

Complete nucleotide sequence of the intergenic spacer between 25S and 17S rDNA in *Miscanthus sinensis* var. *glaber*

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Abstract. The nucleotide sequence of the intergenic spacer (IGS) between the 25S and 17S ribosomal RNA genes of *Miscanthus sinensis* var. *glaber* was determined. The spacer length (2,178 bp) in *Miscanthus* is close to that of rice IGS (2,140 bp). Many repeated elements with consensus lengths of 34 and 59 base pairs are found in the nontranscribed region (NTS), upstream of the putative transcription initiation site (TIS). Subrepeats B with four copies are longer and better conserved than subrepeats A with ten copies. The sequences of the external transcribed region (ETS) show high identity, ranging from 66% to 76%, among *Miscanthus*, rice, maize, and wheat.

Keywords: Intergenic spacer (IGS); *Miscanthus sinensis* var. *glaber*; rDNA gene; Repeated elements; Transcription initiation site.

Introduction

The ribosomal DNA family with units coding for 17S, 5.8S, and 25S RNAs are tandemly arranged in plant nuclear genomes. The intergenic spacer (IGS), located between the 25S and 17S rRNA genes and consisting of a non-transcribed region (NTS) and an external transcribed spacer (ETS), has been found useful for population genetics (Karvonen and Savolainen, 1993) and pathogenic studies (Appel and Gordon, 1996) as a molecular marker. In plants, the structural and functional characterization of this spacer region has been studied in species belonging to gymnosperms, e.g., white spruce (Beech and Strobeck, 1993); dicots, e.g., *Daucus carota* (Suzuki et al., 1996), *Eruca sativa* (Lakshmikumaran and Negi, 1994), and potato (Borisjuk and Hemleben, 1993), and also in monocots, e.g., wheat (Lassner and Dvorak, 1986) and maize (McMullen et al., 1986). Subrepeats characterizing the intergenic spacer region play a critical role in regulating rRNA transcription according to experimental studies on *Daucus* (Suzuki et al., 1996), maize (McMullen et al., 1986), and *Arabidopsis* (Gruendler et al., 1991). Although concerted evolution has been shown to be a common evolutionary phenomenon for the homogeneity of the numerous rDNA repeats (Lassner and Dvorak, 1986), length polymorphisms caused by the variation in the numbers of such subrepeats are commonly found in plant populations such as *Eruca* (Lakshmikumaran and Negi, 1994) and maize (Rocheford, 1994).

Here we present the entire nucleotide sequence of the intergenic spacer between the 3' end of the gene for 25S rRNA and the 5' end of the gene for 17S rRNA in *Miscanthus sinensis* var. *glaber* (Poaceae), one of the commonest weeds in Taiwan (Chou and Ueng, 1991) and a relative of *Sorghum* and *Saccharum*.

Materials and Methods

Specimen and DNA Extraction

Plants of *Miscanthus sinensis* var. *glaber* were collected from Taiwan's Southern Cross-Island Highway at an elevation of 750 m. Fresh leaf tissue was ground in liquid nitrogen and kept in a freezer at -70°C. Genomic DNA was extracted following a CTAB methodology (Doyle and Doyle, 1987).

PCR Amplification and T-A Cloning

PCR amplification was carried out using a pair of primers (Figure 1), which were designated according to the conserved sequences of 25S and 17S rRNA genes of rice [Takaiwa et al., 1990; EMBL accession no. X54194] and maize [McMullen et al., 1986; EMBL accession no. X03990], on a MJ PTC-100 Thermal Cycler. A one-hundred µl reaction was performed by adding 4 U Taq polymerase (Promega). PCR amplification conditions were as follows: 30 cycles of 94°C denaturing for 45s, 49°C annealing for 1 min, and 72°C extension for 3 min, followed by 72°C extension for 10 min and 4°C for storing. PCR products were separated and eluted using agarose gel purification (BM) and ligated to a pT7 Blue T-vector (Novagen). Plasmid DNA was purified using a Wizard Plus SV kit (Promega) and quantified.

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⁴The nucleotide sequence data will appear in the EMBL nucleotide sequence databases under the accession number AJ001978.

1 25S rRNA ←-| 70
 CCCC~~CGT~~CGCACGGATTGTCCTCCCCCTCCACTCTCGCGGGTTGAAGTCAGGCTGGGAGTCCGCGTG
 primer 25S
 71 140
 ACCGTAGCCGACCCAAGTTCATATGGTCA~~AAAAACAGCAGTTCGGCCAAGGCGTCTGGGCCAAAAACAGC~~
 A1 | A2
 141 210
 AGTTCGGCCGAGGCGTCCGTAAGCAGCCCAAGTCCC~~CGGAAAGCCGAGTGTCTGACGGTTGACGGGAT~~
 B1
 211 280
 TCGGCTTGGGCA~~AAAAACAGCAGCTCGGCCGAGCGACATCAGCCAAAAACAGCAGTTCGGCCGAGGCGTTC~~
 A3 | A4
 281 350
 C~~GT~~AAGCAGCCCAAGTCCC~~CGGAAAGCCGGGTTTCGTACGCGTGACTGATGTTCGGCTTGGGCAAAAAACA~~
 B2 | A5
 351 420
 CAGCTCGGCCAAGCGTCCAGGCCAAAAACAGCAGTTCGCCGAGCGTCCC~~CGTGAGCAGCCCAAGTCCC~~
 | A6 B3
 421 490
 AAACCGGGTGTCTGACGCGTGCAGGATGTGCGGCTTGGGCA~~AAAAACAGCAGCTCGCCBAGGCATCCTGGC~~
 A7 | A8
 491 560
 CAAAAACAGCAGTTCGCCGCGCGTCCGTGAGCAGCCCAAGTCCC~~CGGAAAGCCGGGTTTCGTACGCGTG~~
 B4
 561 630
 ACGGATGTGCGGCTTGGGCA~~AAAAACAGCAGCTCGGCCGAGCGACATCGGTTCCGGCAAGCCGGTCCGCGCT~~
 A9
 631 700
 CGGGCTACACACGACTTAAGTGCCACCGCACCGTACACCACAGGGGCTAAGCGTGTATGCGCGGTCCGGAT
 701 770
 GGACTTTTTTTTTTGCATGTGCGCGGTTCGGATGGACTTGCCTGTAAGCGTGTGTGCGCGGCGAGACTCCC
 751 840
 CGTGTAAAGTGTATGCCCGGTTCGATGACCTTGGCGTGCATACCCGGTCCACCAGCTGTTACCCACCGTA
 841 910
 CACACGACCGCGTGGTTCGGATGGACTTGCCTGCGTGTATGCGCGGTTGGTGGATGGATGGCGACGAACG
 911 980
 GCTAGGACTCGGACCGCGGTGATGGATATGCCTCACGAGGTACGGGAGAAGGACAATATGACGTCGGG
 981 1050
 ACAAACGATGGCGAAACGGGACCCGGTCCCACCGGGGTTGCCACGGGGCCGGTCCATCCGCGGAAAAAC
 1051 1120
 AACGAATGCGGTGCCCTCCTCGCGGAAACCTGCGTGTCTTGCCGAAACCATTGA~~AAAACTACGTATCA~~
 A10
 1121 1190
 CATGGATGTCTGAGGTCTCAAACACTGAAGAAACCATACAGAAAACCTGACAGAACCTCCTGGTAGTG
 1191 1260
 GGTTTGCCCATCTGGCACATTAGGGGGTGTATGTCGGTTGCCTGGGCGCTCGGGTAAATGCTTCGAAAC
 *
 1261 1330
 CACTAGTGTTCGATGACCCGCTAATGAGGCTCCACCAAATGTCGGTACGCCCCAGCGCCCGTGGTGC
 1301 1400
 GCAGGCGGCCGTGCCGTAGCTTCGGCTGGCTGCCAACGGGCTGCTGTGCCCCAGCCACATCGCCAT
 1401 1470
 ATCCCGACCCGTGAGTGGCAGAAGTTTTGGTGGCAGCTAGCTAGCTGGTGCCACGTGTGTGAAGTGT
 1471 1540
 TTCGTGACTCAGCCCTCCCTTGTCTGCCGCCAGCTACCGGTGCCGTGCTTCTATGAACGTGCTGCTGTG
 1541 1610
 GGCAGCTGGCTGCGTGGGTGTGTAGCTTGTGTTGAACAGTTTTCGGTGATGCACGCCCTCCCTGGCCGGC
 1611 1680
 CGGCAGCTGTGTTGGCTGCTTCTGTGGCGCTGGCCCGGTTGATGGTTAAGTTTTGGTATCTCCCGTG
 1681 1750
 CTCTGTGGACTTCGGTTGCTGCTCCTCGCAAGCAGACACACCGTGCCTTGC CGGAGTGATGCTTTGGATG
 1751 1820
 GCTTAACCGTCGTGGCTACCTGGCGCATGAGTTGTTTAGACCTGTGACTGCTTGGAGACCCCTACCG
 1821 1890
 ATGTGTGGCCGACTCACGGCGCAGTGTCCGTTTCATTCGCGGGGCATGTTTTGCCTGCTGCATTGACAA
 1891 1960
 GTCGTTCTGCTCTACCTGTTGCATTTGGAAGTTGTGCTCATTACATGTTGTTTACGTCGAGAACATCCTC
 1961 2030
 TGGGTGTGGCTCGTCGGCTACAAGCGCTTGTGCGTTCGCCCGTGTGCCGTCGCCCATGTCCGCGAGCG
 2031 2100
 CTAAGGACACCTCGCTTGTGCTCTTGGTCTTGGATGAGGCTGATGCTGAAGGCTGGAGGGCGTTACGCTC
 2101 2170
 CCCGTGCCATAAAATTCAGTCCGTTTGGAAACAACACAGCCAGCTCCTGGCCGATGCCTCGTGTGGAGG
 2171 2212
 CTGGTGTGGCTCGTCGATGAGGACGCTGCTACCTGGTTGATC
 primer 17S |→ 17S rRNA

Figure 1. Nucleotide sequence of the intergenic spacer (IGS) between 25S and 17S rRNA genes of *Miscanthus sinensis* var. *glaber*. Subrepeats A (A1 to A10) and B (B1 to B4) are underlined and indicated. Positions of rRNA genes, i.e. the 3' end of 25S rRNA and 5' end of 17S rRNA, and the primers for amplification are indicated. The putative initiation transcription site (ITS) is printed in bold letters and indicated by an asterisk.

Nucleotide Sequencing

Cycle sequencing with Taq polymerase was performed by ³²P radioactive labeling using the fmol™ Sequencing System (Promega). The two strands were sequenced from both ends, using primers for PCR amplification and three internal primers (not shown), with overlaps of about 100 bp.

Results and Discussion

The intergenic spacer with 2,178 base pairs (Figure 1) of *M. sinensis* var. *glaber* was amplified and sequenced. Like many other higher plants, the intergenic spacer region of *Miscanthus* is characterized by repeated sequences, which are located upstream of a putative RNA polymerase I transcription initiation site (TIS) (Figure 1) (Borisjuk and Hemleben, 1993). At least two classes of repeated sequences can be identified in this region, designated as A subrepeats and B subrepeats (Figure 1 and Figure 2). The consensus length of subrepeats A (34 bp) is shorter than that of subrepeats B (59 bp). A conserved short sequence AAAAAC (7–12; Figure 2) distributed in the ten copies of the subrepeats A may act as a promotor for transcription (cf. Borisjuk and Hemleben, 1993). A three-base deletion and two other single deletions make the A10 sequence shorter and less similar (55.9% identity to the consensus sequence) to the other copies. Subrepeats B (159–574) with four copies are not only longer but also better conserved (93.2% to 96.6%) relative to subrepeats A (55.9% to 94.1%). That no repeated element was found

in the region downstream of TIS in *Miscanthus* is congruent with reasearch on potato (Borisjuk and Hemleben, 1993) and maize (McMullen et al., 1986) IGSs.

Similar IGS lengths are found in *Miscanthus* (2,178 bp) and rice (2,140 bp), which are only two-thirds the lengths (3,018 bp) in maize. Owing to weak functional constraints, the sequences of the nontranscribed spacer (NTS), slightly upstream of the putative transcription initiation site, show high divergences among *Miscanthus*, rice, maize, and wheat. Quite a low NTS region identity is shared by the above grasses. In contrast, the external transcribed spacer (ETS), especially the region lying upstream from the 5' end of 17S rRNA gene, has better conserved nucleotide sequences. The identities based on computer alignment are 76.0%, 71.1%, and 66.3% between *Miscanthus* and maize, wheat, and rice, respectively. The difference in the degree of conservation between ETS and NTS has thus made the intergenic spacer region useful for elucidating either the phylogenetic relationships of species complex (Lanadu et al., 1992) or the genetic diversity at population level (Karvonen and Savolainen, 1993).

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	1	10	20	30	34
CONSENSUS	TGGGC	AAAAACAGCAGT	TTCGG	CCGAG	G-CGTCC
A1	...	T	A
A2	C		
A3		C	-...A.A
A4	.CA.	C		T
A5	A	C	A	-.....
A6	A.-	C	-	-.....
A7		C	-	A.....A
A8	C	-	-.....
A9		C	C.A.A.G
A10	..---	TA.-	ATCA	-AT...AT....

CONSENSUS:	1	10	20	30	40	50	59
	GTAAGCAGCCCAAGTCCCGGGAAAGCCGGGTGTCGTACGCGT						GACGGATGT
	G			T			G
B1	..A	A	G	T-
B2	..A	-	T	T-
B3	..G	A	--	G	G.....
B4	..G		T		G.....

Figure 2. Sequence comparison of subrepeats A and B shown in Figure 1. Dots (•) indicate the same nucleotides and gaps (-) are introduced to maximize homology. Consensus sequences were determined from the repeated elements.

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25S / 17S rDNA intergenic spacer region in *Pinus sylvestris* L.

Genetic diversity of the 25S/17S rDNA intergenic spacer region in *Pinus sylvestris* L.

¹ *Pinus sylvestris* L. var. *maritima* Mill.

² *Pinus sylvestris* L. var. *resinosa* Ait.

The 25S/17S rDNA intergenic spacer region of *Pinus sylvestris* L. was amplified by PCR. The length of the spacer region was 2,140 bp in *Pinus sylvestris* L. var. *maritima* Mill. (ETS) and 34/59 bp in *Pinus sylvestris* L. var. *resinosa* Ait. (ETS). The spacer region was highly polymorphic. The length of the spacer region was 10 bp in *Pinus sylvestris* L. var. *maritima* Mill. (ETS) and 66% of the spacer region was 76% of the spacer region in *Pinus sylvestris* L. var. *resinosa* Ait.

The intergenic spacer region (IGS) of the 25S/17S rDNA in *Pinus sylvestris* L.