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NUCLEOTIDE SEQUENCE OF A 5.8S RIBOSOMAL RNA GENE AND INTERNAL TRANSCRIBED SPACER OF *IMPERATA CYLINDRICA* (L.) BEAUV. VAR. *MAJOR* (COGONGRASS)

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The entire nucleotide sequence of internal transcribed spacer (ITS) region between 18S and 26S ribosomal RNA (rRNA) genes of Cogongrass [Imperata cylindrica (L.) Beauv. var. major (Nees) Hubb] was amplified by polymerase chain reaction (PCR). The primers for PCR,* IT1-5'CGTAACAAGGTTTCC 3' *and* ITS2-5' AGTTTCTTCTCCTCC *were designed for amplification and sequencing. Comparing the sequence of ITS in Cogongrass with that of other higher plants, the length of PCR product was 693 bp, in which there was 67 bp for 26S rRNA, 42 bp for 18S rRNA, and 584 bp for the length of ITS region. The ITS region comprises 205 bp for ITS1, 163 bp for 5.8S rRNA and 216 bp for ITS2. In addition, between ITS1 and ITS2 there were five type repeat sequences (6 to 8 bp for each).

Key words : Cogongrass, ITS, rRNA, repeat sequence

INTRODUCTION

In higher eukaryotes, ribosomal RNA genes (rRNAs) are organized as families with repeated genes in tandem arrays at the nucleolar organizer regions of chromosomes. The copy number of repeated genes in rDNAs usually reveals from 100 to 1,000 copies per diploid cell in animals, while it carries from 500 to 40,000 copies per diploid cell in plants. Each repeating unit commonly consists of the transcribed region coding for 5.8S, 18S and 26S rRNAs and the intergenic spacer, which consists of the transcribed spacer and non-transcribed spacer regions between 18S and 26S rDNAs (Kato et al. 1990, Perry and Palukaitis 1990, Appels et al. 1980, Waldron et al. 1983, Rogers and Bendich 1987, Taira et al. 1988). The precursor RNA (transcription unit) undergoes a series of processing mature 5.8S, 18S and 26S rRNAs (Cordesse et al. 1990, D'Ovidio 1992). The 18S rRNA gene of each transcription unit is separated from the

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26S rRNA by a non-transcribed intergenic spacer (IGS), whereas the 5.8S rRNA gene is separated from the 18S rRNA gene and from the 26S rRNA gene by internal transcribed spacers (ITS), named ITS1 and ITS2, respectively (Takaiwa et al. 1985a, Barker et al. 1988, D'Ovidio 1992).

The mature 18S, 5.8S and 26S rRNAs and ribosomal protein assemble ribosome subunits. Therefore, rRNA plays a central role in protein biosynthesis, and it is important for growth, development and reproduction of organisms (Stern et al. 1989). In addition, the nucleotide characterization of the ITS1 and ITS2 should play an important role in rRNA processing and in the control of ribosomal production (D'Ovidio 1992, Baldwin 1992, Liu and Schardl 1994). Regarding phylogeny, different regions of the rDNA can be used to examine lineages with different levels of divergence (Suh et al.1993). One of the advantages of rDNA as a phylogenetic tool is that the repeat unit consists of several regions that have different rates of sequence evolution. As a result of functional and structural constraints, rRNAs show strong evolutionary-conservation. Therefore, 5.8S, 18S and 26S rRNAs sequence are useful for elucidating phylogenetic relationships among diverse organisms (Clark 1987, Pace et al. 1986). On the contrary, the IGS and ITS region show much divergence, suggesting that it is useful for comparisons among closely related organisms or for the study of microevolutionary process among populations, or even within population (Baldwin 1992, 1993, Schaal and Learn 1988, Ritland and Straus 1993, Liu and Schard*l* 1994).

MATERIALS AND METHODS

Total DNA extraction

Total cellular DNA was prepared from the fresh leaves of Cogongrass [Imperata cylindrica (L.) Beauv. var. major (Nees) Hubb] (Shure et al. 1983). Approximate amount of DNA yields was determined by a spectrophtometer (Hitachi U-2001), then the DNA sample was stored in a freezer at -20 °C.

PCR primers

Oligonucleotides used for PCR priming were designed from conserved regions of the 5'end of 18S rDNA sequence and the complementary sequence of 3'end of 26S rDNA from sequences previously described (Takaiwa et al. 1984, 1985b, Kiss et al.1989a, Kiss et al. 1989b). Two primer sets for amplifing ITS of rDNA were designated as IT1-5' CGTAACAAGGTTTCC 3' and IT2-5' AGTTTCTTCTCCTCC 3' (Fig. 1).

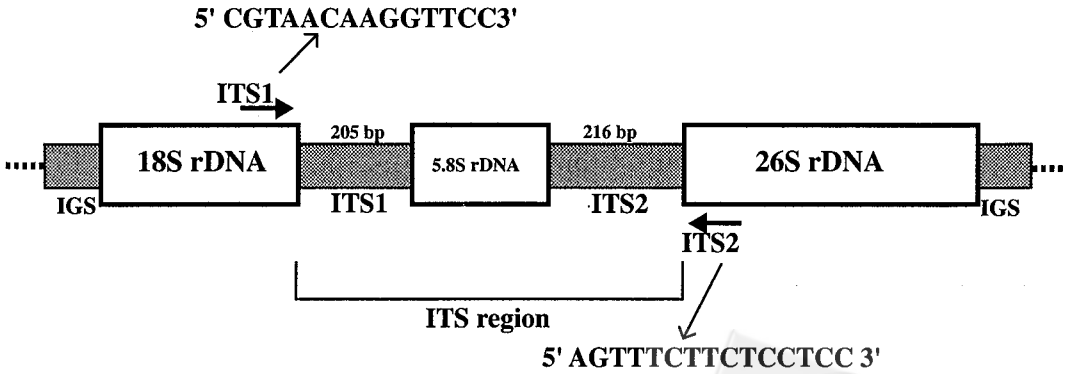

Fig. 1. The structure of nuclear DNA in Cogongrass. The positions of internal transcribed spacer (ITS) regions relative to 18S, and 26S rRNA genes and the intergenic spacer (IGS). Relative positions of primers used for PCR and sequencing are indicated.

PCR amplification

PCR reaction was performed by using a 50 μl mixture, containing 10 mM Tris-HCl pH 9.0, 50 mM KCl, 2 mM $MgCl_2$, 0.01% BSA, with four dNTPs (0.2 mM each), primers (0.5 μM each), 2.5 units of Taq DNA polymerase (Promega) and 80 ng genomic DNA, and 50 μl volume mineral oil. Amplification reactions were done in a dry-block 2 step thermal cycles. In the first step, the mixture was incubated at 94°C for 5 min, 10 cycles of denaturation at 94°C for 45 sec, annealing at 52°C for 20 sec and extension at 72°C for 1 min. Second step was conducted by processes as follows: 30 cycles of denaturation at 94°C for 45 sec, annealing at 50°C for 20 sec and extension at 72°C for 1 min, then, a final extension for 10 min at 72°C. Those reaction samples were detected by agarose gel electrophoresis of 10μl of PCR products (0.8%, w/v in TAE), staining by 0.5 μg/ml of ethidium bromide and photographed under the exposure of UV light.

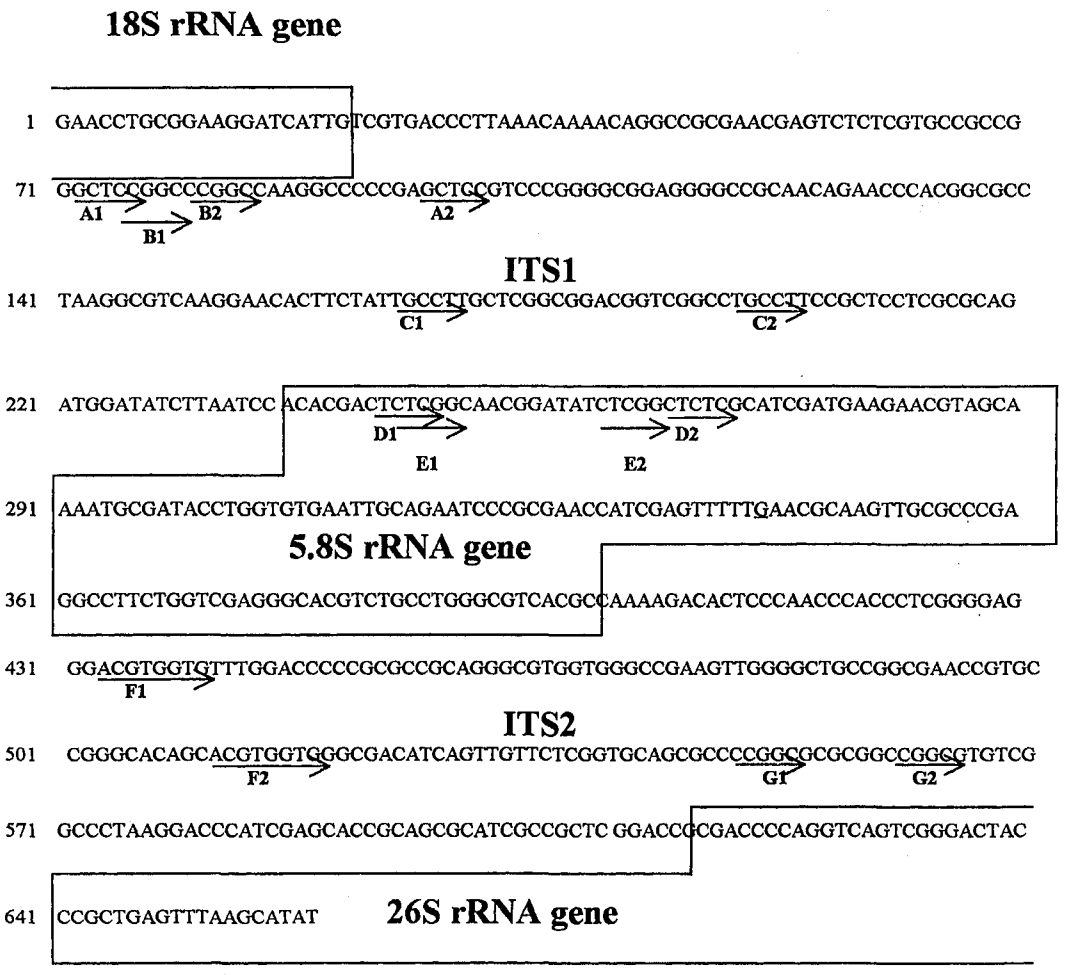

Fig. 2. Nucleotide sequence of the Cogongrass 18S-26S rRNA region. 3' end of 18S rRNA gene, 5.8S rRNA gene and 5' end of 26S rRNA gene are boxed. The arrows show the repeat sequence (type A to G). The nucleotide sequence data reported will appear in GenBank under the accession number AF 092512.

DNA cloning and sequencing

PCR product of ITS in *I. cylindrica* was recovered by glassmilk, cloned into T-vector (Promega) and transformed into the *E.coli* strain 'JM109' competent cell (Promega). After bacteria culture and plasmid extraction (Boehringer Mannheim, Qiagen-tip 20), the DNA sample was sequenced by the dideoxy chain-termination method using the Auto Read Sequencing Kit (Pharmacia). The aforementioned reaction protocols were recommended by the manufacturers.

RESULTS AND DISCUSSION

DNA fragment amplified from PCR using primers IT1 and IT2 was completed by using the DNA sample of Cogongrass. It showed a single band product when separated on an agarose gel. The band was sequenced with the amplification primers, and the length of sequence was 693 bp. Comparing nucleotide sequence as previously reported, it comprises 67 bp for 26S rRNA gene, 42 bp for 18S rRNA gene, and 584 bp for ITS region, including 205 bp for ITS1, 163 bp for 5.8S rRNA and 216 bp for ITS2 (Fig. 1 and 2). The length and G+C content of ITS region of Cogongrass were typically corresponding to the region of other higher plants. Several reports of ITS in higher plant are summarized in Table 1. In the previous reports of ITS region of higher plants, the length of 5.8S rRNA was ranged 162-167 bp, and the lengths of ITS1 and ITS2 were variable. The length ranged from 196 to 272 bp and from 186 to 239 bp for ITS1 and ITS2, respectively. There is neither a specific note that whether the length of ITS1 should be longer or shorter than that of ITS2 in higher plants nor the G+C content for these two sequences. The lengths of sequences for ITS1, ITS2 and G+C content revealed diverse for both mono- and dicotyledon (Table 1).

The ITS1 sequences are too diverse to unambiguously align among different families. In spite of this variability, it still reveals conserved sequence, GGCRY-(4 to 7n)-GYGYCAAGGAA (where Y=C or T, R=G or A), which are present in ITS1 of many higher plant species. The above conserved motif within ITS1 may have a key function in processing of rRNA gene transcripts (Liu and Schardl 1994). In Cogongrass, we also found GGCGC-GCGTCAAGGAA sequence in ITS1 (Fig. 2). Furthermore, we found several type repeat sequences (6 to 8 bp for each) in ITS1, 5.8S and ITS2 region, there were three type 6 bp repeat sequences (type A,B and C), two type repeat sequences [type D (6 bp) and E (7 bp)], and two type repeat sequences [type F (8 bp) and type G (6 bp)], respectively (Fig. 2). Although there were numberous observations of IGS length variation within plant species and an individual plant, by way of containing the numbers of different repeat sequence (Waldron et al. 1983, Rogers and Bendich 1987, Kato et al. 1990, Beech and Strobeck 1993, Borisjuk et al. 1994). However, few reports discussed the ITS length variation of rDNA. Sun et al. (1994) sequenced five clones of *Sorghum nitidum*. Two of them have a deletion of about 40 bp in ITS2 region. Venkateswarlu and Nazar (1991) indicated that CTCTCTCCT and CCG repeat sequence in ITS1 and CGC repeat sequence in ITS2 were revealed in tobacco. The copy numbers of ITS sequences might be changed by gene conversion or unequal crossing over (Beech and Strobeck 1993). Karvonen et al. (1994) also demonstrated that the length variation of ITS within and between species of *Picea* was common.

In evolutionary view, different regions of rDNA can be used to examine phylogeny to see the different levels of divergence (Suh et al. 1993). ITS1 and ITS2 are suitable to construct phylogeny at the genetic or species levels (Baldwin 1992, Schilling and Panero 1996, Suh et al. 1993, Bogler and Simpson 1996, Sun et al. 1994, Nickrent et al. 1994,

Table 1. Presented Cogongrass and other higher plants reported in the length and G+C content in ITS of rDNA.

Name	Family	ITS 1		5.8S rRNA		ITS 2		Source of sequence
		Length (bp)	G+C (%)	Length (bp)	G+C (%)	Length (bp)	G+C (%)	
Monocots								
Imperata cylindrica	Gramineae	205	65.9	163	57.1	216	71.8	This study
Oryza sativa	Gramineae	196	72.7	163	59.5	232	59.5	Takaiwa 1985a
Bromus inermis	Gramineae	219	55.3	163	58.9	215	59.1	Hsaio 1993
Sorghum bicolor	Gramineae	209	56.5	163	57.1	216	68.5	Hsaio 1993
Triticum aestivum	Gramineae	222	62.2	163	59.5	217	61.3	Chatterton et al. 1992
Agave dasylirioides	Agavaceae	250	65.2			239	70.2	Bogler and Simpson 1996
Dicots								
Populus deltoides	Salicaceae	214	66.8	164	55.4	207	69.5	D'Ovidio 1992
Mimulus glaucescens	Scrophulariaceae	206	44.0	162	50.6	225	47.0	Ritland and Straus 1993
Daucus carota	Apiaceae	215	49.4	164	54.2	224	52.3	Yokota et al. 1989
Apioideae	Apiaceae	204-221	49.1-57.7			216-226	42.7-59.6	Downie and Katz-Downie 1996
Vicia faba	Leguminosae	235	51.9	164	50.6	208	49.6	Yokota et al. 1989
Arceuthobium americanum	Viscaceae	209	34.0	167	41.9	227	30.4	Nickrent et al. 1994
Paeonia	Ranunculaceae	267	54.3-56.6	164	53.7	220	57.2-59.5	Sang et al. 1995
Gossypium	Malvaceae	293-294	58.0			210-226	61.0	Wendel et al. 1995
Madiinae	Compositae	255-261	47.7-51.4	164	51.2-53.7	216-223	49.5-53.0	Baldwin 1992
Vigna radiata	Leguminosae	205	60.0	163	52.8	220	59.1	Schiebel and Hemleben 1989
Nicotiana rustica	Solanaceae	216	69.4	163	55.2	217	65.4	Venkateswarlu and Nazar 1991
Lycopersicon esculentum	Solanaceae	236	62.3	163	55.2	224	68.3	Kiss et al. 1988
Sinapis alba	Cruciferae	265	50.6	163	52.8	188	54.3	Rathgeber and Capesius 1989
Canella winterana	Canellaceae	272	62.5	163	55.8	209	62.6	Youngbae et al. 1992
Cucumis melo	Cucurbitaceae	216	55.6	163	58.9	237	60.2	Kavanagh and Timmis 1988

Downie and Katz-Downie 1996). However, it is too divergent to construct the phylogeny based on ITS1 and ITS2 sequence in high level phylogeny (Bayer et al. 1996). In addition, the primers between 18S and 26S rRNA are easy to be designed to amplify the ITS region, because of the more conserved region of the evolutionary processes in 18S and 26S rRNA genes.

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