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Phylogenetic relationships of turfgrasses as revealed by restriction fragment analysis of chloroplast DNA

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Abstract. Chloroplast DNAs (cpDNAs) were analyzed in order to clarify the phylogenetic relationships among turfgrasses. Physical maps of cpDNAs from *Agrostis stolonifera* and *Zoysia japonica*, which are representative species of cool (C3 type) and warm (C4 type) season turfgrasses, respectively, were constructed with four restriction enzymes, i.e., *Pst*I, *Sal*I, *Sac*I, and *Xho*I. The genome structures of these cpDNAs were found to be similar to each other in terms of genome size and gene orders, showing thereby a similarity to other grass cpDNAs. CpDNAs of 5 species of cool season turfgrasses and 6 species of warm season turfgrasses as well as four species of cereals, distributed among 14 genera of Gramineae, were digested with *Pst*I, *Xho*I, and *Bam*HI, and their restriction fragment patterns were compared. Their genome sizes were estimated to be 135–140 kbp. Each species showed characteristic RFLP patterns. On the basis of the frequency of commonly shared fragments, a dendrogram showing the phylogenetic relationships among their cpDNAs was constructed. This dendrogram shows that turfgrasses can be divided into three major groups; these correspond to the subfamilies. Cool and warm season turfgrasses are clearly distinguishable from each other, and the latter can be further classified into two subgroups that correspond to Eragrostoideae and Panicoideae. Our classification of turfgrasses and cereals by RFLP analysis of cpDNA agreed in principal with their conventional taxonomy, except for the location of *Festuca* and *Lolium*.

Key words: Turfgrass – Chloroplast DNA – Physical map – RFLP analysis – Phylogenetic relationships

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Introduction

Turfgrasses are classified into more than 30 species belonging to 20 genera and three subfamilies of Gramineae (Gould and Shaw 1983). On the basis of their ecological habitats and agronomical aspects, they are divided into two major groups: cool and warm season turfgrasses. As for physiological aspects, cool season turfgrasses have the C3 photosynthetic system, whereas warm season ones have the C4 system (Turgeon 1985).

Although turfgrasses have been subjected to conventional systematic studies, the number of genetic studies carried out on interspecific relationships, have been limited because of restrictions in crosses and the low number of available genetic markers (Weeden and Emmo 1985; Pitman et al. 1987).

The restriction fragment length polymorphism (RFLP) analysis of chloroplast DNAs (cpDNAs) has become a powerful tool for investigating phylogenetic relationships among plant species (Palmer et al. 1988). CpDNA analysis has been successfully applied to clarify interspecific relationships in *Nicotiana*, *Brassica*, *Triticum* and *Aegilops*, *Oryza*, and other species (reviewed by Palmer et al. 1988). In some cases, RFLP analysis of cpDNA is also applicable for the investigation of intergeneric relationships (Palmer et al. 1988). This method is especially useful for plants having a long life span and vegetative propagation habit (e.g., Strauss et al. 1988).

The study presented here was aimed at the evaluation of genetic diversity and phylogenetic relationships among turfgrasses by means of cpDNA analysis. Physical maps of cpDNAs from *Agrostis stolonifera* (cool season turfgrass) and *Zoysia japonica* (warm season turfgrass) were constructed to assess the basic structure of chloroplast genomes in turfgrasses. With reference to the

physical maps, the RFLP data were analyzed in order to estimate phylogenetic relationships among turfgrass species; this information could be utilized in breeding programs.

Materials and methods

Plant materials

The turfgrass strains used for cpDNA isolation included 5 species of cool season turfgrasses and 6 species of warm season ones (Table 1). Four cereals, namely, wheat, barley, maize, and rice, were used as controls. Their taxonomic classifications (Gould and Shaw 1983) are also described in Table 1.

Chloroplast DNA extraction

Intact chloroplasts were isolated from fresh leaves of plants grown in the greenhouse by the discontinuous sucrose gradient method. CpDNAs were extracted from the purified chloroplasts (Ogihara and Tsunewaki 1988).

Physical map construction of turfgrass cpDNAs

The cpDNAs of *A. stolonifera* and *Z. japonica* were digested with *Pst*I, *Sal*I, *Sac*I, and *Xho*I separately or with *Pst*I in combination with *Sal*I, *Sac*I, and *Xho*I as described in the manufacturer's instructions (Takara Shuzo Co). The digested DNAs were transferred onto a Hybond N. nylon membrane (Amersham) by the alkali-method (Reed and Mann 1985) after fractionation by 0.8% agarose gel electrophoresis in TAE buffer (Maniatis et al. 1982). Restriction sites of the two cpDNAs were first estimated by restriction fragment orders and subsequently, confirmed by heterologous hybridizations with wheat cpDNA clones, namely, P3, P4, P5, P6, P7, P9, P10, S3a, 166, 249, and B2 (Ogihara and Tsunewaki 1988), which cover the entire chloroplast genome of wheat. The locations of seven chloroplast genes (see top of Fig. 2) on the physical maps of turfgrass cpDNAs were determined. DNA fragments of tobacco cpDNAs corresponding to each gene were extracted from agarose gel (Vogelstein and Gillespie 1979) after digestion with the appropriate restriction enzymes (Shinozaki et al. 1986).

The procedures used for the labeling of the probe DNAs, Southern hybridization, and the detection of the hybridized signals have been described previously (Katayama et al. 1991).

Restriction fragment analysis of chloroplast DNA in turfgrasses

CpDNAs of 15 species were digested with three restriction endonucleases, *Pst*I, *Xho*I, and *Bam*HI, and fractionated by 0.8% agarose gel electrophoresis. The sizes of the digested fragments were estimated by comparison with commercial size markers. The genome size of cpDNA in turfgrass species was calculated by summing up the number of restriction fragments.

The genetic similarity of chloroplast genome types between a given paired species was estimated on the basis of the frequency of commonly shared restriction fragments. A dendrogram was then constructed according to the unweighted pair grouping (UPG) method (Sneath and Sokal 1973).

Results

Chloroplast genome size of turfgrass species

The sizes of the chloroplast genomes of the turfgrass species listed in Table 1 were estimated from restriction fragment patterns. The molecular sizes and copy number of individual restriction fragments of turfgrasses and cereal crops obtained by *Pst*I digestion are presented in Table 2. Since similar data on the molecular sizes of turfgrass cpDNAs were obtained with other restriction enzymes (data not shown), the genome sizes of turfgrass cpDNAs were inferred to be 135-140 kbp. The sizes of the chloroplast genomes of *Er. ophiuroides*, *P. notatum*, and *Z. mays*, all of which belong to the subfamily Panicoideae, were larger than those of the other subfamilies (Table 2).

Table 1. Strains of turfgrasses and cereal crops used in the experiment, and their taxonomic classifications[a]

Species	Common name	Strain	Tribe	Subfamily	Code
Cool season turfgrass					
Agrostis stolonifera	Bentgrass	Penncross	Agrosteae	Pooideae	1
Lolium multiflorum	Italian ryegrass	Common	Festuceae	Pooideae	2
Festuca arundinacea	Tall fescue	Falcon	Festuceae	Pooideae	3
Festuca rubra	Red fescue	Boreal	Festuceae	Pooideae	4
Poa pratensis	Kentucky bluegrass	Ram I	Festuceae	Pooideae	5
Warm season turfgrass					
Cynodon dactylon	Bermudagrass	U-3	Chlorideae	Eragrostoideae	6
Chloris gayana	Rhodesgrass	Catanbora	Chlorideae	Eragrostoideae	7
Eragrostis curvula	Weeping lovegrass	Common	Eragrosteae	Eragrostoideae	8
Zoysia japonica	Zoysiagrass	Common	Zoysieae	Eragrostoideae	9
Eremochloa ophiuroides	Centipedgrass	Common	Andropogoneae	Panicoideae	10
Paspalum notatum	Bahiagrass	Common	Paniceae	Panicoideae	11
Cereal crop					
Triticum aestivum	Common wheat	Chinese Spring	Triticeae	Pooideae	12
Hordeum vulgare	Barley	Jinriki	Triticeae	Pooideae	13
Zea mays	Maize	Jupitor	Maydeae	Panicoideae	14
Oryza sativa	Rice	Nipponbare	Oryzeae	Pharoideae	15

[a] After Gould and Shaw (1983)

Table 2. Number of *Pst*I-digested fragments and their estimated molecular sizes (kbp) of cpDNAs in turfgrasses and cereals

	A. stolonifera		*L. multiflorum*		*F. arundinacea*		*F. rubra*		*P. pratensis*		*C. dactylon*		*Ch. gayana*		*E. curvula*	
	Molecular size	Copy no.	Molecular size	Copy no.	Molecular size	Copy no.	Molecular size	Copy no.	Molecular size	Copy no.	Molecular size	Copy no.	Molecular size	Copy no.	Molecular size	Copy no.
	19.6	1	19.6	1	19.6	1	19.2	1	19.2	1	33.3	1	33.3	1	23.3	1
	19.2	1	18.3	1	18.3	1	18.3	1	15.8	2	15.3	1	19.6	1	15.8	1
	15.3	1	16.2	1	16.2	1	15.5	1	13.5	1	13.5	1	13.5	1	13.5	2
	12.6	1	15.3	1	15.3	1	15.3	1	11.0	1	12.6	1	11.0	1	12.2	1
	11.0	1	12.6	1	12.6	1	11:0	1	9.5	1	11.0	1	10.3	1	11.0	1
	8.4	3	11.0	1	11.0	1	9.5	1	8.4	2	9.3	1	9.3	1	8.4	2
	7.8	1	8.4	3	8.4	3	8.3	2	5.7	2	8.4	2	8.4	2	7.4	1
	5.7	1	5.7	1	5.7	1	6.0	1	5.3	1	5.2	1	5.3	1	5.3	1
	5.3	1	5.2	1	5.2	1	5.7	1	4.6	1	5.1	1	5.1	1	5.1	1
	4.8	1	4.4	1	4.4	1	5.2	1	4.4	1	4.9	1	4.9	1	4.8	1
	4.4	1	1.9	1	1.9	1	4.4	1	3.9	1	4.4	1	3.1	1	3.9	1
	3.9	1	0.6	1	0.6	1	4.3	1	2.8	1	3.9	1	2.6	1	2.8	1
	1.9	1					2.8	1	1.6	1	1.9	1	0.6	1	0.6	1
	0.6	1					0.9	1	0.6	1	0.6	1				
Total	137.3 kpb		136.0 kbp		136.0 kbp		135.5 kbp		136.2 kbp		137.7 kbp		135.4 kbp		136.0 kbp	

	Z. japonica		*Er. ophiuroidea*		*P. notatum*		*T. aestivum*		*H. vulgare*		*Z. mays*		*O. sativa*	
	Molecular size	Copy no.	Molecular size	Copy no.	Molecular size	Copy no.	Molecular size	Copy no.	Molecular size	Copy no.	Molecular size	Copy no.	Molecular size	Copy no.
	33.3	1	18.3	1	23.3	1	33.3	1	20.8	2	18.3	1	19.2	1
	23.3	1	15.8	1	22.5	1	19.6	1	18.8	1	16.8	1	16.2	1
	19.6	1	14.5	1	18.3	1	14.5	1	14.0	1	15.5	1	15.0	1
	16.2	1	10.8	1	13.5	1	12.6	1	12.6	1	14.5	1	14.4	1
	14.5	1	10.3	1	11.0	1	11.0	1	11.0	1	12.6	1	10.9	1
	8.4	2	8.6	1	5.3	3	8.4	2	10.1	1	11.0	1	10.1	1
	5.3	1	7.9	1	5.1	1	8.1	1	8.4	2	8.4	1	8.4	2
	4.8	1	6.6	1	4.6	2	5.6	1	5.6	1	5.3	3	7.9	1
	2.8	1	5.3	2	3.9	2	5.3	1	5.2	1	5.1	1	5.6	1
	0.6	1	5.2	1	3.2	2	5.2	1	0.6	1	4.8	1	5.0	1
			5.0	1	3.1	2	1.9	1			3.9	1	4.7	1
			4.7	2	0.6	1	1.4	1			3.4	2	3.9	1
			3.9	1			0.6	1			3.1	1	2.1	1
			3.4	1							1.9	1	1.9	1
			3.1	2							0.6	1	0.6	1
			1.9	1										
			0.6	1										
Total	137.2 kpb		139.0 kbp		139.8 kbp		135.9 kbp		136.3 kbp		139.0 kbp		134.3 kbp	

Physical maps of chloroplast genomes in A. stolonifera and Z. japonica

The order of the restriction fragments in each turfgrass was determined by double digestions and confirmed by Southern hybridization with 11 wheat cpDNA clones as probes. For example, hybridization patterns of cpDNAs in *A. stolonifera* and *Z. japonica* with wheat clone P4 are shown in Fig. 1. Complete physical maps of *A. stolonifera* and *Z. japonica* cpDNAs were constructed based on the data obtained with the four restriction enzymes (Fig. 2). The structures of the chloroplast genomes of *A. stolonifera* and *Z. japonica* were basically the same as those of other grasses, containing an approximately 82-kbp large single-copy region, an approximately 13-kbp small single-copy region, and approximately 21-kbp inverted repeats (IRs).

The locations of seven chloroplast genes were assigned on the physical maps (Fig. 2). The gene arrangements of turfgrass cpDNAs were the same as those of other grass cpDNAs in terms of Southern hybridization.

Chloroplast genome types in turfgrasses revealed by restriction fragment patterns

The restriction fragment patterns of cpDNAs were highly variable among the 15 species. Fourteen restriction fragment patterns were distinguishable by the 60 different fragments generated after *Pst*I digestion (Table 2) and 54 different fragments obtained with *Xho*I. CpDNAs of *L. multiflorum* and *F. arundinacea* showed the identical fragment pattern upon *Pst*I or *Xho*I digestion. However, *L. multiflorum* and *F. arundinacea* were distinguishable by 78 different fragments after *Bam*HI digestion. In total, 15 different chloroplast genome types were detected among the 15 species, each having a unique chloroplast genome (Fig. 3a, b).

Phylogenetic relationships betwen cpDNAs of turfgrasses and cereals

CpDNAs of cool season turfgrasses produced similar restriction fragment patterns, and these were clearly different from those of warm season turfgrasses. The number and frequency of cpDNA fragments shared in common between all pairs of 11 turfgrass and four cereal species are given in Table 3. Based on the frequency of commonly shared fragments, a dendrogram was constructed by the UPG method (Fig. 4). The turfgrass species were divided into three major clusters, which correspond to the subfamilies of Gramineae. The five cool season turfgrasses in Pooideae were relatively close to each other, consisting of a cluster at 44.8% similarity level. On the other hand, the four species of warm season turfgrasses belonging to Eragrostoideae were clustered at a 57% similarity level. Two other species of Panicoideae

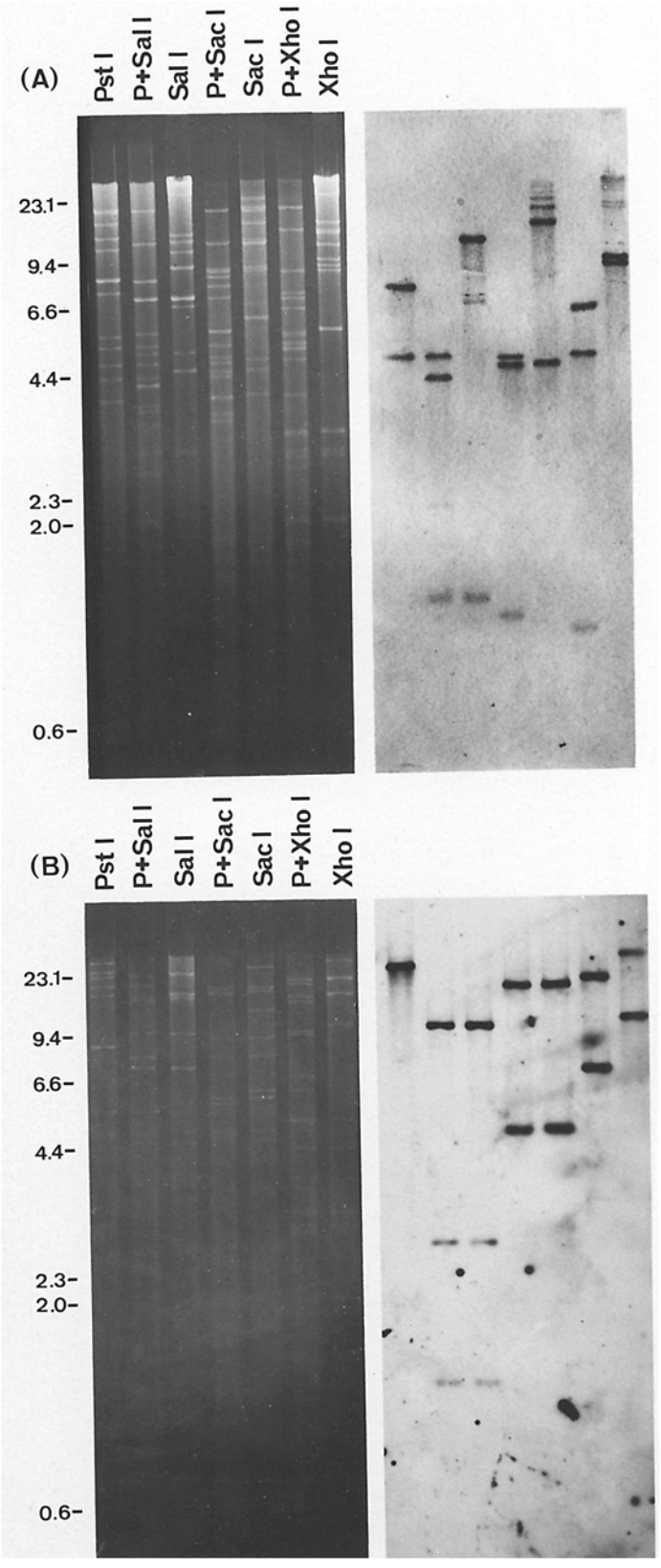

Fig. 1 A, B. Southern hybridization patterns of cpDNAs of *A. stronifera* (**A**) and *Z. japonica* (**B**) probed with the P4 fragment of wheat cpDNA

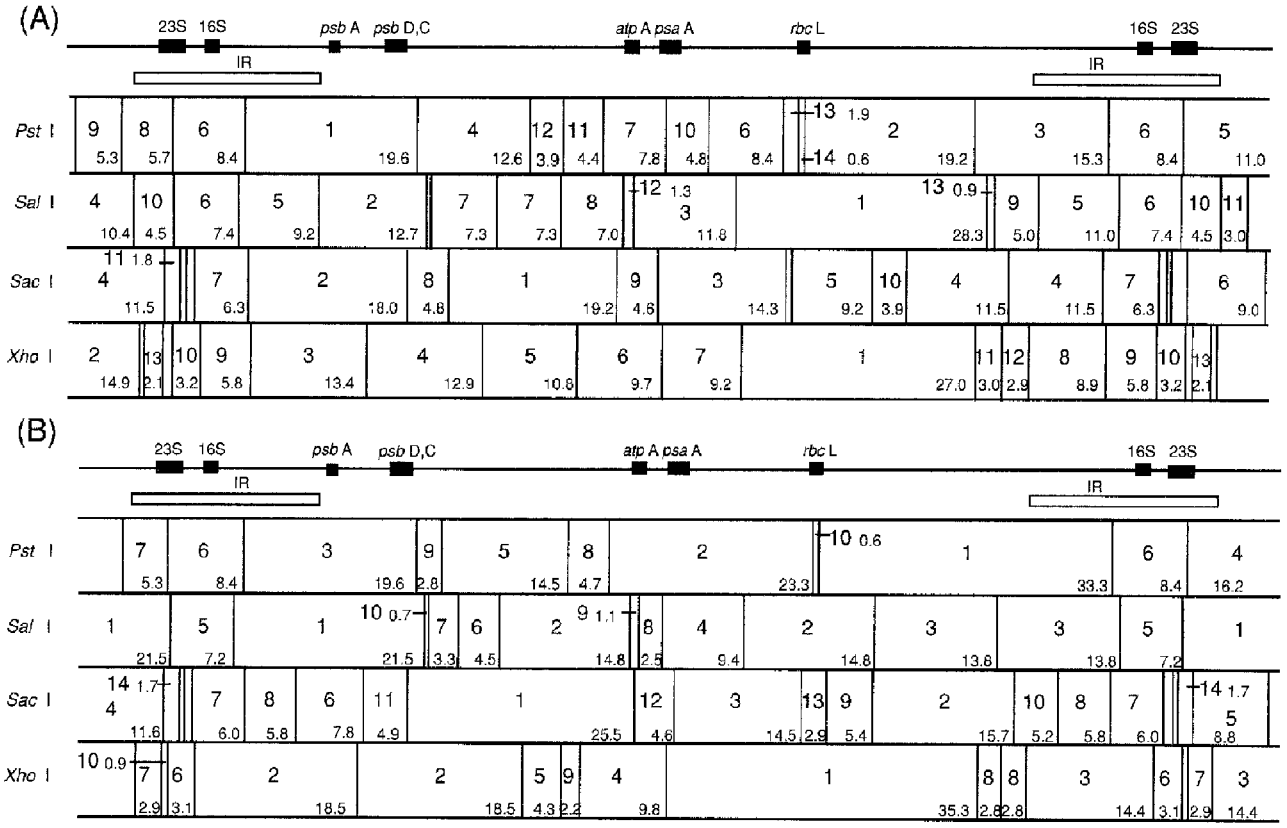

Fig. 2A, B. Physical map of cpDNAs of *A. stolonifera* (**A**) and *Z. japonica* (**B**) showing the recognition sites of four restriction enzymes, *Pst*I, *Sal*I, *Sac*I, and *Xho*I. *Numbers in bold type* indicate the fragment codes, with the fragment sizes (kbp) in *small numbers*. Gene locations and positions of inverted repeats (*IR*) are also shown

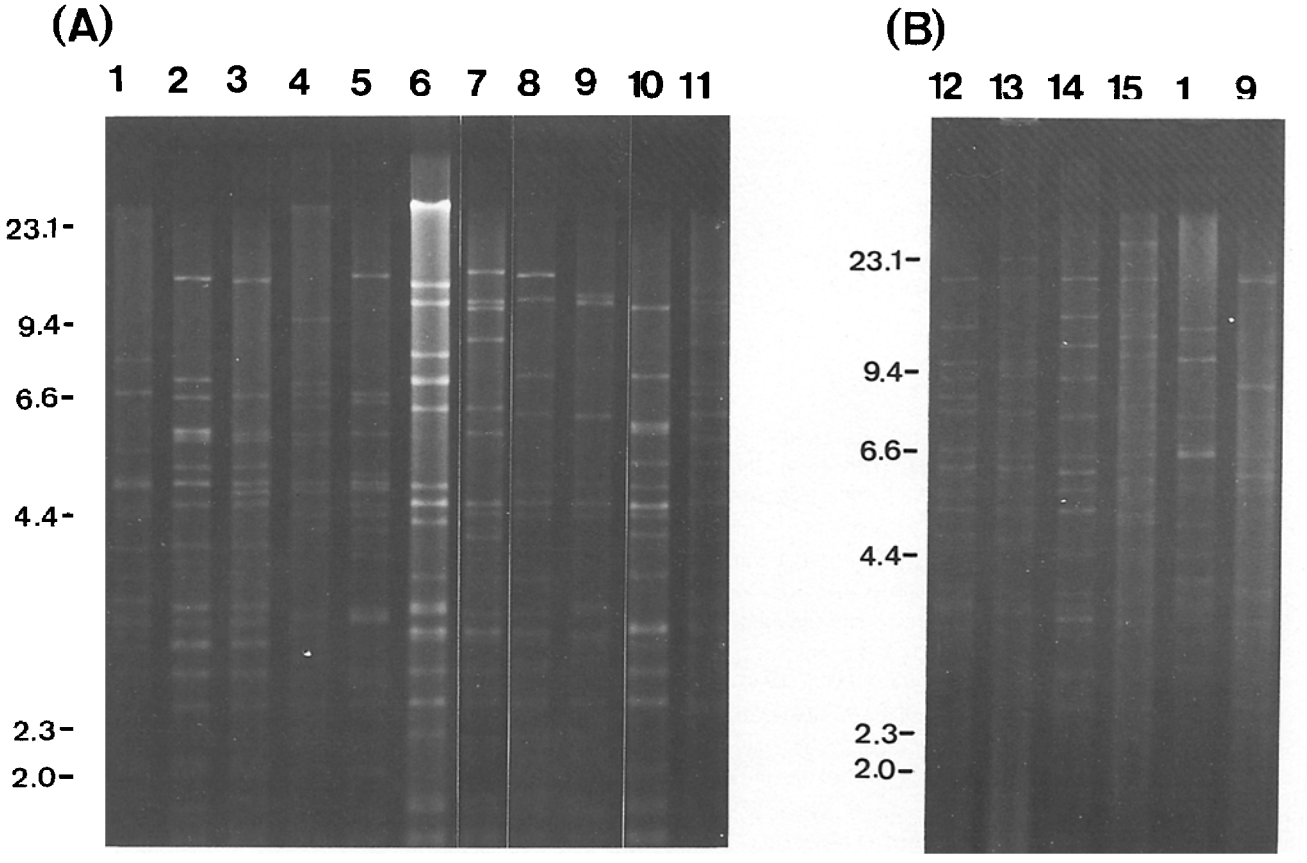

Fig. 3A, B. *Bam*HI fragment patterns of cpDNA in 11 turfgrasses (**A**), and four cereals (**B**). The numbers *above* the lanes indicate the codes of the materials shown in Table 1

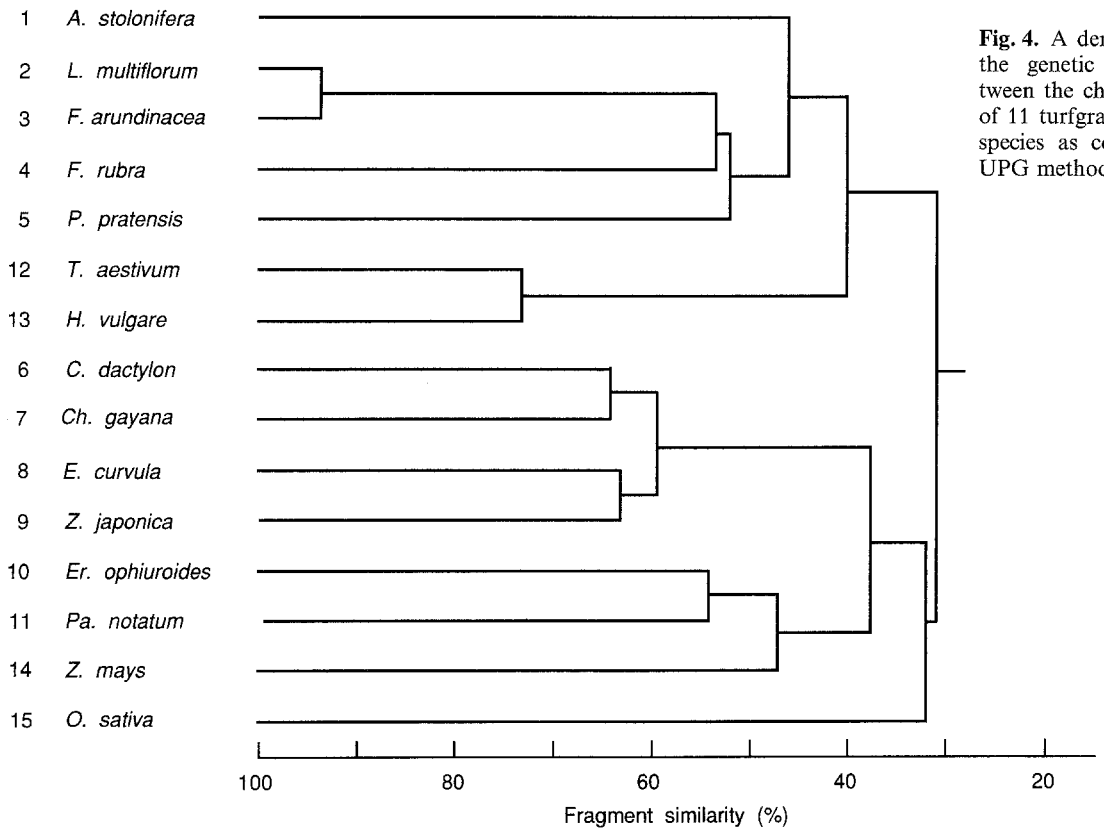

Fig. 4. A dendrogram showing the genetic relationships between the chloroplast genomes of 11 turfgrass and four cereal species as constructed by the UPG method

Table 3. Numbers of shared restriction fragments (lower left half of the table) and the frequency (%) of common fragments (upper right half) between strains

Code of[a] strains	Code of strains[a]															Average
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1	*68*[b]	50.8	53.0	36.6	45.6	36.2	32.3	33.6	32.0	29.2	26.6	43.4	39.4	36.6	33.8	37.8
2	66	*62*	93.7	51.2	52.3	46.3	33.9	35.3	31.9	30.5	32.8	43.9	43.0	35.2	32.3	43.8
3	70	118	*64*	55.1	53.0	47.2	35.0	38.0	34.7	30.1	30.6	46.4	45.5	34.6	33.3	45.0
4	48	64	70	*63*	51.9	32.8	26.9	28.3	28.3	33.3	34.1	29.0	32.8	33.3	27.2	35.8
5	62	68	70	68	*68*	36.2	25.8	36.8	28.8	33.6	28.1	35.7	40.9	33.6	27.7	37.9
6	46	56	58	40	46	*59*	64.3	60.3	58.6	34.8	32.8	30.0	28.8	42.6	33.1	41.7
7	40	40	42	32	32	74	*56*	56.6	63.7	36.8	39.7	30.8	27.8	37.0	27.1	38.4
8	42	42	46	34	46	70	62	*57*	63.2	42.9	42.7	25.0	31.0	40.0	28.6	40.2
9	40	38	42	34	36	68	72	72	*57*	34.9	41.0	35.6	34.5	35.0	33.6	39.7
10	40	40	40	44	46	40	46	54	44	*69*	54.3	26.2	25.0	50.0	39.7	35.8
11	34	40	38	42	36	42	46	50	48	70	*60*	28.1	25.2	43.9	27.9	34.8
12	56	54	58	36	46	36	36	30	42	34	34	*61*	73.3	32.3	24.4	36.0
13	50	52	56	40	52	34	32	36	40	32	30	88	*59*	27.9	21.5	35.5
14	48	44	44	42	44	52	44	48	42	66	54	40	34	*63*	40.0	37.3
15	44	40	42	34	36	40	32	34	40	52	34	30	26	50	*62*	30.7

[a] Codes are found in Table 1; [b] The total number of restriction fragments is given in italics

were grouped together at a 54.1% similarity level. The four cereals used in the experiment also clustered with the turfgrasses.

Discussion

It is widely accepted that the genome structure of chloroplast is conserved among divergent plants (Palmer et al. 1988). This conservatism of cpDNA enables us to study the phylogenetic relationships of diverse plants at various taxonomic levels. We estimated the sizes of the chloroplast genomes of the turfgrasses to be between 135 kbp and 140 kbp. This range is comparable to that found in the cereals and is approximately 20 kbp smaller than that of other monocots and tobacco (Katayama et al. 1991). The size of the IR region of turfgrass cpDNA was esti-

mated to be 21 kbp, whereas the large single-copy region was calculated to be about 82 kbp, and the small single-copy region, 13 kbp. These estimations indicate that the structural features of the turfgrass chloroplast genomes are comparable to those of cereals, such as wheat, barley, rye, oat, maize, and rice, and different from those of other monocots and tobacco (see Katayama et al. 1991).

Although the gene contents in the chloroplast genome are highly conserved among plants (Palmer et al. 1988), it is known that the chloroplast genome of cereals harbors three major inversions (Howe et al. 1988; Hiratsuka et al. 1989), which is in contrast to the situation in the most frequently found chloroplast genomes in angiosperms, such as that of tobacco. In our experiment, seven genes were mapped on the physical map of *A. stolonifera and Z. japonica* cpDNAs, four of which are localized at the borders of the major inversion. Mapping data showed that the gene orders of two turfgrass cpDNAs are the same as those of wheat (Ogihara and Tsunewaki 1988) and rice (Hiratsuka et al. 1989). Our results suggest that the inversions of cpDNA found in cereals might be common occurrences among grasses. Accordingly, the RFLP analysis of cpDNAs in grasses should be reliable for the investigation of their phylogenetic relationships.

RFLP analysis revealed a wide variation among the cpDNAs of turfgrasses. Phylogenetic relationships deduced from the similarity of restriction fragment patterns (Fig. 4) showed that the cool and warm season turfgrasses constituted two major groups, with the latter subdivided into two subgroups corresponding to subfamilies. The phylogenetic relationships of the turfgrass species deduced from our study (Fig. 4) are principally in accordance with their classification based on morphological characters (Tateoka 1957). It should be emphasized that RFLP analysis of cpDNAs allowed the estimation of their relative relatedness.

However, one clear discrepancy was disclosed, and that is the relationship of *F. arundinacea* to *F. rubra* and *L. multiflorum*. Our results indicate that *F. arundinacea* is much closer to *L. multiflorum* than to *F. rubra*. This discrepancy has already been noted by Lehvaslaiho et al. (1987). Our results also strongly suggest that the cytoplasm of *F. arundinacea* was derived from a *Lolium* species. In fact, intergeneric hybrids have been, produced between *Lolium* and *Festuca* (Buckner et al. 1961), and these showed meiotic chromosome pairing (Humphreys 1989).

The present results provide an overall phylogenetical picture of turfgrass species and will be utilized in their future breeding programs.

References

Buckner RC, Hill HD, Burrus II PB (1961) Some characteristics of perennial and annual ryegrass × tall fescue hybrid and of the amphidiploid progenies of annual ryegrass × tall fescue. Crop Sci 1:75–80

Gould FW, Shaw RB (1983) Grass systematics. Texas A & M University Press, Dallas

Hiratsuka J, Shimada H, Whittier R, Ishibashi T, Sakamoto M, Mori M, Kondo C, Honji Y, Sun CR, Meng BY, Li YQ, Kanno A, Nishizawa Y, Hirai A, Shinozaki K, Sugiura M (1989) The complete sequence of rice (*Oryza sativa*) chloroplast genome: intermolecular recombination between distinct tRNA genes accounts for a major plastid DNA inversion during the evolution of cereals. Mol Gen Genet 217:185–194

Howe CJ, Barker RF, Bowman CM, Dyer TA (1988) Common features of three inversions in wheat chloroplast DNA. Curr Genet 13:343–349

Humphreys MW (1989) The controlled introgression of *Festuca arundinacea* genes into *Lolium multiflorum*. Euphytica 42:105–116

Katayama H, Sasakuma T, Ogihara Y (1991) Physical map of chloroplast DNA of the onion *Allium cepa* L., showing the location of photosynthesis-related genes. Jpn J Genet 66:421–431

Lehvaslaiho H, Saura A, Lokki J (1987) Chloroplast DNA variation in the grass tribe Festuceae. Theor Appl Genet 74:298–302

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning – A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Ogihara Y, 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

Palmer JD, Jansen RK, Michaels HJ, Chase MW, Manhart JR (1988) Chloroplast DNA variation and plant phylogeny. Ann Mo Bot Gard 75:1180–1206

Pitman MW, Burson BL, Bashaw EC (1987) Phylogenetic relationships among *Paspalum* species with different base chromosome numbers. Bot Gaz 148:130–135

Reed KC, Mann DA (1985) Rapid transfer of DNA from agarose gel to nylon membranes. Nucleic Acids Res 13:7207–7221

Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida T, Matsubayashi T, Zaita N, Chunwongse J, Obokata J, Yamaguchi-Shinozaki K, Ohto C, Torazawa K, Meng BY, Sugita M, Deno H, Kamogashira T, Yamada K, Kusuda J, Takaiwa F, Kato A, Tohdoh N, Shimada H, Sugiura M (1986) The complete nucleotide sequence of tobacco chloroplast genome: its gene organization and expression. EMBO J 5:2043–2049

Sneath P, Sokal R (1973) Numerical taxonomy. Freeman, San Francisco

Strauss SH, Palmer JD, Howe GT, Doerksen AH (1988) Chloroplast genomes of two conifers lack a large inverted repeat and are extensively rearranged. Proc Natl Acad Sci USA 85:3898–3902

Tateoka T (1957) Miscellaneous papers on the phylogeny of Poacea. X. Proposition of a new phylogenetic system of Poacea. J Jpn Bot 32:275–287

Turgeon AJ (1985) Turfgrass management. Prentice-Hall, Englewood Cliffs, N.J.

Vogelstein B, Gillespie D (1979) Preparation and analytical purification of DNA from agarose. Proc Natl Acad Sci USA 76:615–619

Weeden NF, Emmo AC (1985) Isozyme characterization of kentucky bluegrass cultivars. Can J Plant Sci 65:985–994