorphism that we observed in *Ae. speltoides* was apparently higher than those previously reported in *Triticum* species. The other four Sitopsis species had almost the same levels of intraspecific variations as the *Triticum* species. There are two expla-

Table 3 The percentages of shared bands (above the diagonal) and the genetic distances ($\times 10^3$, below) between all pairs of the 22 accessions. Genetic distance was calculated according to Nei (1987). Total number of the bands observed in each accession is shown on a diagonal (underlined)

Accession	Spe 1	Spe 2	Spe 3	Spe 4	Spe 5	Spe 6	Spe 7	Lon 1	Lon 2	Lon 3	Sea 1	Sea 2	Sha 1	Sha 2	Sha 3	Bic 1	Bic 2	Bic 3	CS	Dur	Squ	Ura
Spe 1	245	32	41	33	39	31	34	13	12	13	10	12	13	14	11	12	14	13	17	18	15	15
Spe 2	68	239	38	36	33	27	30	12	16	14	13	13	16	17	16	18	17	18	16	16	15	11
Spe 3	52	58	252	40	42	33	37	15	16	15	14	16	18	15	16	17	16	17	19	19	19	13
Spe 4	67	61	54	195	40	38	35	12	13	13	12	13	14	15	14	13	13	13	18	18	16	18
Spe 5	55	66	52	54	231	38	49	16	17	18	11	11	14	14	12	12	13	14	21	17	16	20
Spe 6	70	79	66	57	57	204	38	16	12	15	13	11	15	17	15	14	16	15	21	19	13	17
Spe 7	64	72	60	62	42	58	208	16	16	15	11	11	15	13	14	16	15	15	20	16	14	17
Lon 1	131	137	117	136	116	114	114	229	62	63	22	24	60	50	46	36	37	36	16	17	16	12
Lon 2	134	116	117	131	109	132	114	27	258	67	24	26	59	56	52	34	37	36	19	20	18	20
Lon 3	128	124	118	128	107	119	121	27	23	260	23	24	56	54	48	33	34	34	18	18	17	16
Sea 1	145	130	125	137	143	130	142	94	88	89	202	89	22	23	22	26	27	25	16	15	15	16
Sea 2	133	127	115	130	139	140	141	86	82	88	7	212	26	24	24	26	27	27	17	15	16	15
Sha 1	131	113	107	124	125	117	118	29	30	34	92	82	265	62	64	32	36	35	19	16	17	16
Sha 2	124	109	119	118	122	110	129	41	34	36	90	87	28	254	68	32	36	32	17	17	16	15
Sha 3	142	114	115	124	135	121	122	46	37	43	93	88	26	22	223	30	32	31	17	16	14	15
Bic 1	134	107	112	127	132	121	114	61	64	66	81	81	68	69	73	252	81	75	18	18	19	15
Bic 2	126	109	114	130	126	114	121	59	59	64	79	79	60	61	69	12	258	77	17	19	17	16
Bic 3	130	108	111	127	126	118	121	61	60	65	83	81	64	69	71	16	15	284	19	19	16	15
CS	109	113	102	107	95	95	101	115	102	107	113	110	103	109	109	108	110	102	311	60	35	32
Dur	106	114	101	106	111	103	113	112	101	105	117	117	114	111	115	106	102	101	29	292	19	43
Squ	118	117	102	114	114	126	125	114	108	109	119	115	110	114	123	104	111	114	64	103	227	17
Ura	121	141	128	107	101	108	109	131	99	115	114	118	116	118	119	120	117	118	68	49	108	219

Table 4 The average genetic distance between accessions (= nucleotide diversity) of each Sitopsis species

Species	Number of accession pairs	Average genetic distance
Ae. speltoides	21	0.061
Ae. longissima	3	0.026
Ae. searsii	1	0.007
Ae. sharonensis	3	0.028
Ae. bicornis	3	0.014

nations possible for the high level of intraspecific variation in *Ae. speltoides*. One is the difference in breeding system, i.e. *Ae. speltoides* undergoes outbreeding whereas other Sitopsis and *Triticum* species undergo inbreeding. Outbreeders are expected to have a higher level of intraspecific variation than inbreeders. The present result supports this expectation. Another possibility is a difference in the time of their origins. *Ae. speltoides* might be older than other species. Further investigations are necessary to determine which possibility, or both, are responsible for the observed differences in intraspecific nucleotide diversity.

Interspecific relationship

The five Sitopsis species were divided into two distinct groups (group 1 and group 2 in Figs. 2 and 3). This agrees with the classification of Sitopsis into two subsections, Truncata (*Ae. speltoides* alone) and Emarginata (*Ae. longissima*, *Ae. searsii*, *Ae. sharonensis* and *Ae. bicornis*) (Eig 1929). Among the four species of the subsection Emarginata, *Ae. searsii* is very much different from the other three species. This species has been established recently based on geographical distribution and possession of an extra satellited chromosome pair (Feldman 1978). Its morphological characteristics are very similar to those of *Ae. longissima*. Contrary to a morphological similarity to *Ae. longissima*, *Ae. searsii* is more distant from the former than is *Ae. sharonensis* with respect to the nuclear RFLP. The net distance between *Ae. longissima* and *Ae. sharonensis* was the shortest (0.007) of all those observed between the five Sitopsis species.

The trees shown in Figs. 2 and 3 agree well with that obtained from wheat chloroplast (ct) DNA study (Ogihara and Tsunewaki 1988). In the classification based on ctDNA, the Sitopsis species were also divided into two distinct groups, one containing *Ae. speltoides* and the other consisting of three species of subsection Emarginata (*Ae. longissima* was not studied). The similarity between the phylogenetic relationships based on the RFLPs of the nuclear and chloroplast DNAs suggests that the variations in nuclear and organellar DNAs in the species of the Sitopsis section occur along parallel lines.

Table 5 The net distances between five Sitopsis species

	Ae. speltoides	*Ae. longissima*	*Ae. searsii*	*Ae. sharonensis*
Ae. longissima	0.079			
Ae. searsii	0.100	0.072		
Ae. sharonensis	0.075	0.007	0.071	
Ae. bicornis	0.083	0.042	0.071	0.044

Table 6 The approximate net distances between the Sitopsis species and *T. aestivum* or *T. durum*

	T. aestivum	*T. durum*
Ae. speltoides	0.073	0.078
Ae. longissima	0.095	0.093
Ae. searsii	0.109	0.114
Ae. sharonensis	0.092	0.099
Ae. bicornis	0.100	0.096

If the rate of nucleotide substitution per site per year between two DNA sequences (λ) is available, the time of divergence of the two DNA sequences (T) can be estimated by the formula, $d = 2\lambda T$ (Nei 1987). Wolfe et al. (1987) estimated an average synonymous substitution rate in plant nuclear genes as $0.5–3 \times 10^{-8}$ per site per year. By taking this value as λ and the net distance between the species (Table 5) as d, we can estimate the time of divergence. The time of divergence between the two Sitopsis subsections is estimated to be $1.4–8.4 \times 10^6$ years, and the time of interspecific divergence among the four Emarginata species, $1.2–7.0 \times 10^6$ years. These estimates indicate that divergence among the Emarginata species may have occurred at almost the same time as that of the subsectional divergence. This result may suggest that *Ae. speltoides* and other four Sitopsis species were established at almost same time.

Relationship between Sitopsis species and Emmer and Common wheats

The average distances between the five Stopsis species and *T. aestivum* and *T. durum* are shown in Table 6. Common wheat is hexaploid with three different genomes, A, B and D. Its A and B genomes are derived from Emmer wheat and the D genome is from *Ae. squarrosa* (Kihara 1944; McFadden and Sears 1946). The A genome of Emmer wheat is from *T. urartu* (Dvořák et al. 1990; Takumi et al. 1993). Although there are diverse opinions as to what was the B genome donor to polyploid wheats, *Ae. speltoides* is the most possible candidate (Sarkar and Stebbins 1956; Tsunewaki 1989; Dvořák and Zhang 1990). If so, *Ae. speltoides* should have as short distance as *T. urartu* to Emmer and Common wheats. In fact, the net genetic distance between *Ae. speltoides* and Emmer wheat (0.078) was similar to that between *T. urartu* and Emmer wheat (0.043). Similarly, the net genetic distance between *Ae. speltoides* and Common wheat (0.073) was almost the same as that between *T. urartu* and Common wheat (0.068). Among the Sitopsis species *Ae. speltoides* had the shortest distance to both Emmer and Common wheats. From all those results, it is concluded that *Ae.*

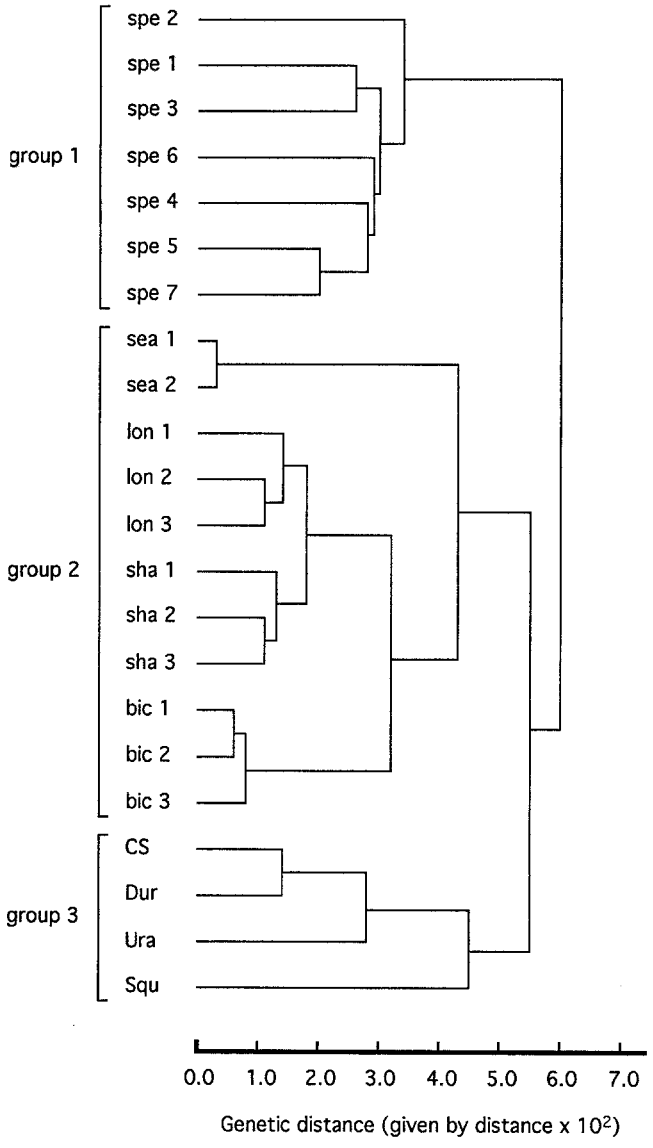

Fig. 2 Dendrogram showing phylogenetic relationships among the 22 accessions of the *Aegilops* and *Triticum* species, constructed using the UPGMA method from the genetic distances given in Table 3. Accession names are shown in Table 1

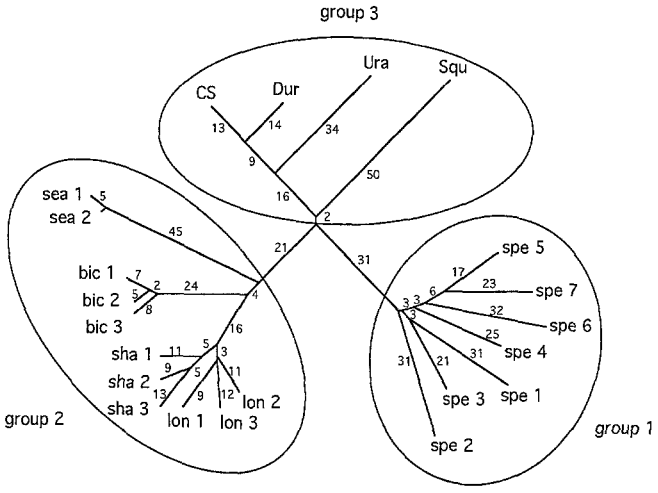

Fig. 3 Phylogenetic network of the 22 accessions, constructed using the NJ method. The *number* indicates the branch length expressed in the number of substitutions per nucleotide ($\times 10^3$). The three groups are shown with different *circles*

speltoides is most probable B genome donor to Emmer and, consequently, to Common wheat.

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