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THE DIVERSITY OF CHLOROPLAST DNA AMONG *TRITICUM* AND *AEGILOPS* SPECIES[1]

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SUMMARY

Chloroplast(ct) DNAs were purified from 35 self-fertile alloplasmic lines of *Triticum* (wheat) or *Aegilops* and six euplasmic lines of common wheats. Fragment patterns of these ctDNAs produced by digestion with eight restriction endonucleases were analyzed by agarose gel electrophoresis. Chloroplast genomes from 41 lines could be classified into 16 types, in total. The above classification of ctDNAs was principally in agreement with that of the plasma types classified from the phenotypic effects based on nucleus-cytoplasm interactions. Most polyploids and their related diploids showed identical restriction patterns indicating the conservatism of the chloroplast genome during speciation. Based on the number of restriction fragments differing between all pairs of plasma types, the phylogenetic relationship of 16 plasma types was determined as shown in Fig. 1. Cytoplasms of A, S^b, S^v, S^1, and D^2 appeared to form a center of evolutionary diversification of the chloroplast genome. The maternal lineage of most polyploid species were deduced from the restriction patterns of ctDNA. For example, the chloroplast genomes of Emmer and Dinkel wheats, and Timopheevi wheats were assumed to have been derived from *Ae. longissima* and *Ae. aucheri*, respectively.

INTRODUCTION

As in most other plant taxa, chloroplast DNA of wheat is a single circular molecule of about 135 kilobase pairs(kb) (Bowman *et al.* 1981) having 21 kb inverted repeat regions in it. In addition, this basic structure of chloroplast genome is well conserved among widely divergent plants (Palmer and Thompson 1981). These facts enable us to carry out restriction fragment analysis of ctDNAs using six base recognition endonucleases which has become a new powerful means for studying phylogenetic relationship among related plant species (Kung *et al.* 1982, and others).

We are fortunate to have alloplasmic lines as well as euplasmic lines in which almost all cytoplasms of *Triticum* and *Aegilops* complex are involved. Using these lines, we carried out restriction fragment analysis of ctDNA with eight restriction enzymes. The results are reported here.

MATERIALS AND METHODS

Intact chloroplasts were isolated from leaves of 35 self-fertile alloplasmic as well as of

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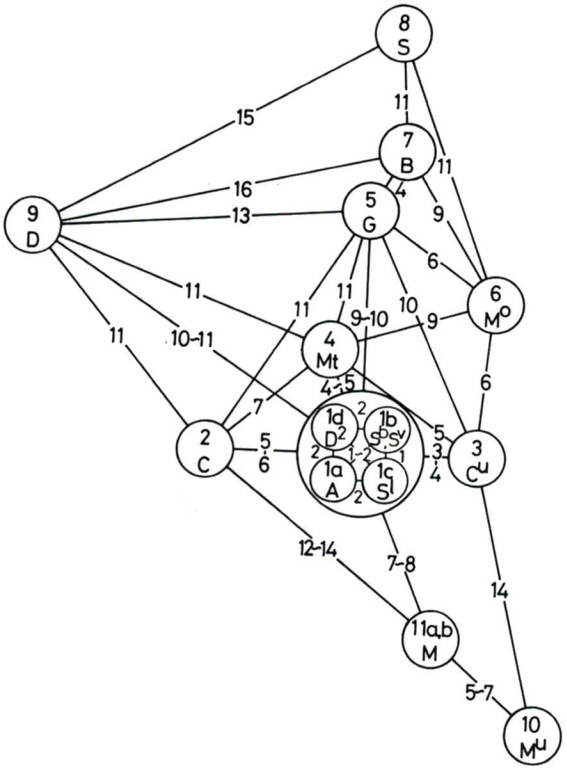

Fig. 1. Relatedness among 11 chloroplast genomes based on their restriction fragment pattern differences.

six euplasmic lines of wheats, including *T. urartu* and *Ae. searsii*, in which the cytoplasms of practically all species of the two related genera were involved. Chloroplasts were purified from crude preparations using the discontinuous Percoll gradient method (Ogihara and Tsunewaki 1982). CtDNAs were extracted according to the method of Kolodner and Tewari (1975). Digestion of ctDNA with *Bam*HI, *Eco*RI, *Hin*dIII, *Kpn*I, *Pst*I, *Sal*I, *Sma*I and *Xho*I was carried out, according to the directions given by their supplier, Takara Shuzo Co. Ltd. The DNA fragments were separated by electrophoresis, using 0.8 or 1.2% agarose slab gel in 40 mM Tris, 20 mM NaOAc and 2 mM EDTA.

RESULTS

Chloroplast genome variations found in 41 wheat-*Aegilops* lines are summarized in Tables 1 and 2. *Sal*I produced 12 fragments, their total size being 135.2 kb as reported by Bowman *et al.* (1981). Three chloroplast genome types were distinguished by this enzyme: One was the standard type, which was found in 35 cytoplasms. The two others had deletions in different fragments; one had a 0.2 kb fragment deletion and was found in three cytoplasms, 04, 28 and 36 (the code numbers of the cytoplasms are given in Table 3). The other type had the deletion of a *Sal*I site between the 6.1 and 1.2 kb fragments which were replaced

Table 1. Chloroplast genome variations found in 41 cytoplasms, using five restriction endonucleases.

Enzyme	No. bands compared	Changed fragments(kb)		Cytoplasms*
		Lost	Gained	
*Sal*I	12	0.23	—	04, 28, 36
		6.1+1.2	7.1	05, 06, 07
*Pst*I	12	0.2	—	11, 21, 22, 52 a, b, c
		0.5	—	06, 07
		0.5, 0.1	—	05
		5.2+1.4	6.8	08
*Eco*RI	13	0.7	—	08, 09, 11, 21, 22, 23, 25, 31, 51, 52 a, b, c
		2.6	—	04, 28, 36
		0.5	—	05, 06, 07
*Bam*HI	21	—	0.9	01, 06, 10, 12, 33, 34, 35, 53, 55, 56, *T. urartu*, *Ae. searsii*
		—	0.6, 2.3	02, 27, 38
		—	0.9	03, 26, 29, 30, 32, 37, 54, 57
		7.0+3.0	10.2	03, 26, 29, 30, 32, 37, 54, 57
		0.2, 3.0	—	13
		—	0.6	13
		7.0+3.0	10.2	31
		0.3	—	11, 21, 22, 52 a, b, c
		0.5	—	08
		0.2, 0.15	—	04, 28, 36
		0.4	—	07
		—	0.9	07
		—	0.9, 2.7	05
*Kpn*I	11	0.2	—	05, 06, 09, 11, 21, 22, 23, 25, 31, 51, 52 a, b, c
		0.3	—	04, 08, 28, 36
		0.4	—	07

* Refer to Table 3 for the cytoplasm code.

by a new fragment of 7.1 kb in the three cytoplasms, 05, 06 and 07. The description of the results obtained with other enzymes is given in a tabular form in Tables 1 and 2.

Based on these restriction fragment patterns of ctDNAs, chloroplast genomes of 41 lines of *Triticum* and *Aegilops* species could be classified into 16 types as shown in Table 3.

DISCUSSION

On variation of the chloroplast genomes in Triticum *and* Aegilops: Of the 165 restriction fragments scored in total, 49 mutational events were revealed (Tables 1 and 2). Of these events, only five resulted from base substitution in the endonuclease recognition sites. All other

Table 2. Chloroplast genome variations found in 41 cytoplasms, using three restriction endonucleases.

Enzyme	No. bands compared	Changed fragments(kb)		Cytoplasms*
		Lost	Gained	
*Hind*III	19	1.3	—	35, 53, 55, 56
		0.3	—	02, 27, 38
		1.1, 0.1	—	09, 23, 25, 51
		1.1	—	31
		1.1, 0.1, 0.2	—	11, 21, 22, 52 a, b, c
		1.1, 0.1, 0.4	—	08
		1.2, 0.2	—	04, 28, 36
		0.4, 0.4	—	07
		0.4	—	05, 06
*Sma*I	17	1.7	—	01, *T. urartu*
		0.3	—	02, 27, 38
		20.8	14.7+5.8	03, 13, 26, 29, 30, 32, 37, 54, 57
		—	0.3	09, 23, 25, 51
		7.3+5.0	11.5	09, 23, 25, 51
		5.0	2.9+2.2	09, 23, 25, 51
		20.8	14.7+5.8	31
		0.8	—	31
		—	0.3, 0.1	11, 21, 22, 52 a, b, c
		7.3+5.0	11.5	11, 21, 22, 52 a, b, c
		7.3+5.0	11.3	08
		5.0	2.9+2.2	08
		1.1	—	04, 28, 36
		0.1	—	05, 06
		0.1, 0.5	—	07
*Xho*I	14	0.4	—	10
		0.2	—	02, 27, 38
		13.1+12.6	25.8	03, 26, 29, 30, 32, 37, 54, 57
		1.3	—	09, 11, 21, 22, 23, 25, 31, 51, 52 a, b, c
		1.3, 0.4	—	08
		1.3, 0.2	—	04, 36
		1.3, 0.2	—	28
		13.1	10.6+2.6	28
		0.3, 0.4	—	07
		0.3	—	05, 06

* Refer to Table 3 for the cytoplasm code.

restriction fragment changes can be attributed to size variation caused by deletion or insertion. Although we have not yet determined the precise location of those mutations on the physical map, except for the *Pst*I and *Sal*I sites (Bowman *et al.* 1981), chloroplast genome

Table 3. Classification of chloroplast genomes of 41 lines of *Triticum* and *Aegilops* based on their restriction fragment patterns.

Chloroplast genome type	Plasma type*	Carrier		
		Diploid	Tetraploid	Hexaploid
1a	A	*T. monococcum* (01) *T. urartu*	—	—
1b	S^b, S^v	*Ae. bicornis* (12) *Ae. searsii*	*Ae. kotschyi* (33) *Ae. variabilis* (34)	
1c	S^l	*Ae. sharonensis* (10)	—	—
1d	D^2	—	*Ae. crassa* 4x (35)	*Ae. juvenalis* (53) *Ae. crassa* 6x (55) *Ae. vavilovii* (56)
2	C	*Ae. caudata* (02)	*Ae. triuncialis* (38) Syn. *triuncialis* (27)	—
3	C^u	*Ae. umbellulata* (03)	*Ae. triuncialis* (26) *Ae. biuncialis* (29, 37) *Ae. columnaris* (30) *Ae. triaristata* 4x (32)	*Ae. triaristata* 6x (54, 57)
4	Mt	*Ae. mutica* (13)	—	—
5	G	*Ae. aucheri* (09)	*T. dic'des nudigl.* (23) *T. timopheevi* (25)	*T. zhukovskyi* (51)
6	M^o	—	*Ae. ovata* (31)	—
7	B	*Ae. longissima* (11)	*T. dic'des spont.* (21) *T. dicoccum* (22)	*T. aestivum* (52a, b) *T. spelta* (52c)
8	S	*Ae. speltoides* (08)	—	—
9a	D	*Ae. squarrosa* (04)	*Ae. ventricosa* (36)	—
9b	D	—	*Ae. cylindrica* (28)	—
10	M^u	*Ae. uniaristata* (07)	—	—
11a	M	*Ae. comosa* (05)	—	—
11b	M	*Ae. heldreichii* (06)	—	—

*) After Tsunewaki (1980).

**) Parenthesis gives the code number of each cytoplasm.

evolution in *Triticum* and *Aegilops* species is characterized by deletion-insertions as the major event, contrasting with base substitution events in *Lycopersicon* and *Solanum* complex (Palmer and Zamir 1982) and *Nicotiana* (Kung *et al.* 1982).

Evolutionary conservatism of chloroplast genomes is strongly supported by the comparison of ctDNAs between diploids and their related polyploid species, which showed, in most cases, identical restriction fragment patterns, except for the chloroplast genomes of *Ae. ovata* and *Ae. crassa* (and *Ae. juvenalis* and *Ae. vavilovii*) for which we can not find any diploids having identical chloroplast genomes. This fact indicates that most chloroplast genomes have differentiated at the diploid level, and that they have not changed appreciably after polyploidization.

Phylogenetic relationship among 16 chloroplast genome types: Tsunewaki (1980) classified 75 cytoplasms of 33 *Triticum* and *Aegilops* species into 12 plasma types on the basis of their biological effects on common wheat characters. Chloroplast genome types which are classifi-

ed by the restriction fragment patterns are principally in agreement with his plasma types, as shown in Table 3.

The number of restriction fragment changes by which every pair of chloroplast genome types differ with each other was calculated, which is assumed to indicate the degree of genetic relativity between the two cytoplasms. Based on this number, the phylogenetic relationship of 12 plasma types was determined as shown in Fig. 1. The chloroplast genomes of five plasma types A, S^b, S^v, S^l and D^2 are closely related with each other. This fact may indicate that they are located in the center of the evolutionary diversification of the chloroplast genome in the two genera.

On the origin of the cytoplasm of individual polyploid species: We were able to find diploid species, the chloroplast genome of which was identical to that of individual polyploid species with a few exceptions (Table 3). The ctDNA of only *Ae. longissima* showed a pattern identical to those of Emmer and common wheats. CtDNAs of *Ae. aucheri* and an *Ae. speltoides* strain were identical to those of the Timopheevi wheat group. This clearly indicates that the cytoplasm donor and consequently a nucleus donor to Emmer and common wheats and to Timopheevi wheats were *Ae. longissima* and *Ae. aucheri* (or some form of *Ae. speltoides*), respectively. CtDNAs of *T. urartu* and *Ae. searsii* were identical with those of *T. monococcum* and *Ae. bicornis*, greatly differing from those of Emmer-common wheats. This verifies that these species did not donate their cytoplasms to Emmer-common wheats. *Ae. speltoides*, *Ae. bicornis*, *Ae. searsii*, *Ae. sharonensis* or *T. urartu* has been suggested as the donors of the B ge-no me to Emmer-common wheats by different workers. The fact that *Ae. longissima* and Emmer-common wheats have identical chloroplast genomes, while they have no homologous nuclear genome, may be due to different rates of evolutionary changes between the nuclear and chloroplast genomes.

Several problems remain; for example, the diploid species having a chloroplast genome identical to that of the *Ae. crassa-juvenalis-vavilovii* group or *Ae. ovata* must be sought. The *Ae. kotschyi-variabilis* group showed a ctDNA identical pattern with *Ae. bicornis*, although their cytoplasms differ in their biological effects. The ctDNA of *Ae. cylindrica* gains an additional *Xho*I site, compared to that of *Ae. squarrosa* and *Ae. ventricosa*: This may be the first example of ctDNA mutation occurring at the polyploid level in the *Triticum-Aegilops* complex.

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