Maternal inheritance of chloroplast DNA in interspecific crosses of *Bromus*

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Abstract

Inheritance of chloroplast DNA (cpDNA) was examined in 41 F_1 progeny obtained from the following interspecific *Bromus* crosses: *Bromus arvensis* (2n = 14) × *B. inermis* (2n = 4x = 28); *B. arvensis* × *B. inermis* (2n = 8x = 56); *B. arvensis* × *B. erectus* (2n = 6x = 42); *B. arvensis* × *B. erectus* (2n = 8x = 56); *B. arvensis* × *B. erectus* (2n =10x = 70). Chloroplast DNA of the parental species was digested with *Bam*HI, *Eco*RI and *Hin*dIII and species-specific restriction fragment length polymorphisms were identified by observation of ethidium bromide stained agarose gels as well as by filter hybridization experiments involving heterologous cloned barley cpDNA probes. The stability of these point mutations was verified by examining the cpDNA restriction patterns of at least 28 individual plants raised from seed of each of the parental species. No intraspecific cpDNA variability was detected. All the F_1 progeny examined exhibited the cpDNA restriction fragment patterns of the female parent. There was no evidence of any paternal or biparental cpDNA inheritance. The results provided evidence for the uniparental-maternal inheritance of cpDNA in the *Bromus* crosses examined.

Additional key words: bromegrass, restriction fragment length polymorphisms.

Introduction

There is a great variation in the mechanisms of plastid DNA inheritance in flowering plants. The three modes of plastid transmission recognized include: *1*) maternal transmission, in which plastids are inherited only from the female parent, *2*) paternal transmission, where plastids are inherited solely from the male parent, and *3*) biparental transmission, in which plastids are inherited from the paternal (pollen) and maternal parent (Harris and Ingram 1991). The majority of flowering plant species are believed to display uniparental-maternal plastid DNA inheritance, while approximately one-third have some degree of biparental inheritance (Sears 1980, Whatley 1982). However, as the interest in understanding the inheritance of plastids has grown, examples of paternal or biparental inheritance of plastids have been reported in several angiosperms taxa including *Pelargonium* (Metzlaff *et al.* 1981), *Nicotiana* (Medgyesy *et al.* 1986), *Medicago* (Lee *et al.* 1988, Schumann and Hancock 1989; Masoud *et al.* 1990), *Iris* (Cruzan *et al.* 1993) and *Lens* (Rajora and Mahon 1995).

Earlier studies on plastid DNA inheritance used *1*) a genetic approach in which phenotypic markers due to plastid mutants were screened in reciprocal crosses, *2*) ultrastructural studies that tried to identify plastids in the male gametophytes, and *3*) epifluorescence microscopy where the DNA fluorochrome DAPI (4',6-diamidino-2-phenylindole) was used to test for the presence of proplastids in pollen (Sears 1980, Whatley 1982, Corriveau and Coleman 1988). Recent studies have employed restriction endonuclease analyses and Southern hybridization of chloroplast DNA as a novel and precise way of determining the mode of plastid transmission. Chloroplast DNA has been used to determine plastid inheritance in a number of plant species (Masoud *et al.* 1990, Rajora and Dancik 1992, Shore *et al.* 1994, Kiang *et al.* 1994, Chong *et al.* 1994, Yao *et al.* 1994, Steinborn

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et al. 1995, Rajora and Mahon 1995). A knowledge of cpDNA inheritance patterns is useful for correctly interpreting phylogenetic data derived from cpDNA, especially in taxa with high levels of polyploidy and hybridization (Harris and Ingram 1991), and of significance for determining the population genetics of organelle genes (Birky *et al.* 1989). The plastid genome carries protein coding genes that are involved in photosynthesis and other biosynthetic pathways (Sears 1983). Chloroplast encoded genes also play a role in herbicide resistance (Souza-Machada 1982). Recent advances in chloroplast transformation in higher plants indicate that chloroplast genes may play an important role in plant improvement (Svab *et al.* 1990, Svab and Maliga 1993). Therefore the mode of plastid inheritance should be of particular interest to plant breeders seeking to manipulate plastid-encoded traits.

Bromus is a large genus of approximately 130 annual and perennial species (Stebbins 1981). The genus includes species with ploidy levels ranging from diploid to decaploid with x = 7. Polyploidy and hybridization have played a major role in the evolution of the genus (Stebbins 1981). Several species of *Bromus* are of economic importance. The most important is *B. inermis*, a cool season forage plant grown in northern USA, western and central Canada and Northern Europe and Asia. *Bromus* is closely related to wheat, oat, barley, and rye and is a potential source of useful alien genes for disease resistance, nutritional value and other agronomic properties for the improvement of the Pooid cereals (Pillay 1995).

The objectives of this study were *1*) to identify RFLPs that characterized the bromegrass species used in the crosses and *2*) to determine the mode of inheritance of cpDNA in F_1 interspecific hybrid progeny obtained from crosses between *B. arvensis* × *B. inermis* (4x, 8x); and *B. arvensis* × *B. erectus* (6x, 8x, 10x).

Materials and methods

Plants: The species used in the crossing program are placed in two different subgenera of *Bromus*. The diploid *B. arvensis* is a member of subgenus *Bromus*, while *B. inermis* and *B. erectus* are placed in subgenus *Festucaria*. Successful reciprocal hybridization between the parental species was not possible. Crosses were successful only when *B. arvensis* was used as the female parent (Table 1*A*). Hybrid plants were verified by chromosome analysis as described by Armstrong (1977). Table 1*B* contains a list of plants that were used to detect the presence of intraspecific cpDNA variation in the parental species. Seeds were sown in soil in plastic pots and germinated in a controlled growth chamber with a 16-h photoperiod and day/night temperatures of 20/15 °C. Individual seedlings were then transplanted to larger pots and grown for 3 to 4 weeks. The leaf material from each plant was harvested, and either used immediately or frozen in liquid nitrogen and stored at -70 °C until use.

Table 1*A*. Crosses and number of progeny used to study chloroplast DNA inheritance in *Bromus*.

Crosses	Number
B. arvensis (70-13) × *B. inermis* (72 -2)	5
B. arvensis (70 -13) × *B. inermis* cv. Baylor (8x)	28
B. arvensis (496) × *B. erectus* (6x)	3
B. arvensis (496) × *B. erectus* (8x)	3
B. arvensis (496) × *B. erectus* (10x)	2

Table 1*B*. List of species, ploidy and number of plants examined for detection of intraspecific cpDNA variation in *Bromus*.

Species	Ploidy	Number
B. arvensis (70-7)	2x	28
B. arvensis (70-13)	2x	28
B. inermis (80-5)	4x	18
B. inermis	8x	28
B. erectus	4x	12
B. erectus	6x	30
B. erectus	8x/10x	14

DNA isolation: Chloroplast DNA was isolated from young leaf tissue of parental type material of *B. arvensis*, *B. inermis* (4x, 8x) and *B. erectus* (8x) as described by Pillay (1993). Total genomic DNA was extracted from 2 - 5 g of either fresh or frozen leaf tissue from single plants by a modified cetyltrimethylammonium bromide (CTAB) method (Pillay 1996). The concentration of the DNA samples was estimated by agarose gel electrophoresis in comparison with a known amount of phage lambda DNA-*Hin*dIII digest.

Restriction enzyme digestion, Southern blotting and hybridization: On the basis of an earlier study (Pillay and Hilu 1995), DNAs from the parental species and the F_1 progeny were digested with the restriction endonucleases *Bam*HI, *Eco*RI and *Hin*dIII according to the suppliers' instructions (*New England Biolabs*, Beverly, USA). The digested DNA fragments were separated on 1 % agarose gels by electrophoresis in 1X TAE buffer. Southern transfer to nylon membranes (*Zeta-Probe*, *Bio-Rad*, Richmond, USA), molecular hybridization and autoradiographic procedures were carried out as described in Pillay (1993).

Hybridization probes: Three clones, P2, P4 and P8 comprising segments of the barley chloroplast genome (Day and Ellis 1985) were used as hybridization probes to identify RFLPs among the parental species. These probes were selected on the basis of a previous study (Pillay and Hilu 1995) and other unpublished data on RFLP analysis in the genus *Bromus*.

Results and discussion

Southern hybridization patterns of the *Bam*HI digests with the P2 probe produced a distinct restriction site mutation that distinguished the cpDNA of *B. arvensis* from those of *B. inermis* and *B. erectus* (Table 2). A unique 6.2 kbp fragment was detected in *B. inermis* and *B. erectus*. This fragment was absent in *B. arvensis*, but was replaced by two smaller fragments of 4.8 kbp and 1.4 kbp. The sum of these two smaller fragments is equivalent to 6.2 kbp, implying that there was a gain of a restriction site in *B. arvensis*. Restriction site loss/gain was determined by comparison with an outgroup species, *Secale cereale* (Pillay and Hilu 1995). When the *Eco*RI digests were hybridized to the P8 clone, unique fragments of 14.0 kbp and 1.7 kbp were detected in *B. arvensis* and a 15.7 kbp fragment was found in *B. inermis* and *B. erectus*. Similarly, the Southern hybridization patterns of the *Hin*dIII digest with P2 and P4, consecutively, produced two mutations that also identified the cpDNA of *B. arvensis* (Table 2). Hybridization with the P4 probe, revealed fragments of 7.3 kbp and 2.9 kbp in *B. arvensis* and *B. erectus* and a larger 10.2 kbp fragment in *B. inermis*. The P2 probe hybridized to fragments of 6.6 kbp and 1.5 kbp in *B. arvensis* (Fig. 1). These fragments were absent in *B. inermis* and *B. erectus*, being replaced by a larger 8.1 kbp band (Fig. 1). The presence or absence of these RFLPs were screened in the 41 F_1 progeny from the interspecific crosses (Table 1*A*). We found that all the progeny plants had the same unique cpDNA fragments as their maternal parent demonstrating uniparental-maternal inheritance of cpDNA. For example, the unique 6.6 and 1.5 kbp fragments of the maternal parent *B. arvensis* is absent in the paternal parent *B. inermis* (Fig. 1). All the F_1 progeny showed the maternal restriction fragments. The 8.1 kbp fragment specific to the male parent (Fig. 1) is absent in the progeny plants. This was also evident in the other *Bromus* crosses (data not shown) suggesting that paternal cpDNA fragments were not transmitted to the offspring. A low frequency of paternal cpDNA transmission sometimes occurs in plants considered to inherit organelles in a strictly maternal way as evidenced in interspecific hybrids of *Nicotiana* and *Epilobium* species (Medgyesy *et al.* 1986, Schmitz and Kowallik 1986). In *Stellaria* and *Zantedeschia* the mode of cpDNA inheritance was maternal in some crosses and maternal, paternal and biparental in others suggesting a possible genotypic effect and/or plastome incompatibility that may promote or permit paternal cpDNA transmission (Chong *et al.* 1994, Yao *et al.* 1994). The crosses used in this study are representative of species placed in different taxa of *Bromus* that are regarded as sections, subgenera or even separate genera. The cpDNA patterns suggest that the species contain distinct cytoplasms. Further, different genotypes and ploidy levels were represented in the crosses. Despite these genetic differences among the

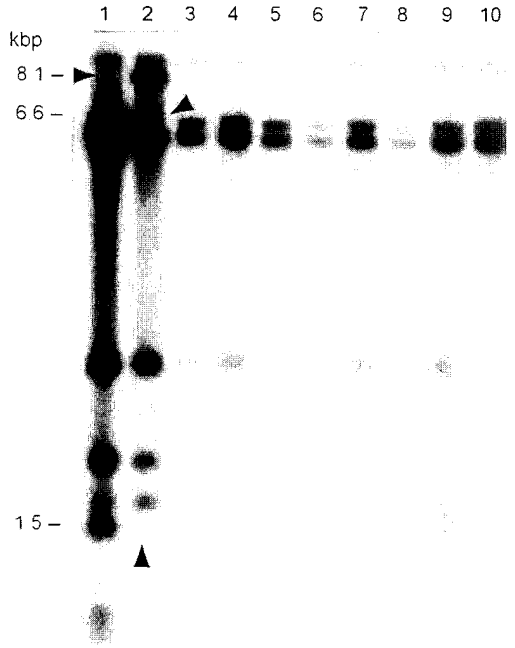

Fig. 1. Maternal inheritance of chloroplast DNA in progeny from *B. arvensis* × *B. inermis* cross. Total DNA was digested with *Hin*dIII and hybridized with the P2 barley cpDNA probe. *Lane 1 - B. arvensis*; *lane 2 - B. inermis*; *lanes 3 to 10* - progeny from the cross. *Arrows* indicate missing fragments in the two parents. The numbers in the left margin represent molecular sizes [kbp] of unique fragments.

Table 2. List of restriction site mutations used to demonstrate the mode of chloroplast DNA inheritance in interspecific crosses in *Bromus*.

Enzyme	Probe	Mutation	Mutated species
*Bam*HI	P2	6.2 = 4.8 +1.4	*B. arvensis*
*Eco*RI	P8	15.7 = 14.0 + 1.7	*B. arvensis*
*Hin*dIII	P2	8.1 = 6.6 + 1.5	*B. arvensis*
*Hin*dIII	P4	10.2 = 7.3 +2.9	*B. arvensis*, *B. erectus*

parents, cpDNA followed a strict pattern of maternal inheritance in all the progeny.

Although the chloroplast genome is considered to be a slowly evolving and highly stable molecule (Olmstead *et al.* 1990), intraspecific cpDNA variation has been observed in many species (Harris and Ingram 1991). In order to demonstrate the stability of the species-specific restriction site mutations of this study, we examined the cpDNA from 28 individual plants of *B. inermis* (8x), 18 plants of *B. inermis* (4x), 28 plants of each of two accessions of *B. arvensis* and 56 plants of *B. erectus* representing 4x, 6x, 8x and 10x levels of ploidy (Table 1*B*). No intraspecific variability was detected in the cpDNAs of these *Bromus* species. The absence of intraspecific variation in cpDNA patterns is illustrated for 26 individual plants of *B. arvensis* (Fig. 2). The absence of intraspecific cpDNA polymorphism in *Bromus* implies that within-species sampling is unnecessary when using cpDNA as a biosystematic tool in congeneric studies of *Bromus* as expressed by Harris and Ingram (1991). Chloroplast DNA polymorphism within a species can be a problem in phylogenetic studies since cladistic analysis of restriction site data are very sensitive to character-state changes (Fitch 1984).

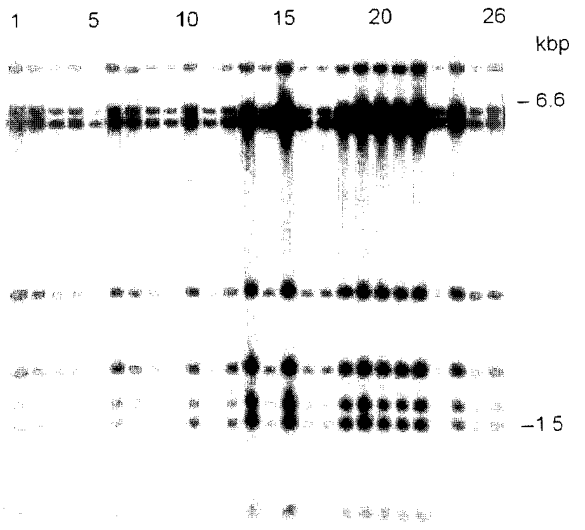

Fig. 2. Southern hybridization patterns showing absence of intraspecific cpDNA variation in *Bromus*. Total DNA from 26 plants of *B. arvensis* was digested with *Hin*dIII and hybridized with the P2 barley cpDNA clone. The numbers represent sizes [kbp] of specific DNA fragments.

There are relatively few studies within the grass family that have used cpDNA restriction analysis to determine the mode of plastid DNA inheritance. Maternal inheritance of cpDNA was demonstrated in *Zea mays* × *Z. perennis* (Conde *et al.* 1979), *Triticum aestivum* × *Secale cereale*, *T. durum* × *S. cereale*, *T. timopheevii* × *S. cereale* (Vedel *et al.* 1981) and *Oryza glaberrima* × *O. rufipogon*, *O. longistaminata* × *O. sativa* and *O. rufipogon* (Dally and Second 1990). However, Dally and Second (1990) reported the presence of some degree of paternal cpDNA inheritance in *Oryza*. Organelle DNA inheritance tends to be more complex in intergeneric hybrids. For example, in *Festuca pratensis* × *Lolium perenne*, all F_1 hybrids exhibited maternal inheritance of cpDNA. However, when the intergeneric F_1 hybrid was backcrossed to *L. perenne* both uniparental-maternal and uniparental-paternal cpDNA inheritance occurred in different backcross hybrids (Kiang *et al.* 1994).

The exact mechanisms for uniparental-maternal inheritance of cpDNA in the *Bromus* crosses are not known. A number of ideas have been proposed for the maternal transmission of plastids in higher plants (Sears 1980, Hagemann and Schroder 1989). These include *1*) exclusion of plastids from the male gamete during spermatogenesis, *2*) the loss of plastids from the male gamete, *3*) the exclusion of plastids during fertilization, and *4*) the degradation of plastids or their DNA following gametic fusion. Plastid transmission can also be affected by the plastid genome (Chiu *et al.* 1988), by plastome-genome interactions (Chiu and Sears 1993) or by the influence of nuclear genes (Tilney-Bassett 1988, Derepas and Dulieu 1992). A growing body of evidence is emerging to show that organelle DNA inheritance may be controlled by different mechanisms in different crosses within different species.

The results of this study demonstrated that the F_1 interspecific hybrid plants of *B. arvensis* × *B. inermis* and *B. arvensis* × *B. erectus* had cpDNA patterns of the maternal *B. arvensis* parent providing clear evidence for uniparental-maternal inheritance of cpDNA in the *Bromus* material studied.

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