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Chloroplast genome organization of bromegrass, *Bromus inermis* Leyss

M. Pillay

Department of Crop and Soil Sciences, Irrigated Agriculture Research and Extension Center, Washington State University, Prosser, WA, 99350, USA

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Summary. A physical map of the *Bromus inermis* chloroplast genome was constructed using heterologous probes of barley and wheat chloroplast DNA (cpDNA) to locate restriction sites. The map was aligned from data obtained from filter hybridization experiments on single and double enzyme digests. Cleavage sites for the enzymes *Pst*I, *Sal*I, *Kpn*I, *Xho*I and *Pvu*II were mapped. The chloroplast genome of *B. inermis* is similar in physical organization to that of other grasses. The circular cpDNA molecule of *B. inermis* has the typical small (12.8 kbp) and large (81.3 kbp) single-copy regions separated by a pair of inverted repeat (21 kbp) regions. The cpDNA molecule of *B. inermis* is collinear in sequence to that of wheat, rye, barley and oats. No structural rearrangements or major deletions were observed, indicating that the cpDNA of *Bromus* is a useful tool in phylogenetic studies.

Key words: Chloroplast DNA-Restriction site-map-*Bromus inermis*

Introduction

Bromus inermis Leyss is an economically important forage species in the temperate zones of North America. It is an upright perennial grass that spreads by rhizomes to form a dense sod on a wide range of soils (Molnar 1988). *Bromus inermis* is native to Eurasia and is also known as Austrian, Hungarian or Russian brome. It was introduced into North America at the turn of the century (Newell 1973) and is now cultivated widely in the United States corn belt and in western and central Canada. It is also grown in Russia, China and Northern Europe (Molnar 1988).

Bromus inermis is an octoploid (AAAABBBB) with a chromosome number of 2n = 56 and has been the subject of few genetic studies because of its high ploidy level (Molnar 1988). Although *B. inermis* can be successfully crossed with several other North American and Eurasian species (Armstrong 1973, 1977, 1981, 1982, 1983, 1984a, b, 1985), its exact parentage is unknown. The chloroplast DNA relationship of *B. inermis* to other diploid and polyploid species of the genus *Bromus sensu lato* has been investigated (Pillay and Hilu 1990).

This paper presents a physical map of the chloroplast genome of *B. inermis*. The availability of a physical map will enable the localization of detailed rearrangements and additions or deletions of similar plastomes in related species of *Bromus*. Restriction site maps not only provide information about genomic evolution, they are also useful to systematists interested in organismic phylogenies (Kellogg 1992).

Materials and methods

Plant material

Seed of *B. inermis* cv 'Manchar' were obtained from Sharp Bros. Seed Co, Healy, Kansas, USA. They were germinated in flats under greenhouse conditions, and after 2 weeks of growth the seedlings were transferred into a dark cupboard in the greenhouse for 2 days to reduce the starch content of the leaves. The leaves were then harvested, frozen in liquid nitrogen and stored at −70 °C in a freezer until use.

DNA extraction, restriction endonuclease digestion and gel electrophoresis

Chloroplast DNA (cpDNA) was extracted using the procedures of Kemble (1987) with the following modifications. In each extraction, 25 g of leaf material was ground with a pestle in a mortar containing liquid nitrogen. The powder was then resuspended in 400–500 ml of the isolation buffer. After lysis of the chloroplasts and the addition of ammonium acetate, the lysate

was placed on ice for about 30 min. This step seemed to help in removal of RNA from the samples during the phenol/chloroform extraction. The DNA was digested singly with *Pst*I, *Sal*I, *Kpn*I, *Xho*I and *Pvu*II following directions provided by the supplier (GIBCO BRL). Double digestions were done with a *Pst*I digest and each one of the other enzymes listed above. These restriction enzymes were used because they cut cpDNA somewhat infrequently and produce a small number of fragments. Such rare-cutting enzymes are appropriate for constructing a complete map of the chloroplast genome (Palmer 1986). The DNA fragments resulting from the digestions were separated by electrophoresis in a 0.8% agarose gel. The DNA was stained in ethidium bromide and photographed under UV light.

Southern transfer and DNA hybridization

The DNA fragments in the gel were nicked (induced single-strand breakage) for 5 min under UV light and transferred directly to Zetaprobe membranes under high alkaline conditions (0.4 *M* NaOH, 1 *M* NaCl) (Reed and Mann 1985). The membranes were baked overnight at 65 °C.

Recombinant plasmids were used as hybridization probes. The probes were a set of ten *Pst*I barley cpDNA clones provided by Dr. A. Day (University of Geneva, Switzerland) and Dr. T. H. N. Ellis (John Innes Institute) and covered a major part of the chloroplast genome of *Bromus*. These probes from barley will be designated as P(number of probe)B in this study. In addition, the positions of some restriction sites and fragments were confirmed by using the wheat *Pst*I fragment P4 and the collective *Bam*HI fragments of wheat, B(11-3), B(2-15) and B(10-18), provided by Dr. C. Bowman (Plant Breeding Institute, Cambridge). The *Bam*HI clones represent partial digestions, the nomenclature being defined by the two *Bam*HI fragments that border the inserted DNA. For example, B(2-15) contains *Bam*HI fragments B2, B26 and B15 of wheat. The wheat clone designations will have a "W" suffix added in this study. The size and approximate location of the barley and wheat probes are shown in Fig. 1.

Entire plasmids were labelled by the nick translation procedure (Rigby et al. 1977) using 50 μCi = 1.85×10^6 Bg of [α-^{32}P]-dCTP and 1 μg of DNA per reaction. Unincorporated [α-^{32}P]-dCTP was removed by centrifugation (Maniatis et al. 1982). The membranes were prehybridized for at least 2 h at 65 °C in the hybridization buffer (3 × SSC, 20 m*M* NaH_2PO_4, pH 7.2, 7% SDS, 10 × Denhardt's and denatured salmon sperm DNA at 100 μg/ml). Hybridization was carried out at 65 °C for 12–20 h in fresh hybridization buffer to which a radiolabelled cpDNA probe was added. After hybridization the membranes were washed for 15 min each at 65 °C 3 times in 3 × SSC/0.5% SDS and 3 times in 1 × SSC/0.1% SDS and once in 0.5 × SSC/0.1% SDS to remove unbound radioactive material. The membranes were air dried briefly, wrapped in Saran wrap and exposed to Kodak XAR-5 X-ray film at −70 °C with an intensifying screen for a few to several hours or days. In some instances, sharper bands were obtained by exposing films at room temperature without the screen. This procedure was useful in identifying bands that were very close together.

Analysis of data

Photographs were used to measure the mobilities of individual DNA fragments, and fragment sizes were estimated using a regression analysis computer program. Molecular weight standards included *Hin*dIII-digested lambda DNA and a 1-kb marker (GIBCO BRL). For comparative purposes, oats, rye and, sometimes, barley cpDNA and total cellular DNA of wheat were co-electrophoresed in the gels. These patterns were compared with data published on wheat (Bowman et al. 1981; Ogihara and Tsunewaki 1988), rye (Murai et al. 1989), barley

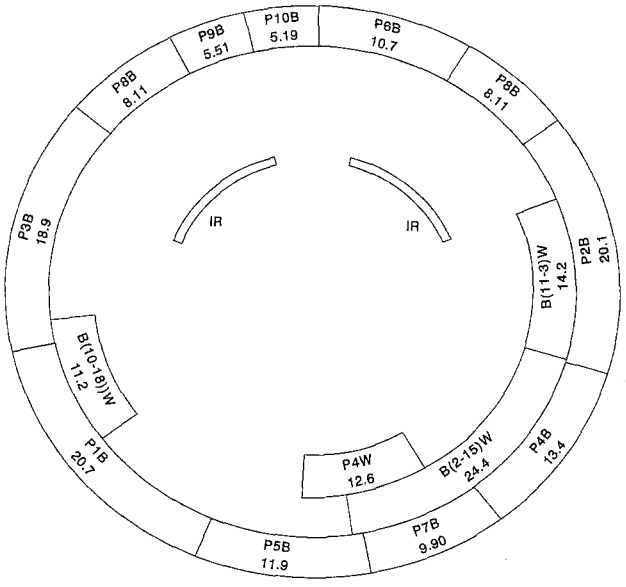

Fig. 1. Approximate location of barley and wheat cpDNA fragments used as probes in this study. The *outer complete ring* represents *Pst*I fragments of barley cpDNA; the *inner segments* represent wheat cpDNA fragments; *P4W* represents a *Pst*I fragment and the others are collective *Bam*HI fragments. The size (kbp) of each probe is indicated. *IR* represents the inverted repeat region

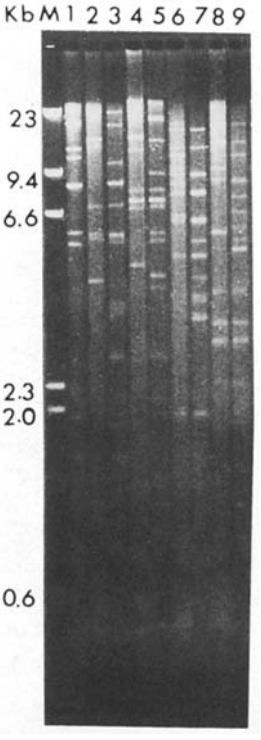

Fig. 2. Restriction endonuclease digests of *B. inermis* cpDNA. Digestion of DNA was carried out with *Pst*I (*lane 1*), *Pvu*II (*2*), *Pst*I and *Pvu*II (*3*), *Sal*I (*4*), *Pst*I and *Sal*I (*5*), *Kpn*I (*6*), *Pst*I and *Kpn*I (*7*), *Xho*I (*8*) and *Pst*I and *Xho*I (*9*). *Numbers* in *left margin* indicate size markers of lambda DNA-*Hin*dIII restriction digest fragments (*M*)

Table 1. *Bromus inermis* cpDNA fragment size, copy number, and barley and wheat probes to which they hybridized after digestion with *Pst*I, *Sal*I, *Kpn*I, *Xho*I and *Pvu*II

Enzyme	Fragment designation	Size (kbp)	Copy number	Probe-fragment hybridization
*Pst*I	P1	33.3	1	P2B, P3B, P4B, B(11-3)W, B(2-15)W
	P2	19.6	2	P1B, P2B, P3B, B(11-3)W, B(10-18)W
	P3	12.6	1	P5B, P7B, P4W
	P4	11.0	1	P6B, P9B
	P5	8.4	3	P4B, P8B, B(2-15)W
	P6	5.6	1	P6B, P9B
	P7	5.3	1	P10B
	P8	1.9	1	B(2–15)W
	P9	1.4	1	P1B, B(10-18)W
	P10	0.6	1	P1B
*Sal*I	S1	27.2	1	P2B, P4B, B(11-3)W, B(2-15)W
	S2	21.8	1	P2B, P3B, B(11-3)W, B(10-18)W
	S3	14.2	1	P2B, P3B, B(11-3)W
	S4	13.6	1	P6B, P9B, P10B
	S5	11.8	1	P5B, P7B, P4W
	S6	7.4	1	P1B
	S7	7.2	3	P1B, P8B
	S8	6.8	1	P1B, P5B
	S9	4.5	2	P6B, P9B
	S10	1.1	1	P5B, P7B
	S11	0.8	1	P1B
	S12	0.6	1	B(11-3)W
*Kpn*I	K1	18.0	1	P6B, P9B, P10B
	K2	15.8	1	P1B
	K3	11.8	1	P2B, P3B, B(11-3)W
	K4	10.8	3	P2B, P3B, P5B, P7B, P8B, P4W
	K5	9.8	1	P4B, B(2-15)W
	K6	8.9	1	P2B, P4B, B(11-3)W, B(2-15)W
	K7	8.3	1	P1B, P5B, P7B, P4W
	K8	7.4	1	P2B, P3B B(11-3)W
	K9	6.3	1	P1B, P3B, B(10-18)W
	K10	5.8	1	P4B, B(2-15)W
	K11	4.9	1	P3B, B(10-18)W
	K12	1.8	2	P6B, P9B
	K13	0.8	2	P6B, P9B
	K14	0.7	1	P5B, P4W
*Xho*I	X1	18.2	3	P1B, P2B, P3B, P4B, P5B, P7B, P4W, B(11-3)W, B(2–15)W, B(10-18)W
	X2	14.9	1	P6B, P9B, P10B
	X3	13.1	1	P2B, P3B, B(11-3)W, B(2-15)W
	X4	9.7	1	P1B, P5B, P7B, P4W
	X5	8.6	1	P2B, P3B, B(11-3)W, B(2-15)W
	X6	5.6	2	P2B, P8B
	X7	3.8	1	P1B
	X8	3.2	2	P8B
	X9	2.9	3	P2B, P6B, P9B, B(11-3)W
	X10	2.8	1	P2B, B(11-3)W
	X11	2.4	1	P1B
	X12	0.9	2	P6B, P9B
	X13	0.6	1	P3B
	X14	0.4	2	P2B, P3B
*Pvu*II	V1	37.6	1	P2B, P4B, B(11-3)W, B(2-15)W
	V2	28.0	1	P1B, B(10-18)W
	V3	14.0	2	P3B, P6B, P8B, P9B, B(11-3)W
	V4	12.8	1	P10B
	V5	6.7	1	P5B, P7B, P4W
	V6	5.4	1	P1B, P5B, P7B, P4W
	V7	5.2	1	P6B, P9B
	V8	4.1	2	P6B, P8B, P9B
	V9	1.4	2	P2B, P3B, B(11-3)W
	V10	0.6	2	P6B, P9B

(Poulsen 1983; Day and Ellis 1985) and oats (Murai and Tsunewaki 1987) and with the data of Enomoto et al. (1985). The *B. inermis* fragments that co-migrated with those of wheat, rye, oats or barley were estimated to be of the same size for comparative reasons.

Results

Fragmentation of B. inermis cpDNA with restriction endonucleases

Figure 2 shows the restriction fragment patterns of the cpDNA of *B. inermis* with the five restriction endonucleases used for mapping. Very intense bands were assumed to contain more than one dose of a sequence of that size. This assumption was confirmed by hybridization. The restriction enzymes *Pst*I, *Sal*I, *Kpn*I, *Xho*I and *Pvu*II produced between 10 and 14 fragments of differing sizes per enzyme. Most of the DNA fragments were over 2 kbp in length. Smaller sized fragments, if not visible in gels, were seen in the autoradiograms. Figure 3 represents a typical autoradiogram obtained from hybridization of a radiolabelled barley clone to *Bromus* cpDNA. The estimated molecular size and copy number of the DNA fragments are listed in Table 1. Chloroplast DNA fragments obtained from digestion by *Pst*I, *Sal*I, *Kpn*I and *Xho*I are designated with the first letter of the enzyme used; fragments from *Pvu*II are designated with "V". The fragments from an enzyme digest are numbered according to decreasing size. Summation of the fragment sizes generated by each enzyme was approximately 136 kbp. Since the enzyme *Pst*I produced the least number of fragments, the chloroplast genome size of *B. inermis* was equated to the sum of these fragments, which amounted to 136.1 kbp.

Construction of the physical map

The data provided in Table 1 were used to construct the physical map (Fig. 4). Alignment of the maps was facilitated by the overlap filter hybridization method (Palmer 1986). Double digestions were used in determining overlapping regions of the *Pst*I map with those of other enzymes.

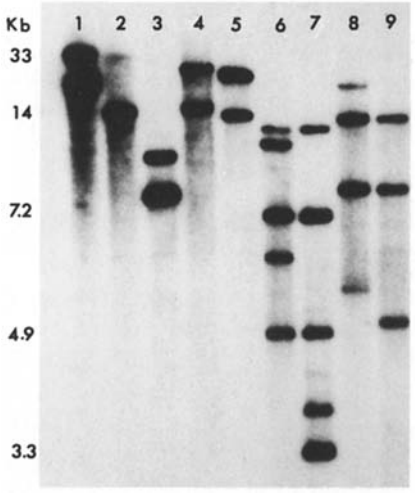

Fig. 3. Autoradiograph resulting from hybridization of [^{32}P]-labelled barley probe P3B to Zetaprobe membrane of *B. inermis* cpDNA. The lanes contain DNA digested with *Pst*I (*lane 1*), *Pvu*II (*2*), *Pst*I and *Pvu*II (*3*), *Sal*I (*4*), *Pst*I and *Sal*I (*5*), *Kpn*I (*6*), *Pst*I and *Kpn*I (*7*), *Xho*I (*8*) and *Pst*I and *Xho*I (*9*). *Numbers* in *left margin* indicate approximate fragment sizes in kbp

Some difficulties were encountered in positioning fragments hybridizing to the following pairs of barley probes: P2B and P3B, P5B and P7B, and P6B and P9B. Figure 1 shows that P2B and P3B as well as P6B and P9B contain similar regions of the inverted repeat. Consequently, these probe pairs hybridized to the same fragments of *B. inermis*, thus causing ambiguity in map construction. The wheat probe B(11-3)W, which overlaps a greater part of P2B, extending into the large single-copy region was useful in partially resolving the ambiguity. Barley probes P5B and P7B always produced identical hybridization patterns. Although the homology between barley and *B. inermis* cpDNA was very high, as judged from the high stringency washing, neither the P5B nor the P7B clones hybridized to the 1.9-kbp or 8.4-kbp *Pst*I fragments of *B. inermis* located in the region occupied by P7B (Figs. 1 and 4). It seems as if the P7B clone does not completely cover the region of the barley chloroplast genome indicated in Fig. 1. In this regard, the wheat clone B(2-15)W provided strong intensities with both the 1.9-kbp and 8.4-kbp fragments. This result confirmed the presence of three copies of 8.4-kbp sequences in the *Pst*I digest of *B. inermis*, as is also found in oats (Murai and Tsunewaki 1987).

The existence of multiple sequences in some restriction bands, some of which were not identical, also complicated the interpretation of physical mapping data. For

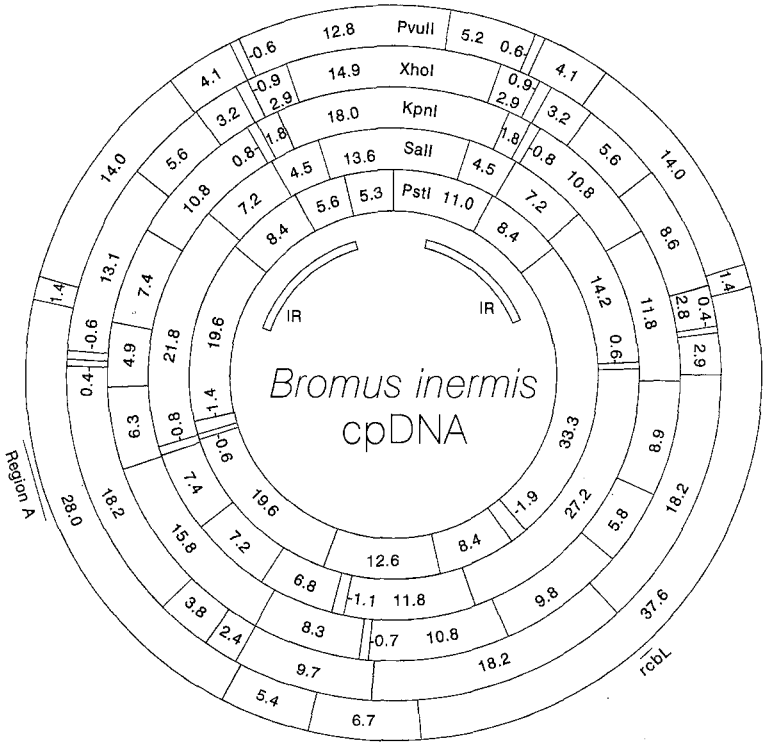

Fig. 4. Restriction site map of *B. inermis* cpDNA showing cleavage sites for *Pst*I, *Sal*I, *Kpn*I, *Xho*I and *Pvu*II. The inverted repeat is designated *IR*. Fragment sizes are shown in kbp. *Region A* indicates *Pst*I and *Sal*I restriction sites unique to *Bromus* and the M^{u} chloroplast genome type of *Triticum/Aegilops*. The position of the *rbcL* gene is inferred from Bowman et al. (1981)

example, the *Xho*I digest produced three nonidentical fragments of 18.2 kbp in a single band. Despite these problems, the data provided in Table 1 were sufficient to produce a physical map of *B. inermis* (Fig. 4).

Discussion

Method of cpDNA extraction

In many plants the isolation of highly pure cpDNA that is easily digested with restriction endonuclease remains an enigma; this is especially true in the grasses (Bowman and Dyer 1982; Baum and Bailey 1989; Dally and Second 1990). Instead of isolating pure cpDNA many laboratories have adopted methods involving the isolation of total DNA and the use of heterologous DNA probes to locate specific fragments. There are advantages and disadvantages associated with these latter methods. One disadvantage, as explained using examples by Green et al. (1986), is the possibility that cpDNA probes might hybridize to homologous nuclear or mitochondrial DNA. Such anomalies, if left unchecked, can introduce gross errors in physical mapping studies. Therefore, the extraction of pure cpDNA from some species is necessary in order to distinguish cpDNA fragments in a total DNA preparation. Pure cpDNA may also be desired for comparative studies in laboratories not equipped for radioisotope work or for the examination of cpDNA sequence homologies among plants. The cpDNA extraction method used in this study (Kemble 1987) yielded highly pure DNA with no contaminating nuclear DNA and little or no RNA as observed from gel electrophoresis. Although the cpDNA isolation procedure used in this study was developed initially for dicotyledons, it can be applied, with modifications, to a wider range of plants. By means of this technique, cpDNA has been isolated from hops (unpublished data) and many grasses including barley, *Brachypodium*, *Cynodon*, *Danthonia*, *Digitaria*, finger millet, maize, oats, *Paspalum*, rye, rice, sorghum and pearl millet. The procedure works with more than one plant group and is highly recommended, especially for grasses.

Physical map of B. inermis

Chloroplast DNA restriction endonuclease maps are currently available for only a few grass species. In subfamily *Pooideae*, to which *B. inermis* belongs, cpDNA physical maps have been constructed for wheat (Bowman et al. 1981; Ogihara and Tsunewaki 1988), barley (Poulsen 1983; Day and Ellis 1985), rye (Murai et al. 1989) oats (Murai and Tsunewaki 1987), *B. tectorum* (Kellogg 1992) and Poa (R. J. Soreng unpublished data). In this study, the cpDNA of *B. inermis* is physically mapped (Fig. 4), and the positions of the cleavage sites for *Pst*I, *Sal*I, *Kpn*I, *Xho*I and *Pvu*II are shown. The cpDNA restriction map of *B. inermis* is similar in physical organization to the chloroplast genomes of other grasses. The chloroplast genomes of grasses are generally characterized by the presence of three inversions (Howe et al. 1988). The circular chloroplast genome of *B. inermis* has the typical small (12.8 kbp) and large (81.3 kbp) single-copy regions separated by a pair of inverted repeat (21 kbp) regions.

With a genome size of 136.1 kbp, the cpDNA of *B. inermis* is similar in size to the cpDNAs of wheat, rye, barley, oats, finger millet and rice (Enomoto et al. 1985). This size corresponds to that of the average chloroplast genome of members of the *Pooideae*, which is reported to be 135 kbp (Soreng et al. 1990). The chloroplast genome of *B. inermis* is smaller than those reported for foxtail millet, maize, Job's tears and sorghum, all of which have cpDNA molecule of approximately 140 kbp (Enomoto et al. 1985).

The cpDNA molecule of *B. inermis* is collinear in sequence to those of wheat, rye, barley and oats, although the restriction patterns of *B. inermis* were more similar to those of wheat and rye than to barley and oats. The proportion of shared restriction fragments (F value of Nei and Li 1979) between *B. inermis* and rye, wheat, barley and oats was 0.76, 0.72, 0.57 and 0.46, respectively. The physical map of *B. inermis* has more homologous restriction sites with rye and wheat than with barley or oats. A detailed comparison of restriction site maps of *B. inermis* with published maps of closely related genera will not be attempted in this paper. However, attention should be drawn to the equivocal arrangement of two large *Kpn*I fragments mapped in wheat (Ogihara and Tsunewaki 1988) and rye (Murai et al. 1989). These two *Kpn*I maps appear to be incorrect. The *Kpn*I map of *B. inermis* (Fig. 4) indicates the correct order of the 8.3-kbp and 10.0-kbp fragments. The studies of Ogihara and Tsunewaki (1988) and Murai et al. (1989) placed these fragments in the reverse order. This author's unpublished *Kpn*I maps of wheat and rye as well as those of Rob Soreng (personal communication) suggest that the arrangement of the 8.3-kbp and 10.0-kbp fragments are identical in *B. inermis*, wheat and rye.

Chloroplast DNA restriction site maps using six restriction endonucleases were recently published for *B. tectorum* (Kellogg 1992). The *B. tectorum* study included maps for four of the enzymes used to map *B. inermis*. However, it is not possible to make a meaningful comparison between most of the restriction maps of the two species of *Bromus* since there are large gaps, indicating unmapped areas, in the mapping studies involving *B. tectorum*.

An interesting observation is made when portions of the *Pst*I and *Sal*I maps of *B. inermis* are compared with those of *Triticum*/*Aegilops*. Ogihara and Tsunewaki (1982) mapped changes that have occurred in the chloro-

plast genomes of *Triticum/Aegilops*. Their study suggests that the M^u chloroplast genome differed from the standard type by having a smaller P10 and a larger S7 fragment. The smaller P10 (4.7 kbp) fragment was the result of a 0.5-kbp deletion in the standard P10 (5.2 kbp) fragment. The larger S7 fragment was the result of a base-pair change in the recognition site between fragment S7 (6.1 kbp) and S9 (1.2 kbp) (Ogihara and Tsunewaki 1982). Since the larger S7 fragment observed by Ogihara and Tsunewaki (1982) was only 7.1 kbp, the authors proposed that a deletion of 0.2 kbp had occurred between fragments S7 and S9. The changes characteristic of the M^u chloroplast genome were present only in *Aegilops uniaristata*. In a later study, Ogihara and Tsunewaki (1988) reported the presence of the 0.2-kbp deletion between S7 and S9 in *Ae. comosa* and *Ae. heldreichii*. Similar restriction site changes are present in this region (Fig. 4, Region A) of the chloroplast genome of *B. inermis*. For example, there is a *Pst*I restriction site 0.6 kbp from the 1.4-kbp fragment in *B. inermis*. Furthermore, no P10 (5.2 kbp) fragment is found in *B. inermis* as is found in wheat; instead, a larger 19.6-kbp fragment is located in this position (Fig. 4). The 19.6-kbp sequence is approximately equal to the sum of two adjacent fragments, P10 (5.2 kbp) and P3 (14.5 kbp), found in the wheat chloroplast genome and is most likely the result of a restriction site loss between the two fragments. The *Sal*I map of *B. inermis* (Fig. 4, Region A) differs from the standard *Sal*I map of wheat by lacking the S9 (1.2 kbp) fragment. A base-pair change in the recognition site similar to that found in species of *Aegilops* is a probable reason for this event. However, there is no deletion in *B. inermis*. Instead, a larger fragment of 7.4 kbp, equal to P9 (1.2 kbp) and P7 (6.2 kbp), is found in this position. The taxonomic implications of these parallel changes between *B. inermis* and *Aegilops* and further details on the structure and evolution of chloroplast genomes in *Bromus*, wheat, barley, rye and oats is in preparation.

The physical map of the chloroplast genome of *B. inermis* has the same general structural form as those of most of the higher plants and is very similar in sequence arrangement to those of other members of the *Pooideae*. The conservative nature of the chloroplast genome in terms of overall size, organization and sequence makes it a powerful tool to address questions of a phylogenetic nature.

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References

Armstrong KC (1973) Chromosome pairing in hexaploid hybrids from *Bromus erectus* × *B. inermis* (2n = 56). Can J Genet Cytol 15:427–436

Armstrong KC (1977) Hybrids of the annual *Bromus arvensis* with perennial *B. inermis* and *B. erectus*. Can J Genet Cytol 21:65–71

Armstrong KC (1981) The evolution of *Bromus inermis* and related species of *Bromus* sect. *Pnigma*. Bot Jahrb Syst 102:427–443

Armstrong KC (1982) Hybrids between the tetraploids of *Bromus inermis* and *Bromus pumpellianus*. Can J Bot 60:476–482

Armstrong KC (1983) The relationship between some Eurasian and American species of *Bromus* section *Pnigma* as determined by the karyotypes of some F_1 hybrids. Can J Bot 61:700–707

Armstrong KC (1984a) Chromosome pairing affinities between old and new world species of *Bromus* section *Pnigma*. Can J Bot 62:581–585

Armstrong KC (1984b) The genomic relationship of the diploid *Bromus variegatus* to *Bromus inermis*. Can J Genet Cytol 26(4):469–474

Armstrong KC (1985) Chromosome pairing in octoploid F_1 and octoploid amphiploid hybrids between *Bromus inermis* and *B. pumpellianus*. Can J Genet Cytol 27:538–541

Baum BR, Bailey LG (1989) Species relationships in the *Hordeum murinum* aggregate viewed from chloroplast DNA restriction patterns. Theor Appl Genet 78:311–317

Bowman CM, Dyer TA (1982) Purification and analysis of DNA from wheat chloroplast isolated in nonaqueous media. Anal Biochem 122:108–118

Bowman CM, Koller B, Delius H, Dyer TA (1981) A physical map of wheat chloroplast DNA showing the location of the structural genes for the ribosomal RNAs and the large subunit of ribulose 1,5-bisphosphate carboxylase. Mol Gen Genet 65:247–262

Dally AM, Second G (1990) Chloroplast DNA diversity in wild and cultivated species of rice (genus *Oryza*, section *Oryza*): cladistic-mutation and genetic-distance analysis. Theor Appl Genet 80:209–222

Day A, Ellis THN (1985) Deleted forms of plastid DNA in albino plants from cereal anther culture. Curr Genet 9:671–678

Enomoto S, Ogihara Y, Tsunewaki K (1985) Studies on the origin of crop species by restriction endonuclease analysis of organellar DNA. I. Phylogenetic relationships among ten cereals revealed by the restriction fragment patterns of chloroplast DNA. Jpn J Genet 60:411–424

Green RM, Vardi A, Galun E (1986) The plastome of *Citrus*: physical map, variation among *Citrus* cultivars and species and comparison with related genera. Theor Appl Genet 72:170–177

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

Kellogg EA (1992) Tools for studying the chloroplast genome in the Triticeae (Gramineae): an *Eco*RI map, a diagnostic deletion, and support for *Bromus* as an outgroup. Am J Bot 79:186–197

Kemble RJ (1987) A rapid, single leaf, nucleic acid assay for determining the cytoplasmic organelle component of rape-

seed and related *Brassica* species. Theor Appl Genet 73: 364–370

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

Molnar SJ (1988) Smooth bromegrass (*Bromus inermis* Leyss). In: Bajaj YPS (ed) Biotechnology in agriculture and forestry vol. 6. Springer, Berlin Heidelberg New York, pp 428–443

Murai K, Tsunewaki K (1987) Chloroplast genome evolution in the genus *Avena*. Genetics 116: 613–621

Murai K, Naiyu Xu, Tsunewaki K (1989) Studies on the origin of crop species by restriction endonuclease analysis of organellar DNA. III. Chloroplast DNA variation and interspecific relationships in the genus *Secale*. Jpn J Genet 64: 35–47

Nei M, Li W-H (1979) Mathematical models for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76: 5269–5273

Newell LC (1973) Smooth bromegrass. In: Heath ME, Metcalfe DS, Barnes RF (eds) Forages: the science of grassland agriculture, 3rd edn. Iowa State University Press, Ames, Iowa, pp 254–262

Ogihara Y, Tsunewaki K (1982) Molecular basis of the genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. I. Diversity of the chloroplast genome and its lineage revealed by the restriction pattern of ct-DNAs. Jpn J Genet 57: 371–396

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 (1986) Isolation and structural analysis of chloroplast DNA. In: Weissbach A, Weissbach H (eds) Methods in enzymology vol 118. Academic Press, New York, pp 167–186

Pillay M, Hilu KW (1990) Chloroplast DNA variation in diploid and polyploid species of *Bromus* (Poaceae) subgenera *Festucaria* and *Ceratochloa*. Theor Appl Genet 80: 326–332

Poulsen C (1983) The barley chloroplast genome: physical structure and transcriptional activity in vivo. Carlsberg Res Commun 48: 57–80

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

Rigby PWJ, Dieckmann M, Rhodes C, Berg P (1977) Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J Mol Biol 113: 237–251

Soreng RJ, Davis JI, Doyle JJ (1990) A phylogenetic analysis of chloroplast DNA restriction site variation in *Poaceae* subfamily *Pooideae*. Plant Syst Evol 172: 83–97