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The Heterogeneity of the Internal Transcribed Spacers (ITS) of rDNAs in *Imperata cylindrica* in Taiwan

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ABSTRACT: Ribosomal DNA for internal transcribed spacers ITS, ITS1, 5.8S rRNA gene, ITS2 and the adjoining regions of the 17S and 25S rRNA genes were obtained from 45 individuals of *Imperata cylindrica* (Cogongrass) in Taiwan via PCR amplification. The length of each PCR product was 692 bp, except for one sample (about 702 bp). All ITS DNA fragments were digested with *Eco*RV, *Cfo*I, *Ban*I, *Hae*III, *Msp*I, *Hinf*I, *Bst*OI, *Sau*96I, *Sty*I and *Eco*109I, respectively. The ITS, including ITS1, 5.8S rRNA gene and ITS2, were very heterogeneous within an individual of Cogongrass in Taiwan. Of 45 individuals of Cogongrass in Taiwan, the ITS1 and ITS2 regions showed at least eight type variants, and 5.8S rRNA gene showed at least five type variants among Cogongrass population in Taiwan based on PCR-amplified RFLP. In conclusion, the ITS repeat sequences from Cogongrass in Taiwan was very high heterogeneous.

KEY WORDS: Cogongrass, ITS, rDNA, Heterogeneity.

INTRODUCTION

Imperata cylindrica (L.) Beauv. var. *major* (Nees) C. E. Hubb. *ex* Hubb. & Vaughan [Cogongrass], an aggressive rhizomatous grass, distributed widely in many parts of the world from tropical to subtropical areas (AL-Juboory and Hassawy, 1980). It can be spread by both seeds and rhizomes (Wilcut *et al.*, 1988). The light thistle-like seeds of Cogongrass are capable of being transported over great distances, leading its wide and great distribution. The amount of seed production is tremendous, and seeds can well adapt the new environment (AL-Juboory and Hassawy, 1980; Dickens, 1974).

In higher eukaryotes, ribosomal RNA genes (rDNAs) 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 usually consists of the transcribed region (coding for 5.8S, 17S and 25S rRNAs) and the intergenic spacer (IGS) which consists of the transcribed spacer and non-transcribed spacer regions between 17S and 25S 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 to be mature 5.8S, 17S and 25S rRNAs (Cordesse *et al.*, 1990; D'Ovidio, 1992). The 17S rRNA gene of each transcription unit is separated from the 25S rRNA gene by an intergenic spacer, whereas the

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5.8S rRNA gene is separated from the 17S rRNA gene and from the 25S rRNA gene by internal transcribed spacers, named ITS1 and ITS2, respectively (Takaiwa *et al.*, 1985; Barker *et al.*,1988; D'Ovidio, 1992).

The mature 17S, 5.8S and 25S rRNA genes and ribosomal protein assemble ribosome subunits. Therefore, ribosomal RNA (rRNA) plays a central role in protein synthesis, and it is important in growth, development and reproduction of organisms (Stern *et al.*, 1989). The nucleotide characterization of the internal transcribed spacers (ITS1 and ITS2) should bring insight into the roles of these regions in rRNA processing and in the control of ribosomal production (D'Ovidio, 1992; Baldwin, 1992; Liu and Schardl, 1994). One of the advantages of rDNA as a phylogenetic tool is that the repeat unit consists of several regions, having different rates of sequence evolution. Therefore, the different regions of rDNAs can be used to examine lineages with the different levels of divergence (Suh *et al.*, 1993). As a result of functional and structural constraints, ribosomal RNAs show strong evolutionary conservation. Therefore, sequences of 5.8S, 17S and 25S rRNAs are useful for estimating phylogenetic relationships among taxonomically diverse organisms (Clark, 1987; Pace *et al.*, 1986). By contraries, the IGS and ITS regions show much more divergence, providing utility for comparison among closely related organisms or for microevolutionary process among populations even within population (Baldwin, 1992, 1993; Schaal and Learn, 1988; Ritland and Straus, 1993; Liu and Schardl, 1994).

In fact, the sequence comparison of the ITS of rDNA has been used to reconstruct the phylogeny of numerous plant families, such as Asteraceae (Baldwin, 1992, 1993; Bayer *et al.*, 1996; Kim and Jansen, 1994; Sang *et al.*, 1995; Schilling and Panero, 1996), Winteraceae (Suh *et al.*, 1993), Agavaceae (Bogler and Simpson, 1996), Poaceae (Sun *et al.*, 1994; Hsiao *et al.*, 1995), Viscaceae (Nickrent *et al.*, 1994), and Apiaceae (Downie and Katz-Downie, 1996). The ITS region has several advantages for phylogenetic sequence analyses, such as (1) the rate of evolution is suitable for studies at the specific and generic levels, (2) it is easy to align because of very little length variation at the generic level in flowering plants, (3) it can offer enough characters to reconstruct phylogeny, and (4) the primers are easy to design to amplify the ITS region (Bayer *et al.*, 1996). Several reports have revealed that the ITS variants (in sequence) were found within an individual by PCR-amplified sequencing (Sang *et al.*, 1995; Boysen *et al.*, 1996) or by restriction fragment length polymorphism (RFLP) (Wendel *et al.*, 1995). The technique of PCR-amplified RFLP was introduced first time to detect the variation of the chloroplast genes *rpo*C_1, and *rpo*C_2 among 14 species of *Astragalus* (Fabaceae) (Liston, 1992). Since then several reports have been appeared to show the genetic variation of chloroplast DNA (Ghareyazie *et al.*, 1995; Tsumura *et al.*, 1996; Lashermes *et al.*, 1996; Ziegenhagen and Fladung, 1997), or specific region of nuclear DNA in plants (Ghareyazie *et al.*, 1995), or mitochondrial DNA in animals (Wilding *et al.*, 1997).

The aim of this study was to elucidate the heterogeneity of the ITS of rDNA from *I. cylindrica* populations in Taiwan based on PCR-amplified RFLP instead of sequencing.

MATERIALS AND METHODS

Sites of sampling

Fifteen sampling sites selected for the present study are located at Fengshan (A), Potzu (B), Fengyuan (C), Chuangwei (D), Hualien (E), Chengkung (F), Yehliu (G), Sarlum (H), Hoshe (I), Kengting (J), Penghu (K), Lutao (L), Lanyu (M), Chuwei (N), and Nankang (O) (Fig. 1).

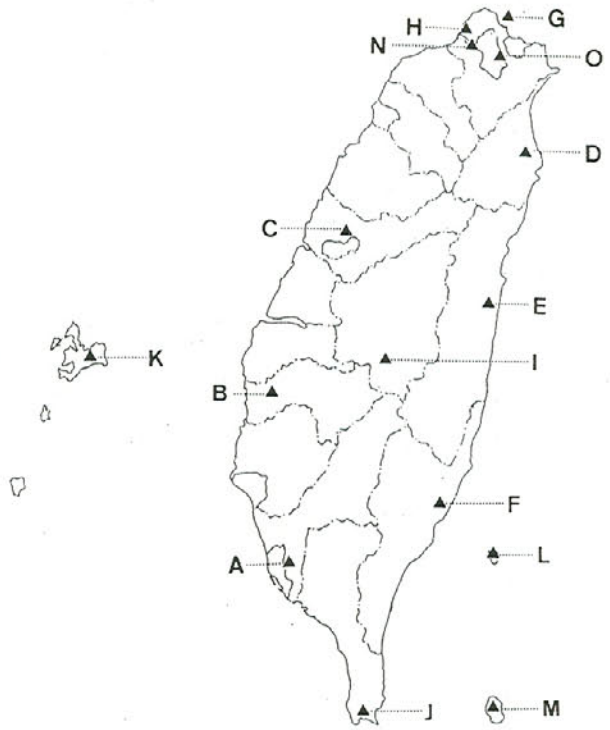

Fig. 1. The sampling locations of *I. cylindrica* in Taiwan. The alphabets indicate that the samples were analyzed in the present study. The abbreviations of sampling sites are: A (Fengshan), B (Potzu), C (Fengyuan), D (Chuangwei), E (Hualien), F (Chengkung), G (Yehliu), H (Sarlum), I (Hoshe), J (Kengting), K (Penghu), L (Lutao), M (Lanyu), N (Chuwei) and O (Nankang).

Plant materials

Rhizomes of Cogongrass collected from the aforementioned sites were transplanted into pots which were set in the greenhouse of the Institute of Botany, Academia Sinica at Taipei, Taiwan.

Preparation of total cellular DNA

Total cellular DNA from the leaves of transplanted Cogongrass was prepared by using an extraction technique modified from that of Shure *et al.* (1983). Point five grams of fresh leaves were harvested and ground to powder with liquid nitrogen in a mortar and pestle, then transferred into a 1.5 ml centrifuge tube containing 700 μL of urea buffer (8.0 M urea, 0.05 M NaCl, 0.05 M Tris-HCl pH 7.5, 0.02 M EDTA, 1% sarcosyl) preheated in 60℃ water. We mixed the sample throughly and incubated it in a water bath at 60℃, inverting the tube constantly. We, then, added 700 μL phenol: chloroform=1:1 (v/v, Tris pH 8.0 saturated), inverting many times gently, and centrifuged the tube at 10,000 rpm (Sigma 2K15; Nr12139) for 10 min at 4℃. After adding 0.7 volume of 2-propanol and 1/10 volume 4.4 M NH_4OAc, the supernatant was transferred to a new centrifuge tube by filtering through two layers of miracloth. The mixture in tube was centrifuged at 10,000 rpm for 10 min at 4℃, and the precipitated DNA was collected. The DNA pellet was resuspended with 400 μL TE (10 mM, Tris-HCl pH 8.0, 1 mM EDTA) and incubated with 5 μg DNase-free RNase (Sigma) for 10 minutes at 65℃. The RNase and remaining protein were extracted with an equal volume of

phenol: chloroform =1:1 (v/v, Tris pH 8.0 saturated) and centrifuged at 10,000 rpm for 10 min at 4℃. The supernatant was transferred to a new tube. Then, DNA was precipitated by the addition of a 1/10 volume 4.4 M NH_4OAc and three volumes of 95 % ethanol. Precipitated DNA was collected by centrifugation at 10,000 rpm for 10 min at 4℃, washed with 70 % ethanol twice, and dried before being redissolved in 200 μL of TE. Approximate amount of yields was calculated by a spectrophotometer (Beckman DU-20), and the DNA samples were stored at -20℃.

PCR amplification

Oligonucleotides used for PCR priming were designed from the conserved regions of the 3'end of 17S rDNA sequence and the complementary sequence of the 5'end of 25S rDNA (Takaiwa *et al.*, 1984, 1985; Kiss *et al.*,1989 a, b). Two primers for amplifying the ITS of Cogongrass rDNA were designated as IT1: 5' CGTAACAAGGTTTCC 3' and IT2: 5' AGTTTCTTCTCCTCC 3' (Fig. 2). PCR reactions were 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, dNTPs (0.4 mM each), primers (0.5 μM each), 2.5 units of *Taq* DNA polymerase (Promega Co.), 80 ng genomic DNA and 50 μL mineral oil. Amplification reactions were done in a dry-block with two-step thermal cycles. In the first step, the mixture was incubated at 94℃ for 5 min, and then 10 cycles of denaturation at 94℃ for 45 sec, annealing at 52℃ for 20 sec and extension at 72℃ for 1 min. In the second step, processes were conducted as follows: 30 cycles of denaturation at 94℃ for 45 sec, annealing at 50℃ for 20 sec, extension at 72℃ for 1 min, and then a final extension for 10 min at 72℃. PCR products were detected by agarose gel (0.8%, w/v in TAE), with 0.5 μg/mL of ethidium bromide, and photographed under the exposure of UV light.

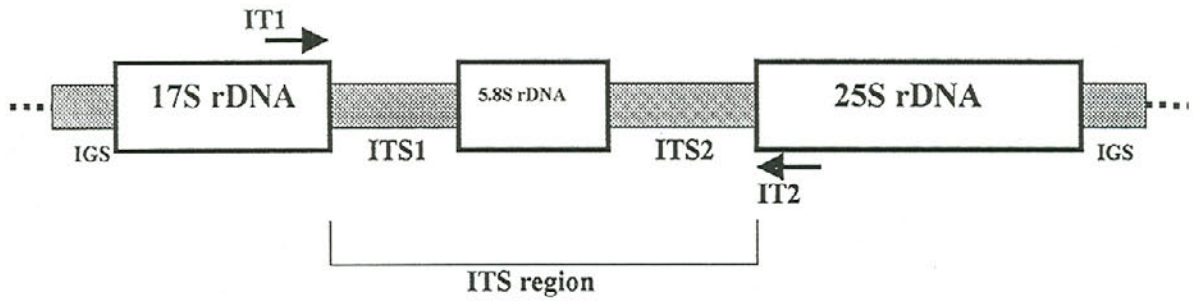

Fig. 2. The structure of ribosomal DNA in plants. The positions of internal transcribed spacer (ITS) regions relative to 17S, 5.8S and 25S rRNA genes and the intergenic spacer (IGS). Relative positions of primers (IT1 and IT2) used for PCR and sequencing are indicated.

Restriction enzyme digestion

DNA fragments amplified by PCR were digested with ten restriction enzymes, namely *Eco*RV, *Cfo*I, *Ban*I, *Hae*III, *Msp*I, *Hinf*I, *Bst*OI, *Sau*96I, *Sty*I and *Eco*109I. All restriction enzymes digested were carried out under the conditions recommended by manufacturers (BRL or Promega Co.), using 5-10 units of enzyme per microgram of DNA, and there were incubated for overnight.

Gel electrophoresis

Approximate 5-10 μg of digested DNA mixed with tracking dye [0.25% bromophenol blue, 40% (w/v) sucrose in water] were electrophoresed overnight at 50V-80V in 2.5% -4.0 % NuSieve 3:1 agarose gels (FMC Co.) with 0.5 μg/mL EtBr. The patterns of bands was examined under the UV light.

DNA labeling and Southern hybridization

The DNA bands on the gels was recovered by glassmilk (BIO 101 Co., Geneclean Kit II). Probes were labeled with digoxigenin. Prehybridization and hybridization were performed with a non-radioactive DNA labeling and detection Kit (Boehringer Mannheim). Detection of hybrid DNAs was achieved by chemiluminescent reaction using AMPPD on Kodak x-omat film. Immunological detection was also done using color solution (containing NBT solution and x-phosphate) on a membrane (Boehringer Mannheim Co.). The aforementioned reaction protocol was recommended by the manufacturers.

DNA cloning and sequencing

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

RESULTS

PCR amplification and the sequence of ITS region

The ITS regions from 45 individuals of Cogongrass were completely amplified during PCR using primers IT1 and IT2. The length of each PCR product is 692 bp, excepted for sample M2 (about 702 bp) (Fig. 3). The sequence of sample A3 represented the ITS (Fig. 4). The PCR product of sample A3 consists of 66 bp of 25S rRNA gene, 584 bp of ITS region, and 42 bp of 17S rRNA gene. The ITS region constitutes 205 bp of ITS1, 163 bp of 5.8S rRNA gene and 216 bp of ITS2. The PCR product of sample A3 was hybridized with the remaining PCR products of other samples, confirming that the sequences were also represented ITS (data not shown).

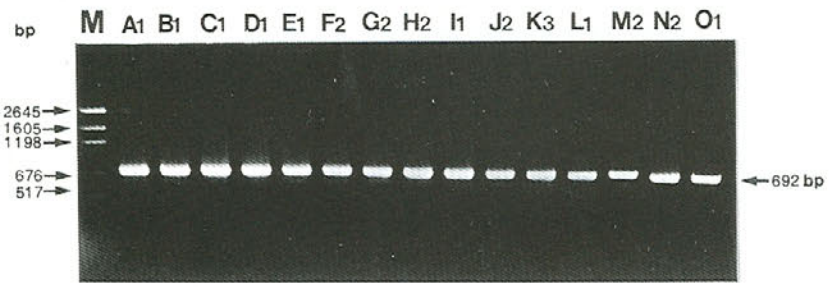

Fig. 3. The ITS region of rDNA in 15 populations of *I. cylindrica* in Taiwan. The ITS region was obtained by the PCR amplification of primers IT1 and IT2. The abbreviations of sampling sites see Fig. 1. The arabic numbers indicate different samples of each population.

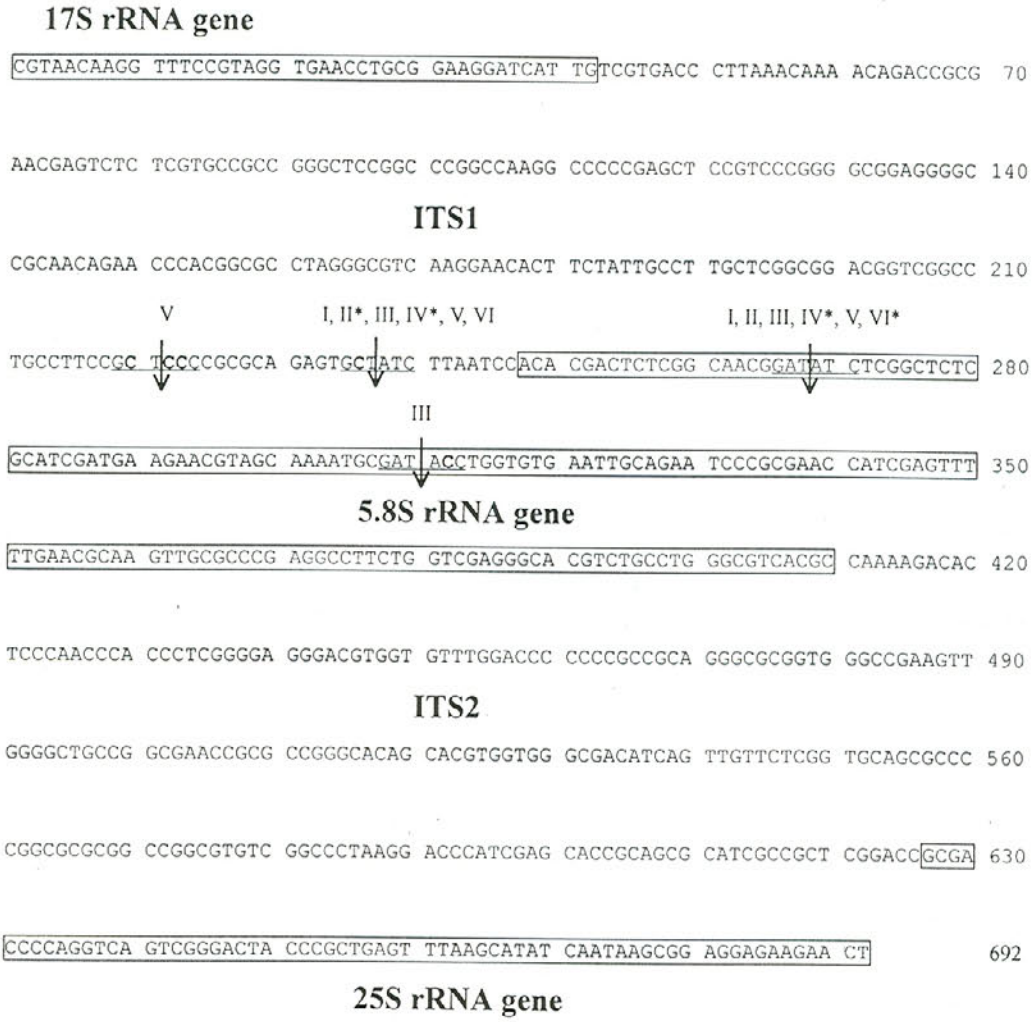

Fig. 4. Nucleotide sequence of the ITS region in Cogongrass (sample A3, belonging to type II). 3' end of 17S rRNA gene, 5.8S rRNA gene and 5' end of 25S rRNA gene were boxed, cutting with *Eco*RV among 45 samples revealed 6 types' pattern, namely type I (237 bp + 31 bp + 424 bp)(main type), type II (268 bp + 424 bp), type III (237 bp + 31 bp + 42 bp + 382 bp), type IV (692 bp), type V (221 bp + 16 bp + 31 bp + 424 bp) and type VI (237 bp + 455 bp). * indicated restriction site destroyed; recognition sites of *Eco*RV were underline; nucleotide substitutions were bold.

The digestion of ITS region

Each PCR product of the 45 individuals was digested with ten restriction enzymes, namely *Eco*RV, *Cfo*I, *Ban*I, *Hae*III, *Msp*I, *Hinf*I, *Bst*OI, *Sau*96I, *Sty*I and *Eco*109I, revealing 91 bands in total. Of them, 83 bands were polymorphic. The dendrogram was obtained by the UPGMA analysis (data not shown). Based on the dendrogram, no population differenciation was detected among the 15 populations in Taiwan. Seven of ten restriction enzymes, namely *Eco*RV, *Cfo*I, *Hae*III, *Msp*I, *Hinf*I, *Bst*OI, and *Sty*I were able to reveal more polymorphic bands in the ITS region of rDNA of Cogongrass, wheras *Eco*l09I digestion was less effective (Table 1). Furthermore, following digestion by restriction enzymes and separation on an agarose gel, minor and major bands were found, indicating that the ITS sequences of rDNAs were not different within an individual.

Table 1. The distribution of polymorphic bands in the ITS region from 45 individuals of Cogongrass, using ten restriction enzymes.

The band patten of various endonuclease digested (Type)	45 accessions of *I. cylindrica*																							
	A			B			C			D			E			F			G			H		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
***Cfo*I**																								
I: 159+69+138+109+35+47+8+45+82	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II: 228+329[b]+53+82																+	+							
III: 228+138+109+82+8+127																							+	+
IV: 475[b]+82+8+45+82																								
V: 228+237[b]+92+53+82[a]																								
VI: 159+69+247[b]+82+8+127																								
VII: 159+69+138+199[b]+45+82																								
VIII: 159+69+138+109+82+8+45+82																								
***Ban*I**																								
I: 533+159[a]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	
II: 390+302[b]																						+	+	+
***Eco*RV (bp)**																								
I: 237+31+424	+	+		+	+	+	+	+	+	+		+				+	+	+	+	+	+	+	+	+
II: 268+424			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+		+	+	+	
III: 237+31+42[b]+382[a]																+								
IV: 692[b]																								
V: 221+16+31+424																			+					
VI: 237+455[b]																								
***Hae*III**																								
I: 99+5+6+29+69+165+109+88 +12+110	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II: 99+5+6+29+69+274[b] +88+12+110																+	+							
III: 65+34+5+6+29+69+165+109 +88+12+110																								
IV: 110+29+69+165+109+88+12+110																								
V: 110+29+69+165+109+88+122																								
VI: 139+69+165+109+88+12+110[a]																								

	I			J			K			L			M			N			O		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
***Cfo*I**																					
I: 159+69+138+109+35+47+8+45+82			+	+	+	+	+	+	+	+	+	+			+	+		+			+
II: 228+329[b]+53+82	+					+												+	+		
III: 228+138+109+82+8+127																					
IV: 475[b]+82+8+45+82		+			+																
V: 228+237[b]+92+53+82[a]													+	+	+						
VI: 159+69+247[b]+82+8+127																+	+				
VII: 159+69+138+199[b]+45+82							+	+	+												
VIII: 159+69+138+109+82+8+45+82																		+			
***Ban*I**																					
I: 533+159[a]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II: 390+302[b]																					
***Eco*RV (bp)**																					
I: 237+31+424	+	+	+		+		+	+	+	+	+	+	+			+	+	+	+	+	+
II: 268+424		+		+	+			+	+	+	+		+		+						
III: 237+31+42[b]+382[a]	+					+								+					+	+	
IV: 692[b]																+	+				
V: 221+16+31+424								+													
VI: 237+455[b]		+																+			
***Hae*III**																					
I: 99+5+6+29+69+165+109+88 +12+110			+	+	+		+	+	+	+	+	+	+		+	+					+
II: 99+5+6+29+69+274[b] +88+12+110	+																		+	+	
III: 65+34+5+6+29+69+165+109 +88+12+110		+																			
IV: 110+29+69+165+109+88+12+110						+															
V: 110+29+69+165+109+88+122																+	+	+			
VI: 139+69+165+109+88+12+110[a]														+	+						

a: There was an approximately 10 bp insertion in sample M2 than others

b: Represent creating or destroying restriction site on 5.8S rRNA gene

Table 1. continued.

The band patten of various endonuclease digested (Type)	45 accessions of *I. cylindrica*																							
	A			B			C			D			E			F			G			H		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
***Bst*OI**																								
I: 313+85+236+58[a]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II: 634[b]+58																+	+							
III: 25+288+85+236+58						+																		
IV: 313+306[b]+15+58																								
***Eco*109I**																								
I: 480+212[a]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
***Hinf*I**																								
I: 74+178+76+364[a]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II: 74+178+440[b]																						+	+	+
III: 252+76+364																								
***Msp*I (bp)**																								
I: 89+7+5+25+372+13+49+11+121	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+		+	
II: 126+372+194																+	+							
III: 89+7+5+25+445+121																			+	+				
IV: 96+5+470+121																						+	+	+
V: 89+7+5+25+71+20+281+13+49+11+121																								
VI: 126+372+62+11+121[a]																								
VII: 89+7+5+25+71+301+62+11+121[a]																								
VIII: 89+422+49+11+121						+																		
***Sau*96I**																								
I: 98+11+28+318+25+101+8+33+70[a]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II: 98+11+346+25+101+8+33+70																								
III: 98+11+346+25+109+33+70																								
***Sty*I**																								
I: 105+587	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II: 395[b]+297																								
III: 69+623[a]																								
IV: 160+532	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	
V: 105+55+532	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	
VI: 178+514																+								

	I			J			K			L			M			N			O		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
***Bst*OI**																					
I: 313+85+236+58[a]			+	+	+	+	+		+	+	+	+	+	+	+	+		+		+	+
II: 634[b]+58	+	+			+														+	+	
III: 25+288+85+236+58																	+				
IV: 313+306[b]+15+58							+	+	+												
***Eco*109I**																					
I: 480+212[a]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
***Hinf*I**																					
I: 74+178+76+364[a]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II: 74+178+440[b]					+																
III: 252+76+364																+	+				
***Msp*I (bp)**																					
I: 89+7+5+25+372+13+49+11+121		+		+	+		+		+	+	+	+	+		+	+		+			+
II: 126+372+194	+	+														+	+	+	+	+	
III: 89+7+5+25+445+121																					
IV: 96+5+470+121																					
V: 89+7+5+25+71+20+281+13+49+11+121			+							+		+									
VI: 126+372+62+11+121[a]						+								+							
VII: 89+7+5+25+71+301+62+11+121[a]													+	+							
VIII: 89+422+49+11+121										+	+	+				+	+				
***Sau*96I**																					
I: 98+11+28+318+25+101+8+33+70[a]		+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+			+
II: 98+11+346+25+101+8+33+70																			+	+	
III: 98+11+346+25+109+33+70	+					+															
***Sty*I**																					
I: 105+587	+	+	+	+	+		+	+	+	+	+	+	+		+	+	+	+	+	+	+
II: 395[b]+297																+	+	+			
III: 69+623[a]						+							+	+	+						
IV: 160+532			+	+	+		+		+	+	+	+	+		+						+
V: 105+55+532			+	+	+		+		+	+	+	+	+		+						+
VI: 178+514																			+	+	

The heterogeneity of ITS 1, 2 and 5.8S rRNA gene

The different types of ITS sequences were revealed within an individual after restriction enzymes digestion (Table 1). For example, based on *Eco*RV digestion, the main type I consists of 237 bp, 31 bp and 424 bp DNA fragments. Of the 45 individuals, 36 revealed type I DNA pattern. The recognition sequence of *Eco*RV was GATATC. Based on the sequence and the electrophoresed DNA pattern of sample A3 digested with *Eco*RV, two DNA fragments of type II were deduced as 268 and 424 bp. Compared with type I pattern, one nucleotide of type II was substituted from A into C on ITS1 region, hence there was one extra restriction site on ITS1 region. Type III consisted of 237, 31, 42 and 382 bp DNA fragments. Compared with type I pattern, one nucleotide substitution was deduced (C changed into T) on 5.8S rRNA gene, one extra restriction site was created on 5.8S rRNA gene. Type IV showed no cutting site on ITS region, therefore, restriction site was destroyed once on 5.8S rRNA gene and ITS1 region, respectively. Type V consisted of 221, 16, 31 and 424 bp DNA pattern, one restriction site created on ITS1 region was deduced. Type VI consisted of 237 and 455 bp DNA fragments, one restriction site destroyed on 5.8S rRNA gene was deduced. Of them, types II, IV and V showed creating or destroying restriction sites on ITS1 region. In total, 32 of the 45 individuals showed the variant ITS1 sequence based on *Eco*RV analysis. Furthermore, types III, IV and VI showed creating or destroying restriction site on 5.8S rRNA gene. In total, 10 of the 45 samples showed variant 5.8S rRNA genes based on *Eco*RV analysis. Of the 45 individuals, 27 showed more than one type of DNA pattern of ITS sequences digested with *Eco*RV, and up to three types of ITS sequences within an individual, including samples F1, G1, I2 and K2 (Figs. 4, 5, and Table 1).

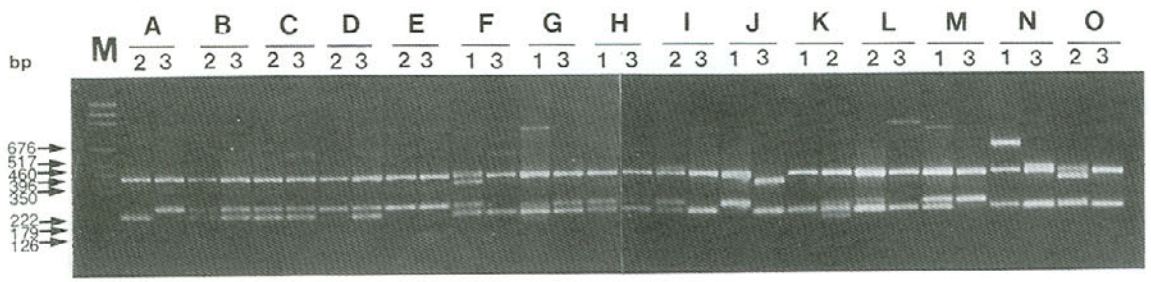

Fig. 5. The band patterns of ITS region of rDNA of *I. cylindrica* obtained by using PCR, digested with *Eco*RV restriction enzyme, and separated by 2.5% NuSieve 3:1 agarose gel. The abbreviations of sampling sites see Fig. 1. The arabic numbers indicate different individuals of each population.

The various types of ITS sequences of rDNAs from the 45 samples of Cogongrass in Taiwan were obtained by restriction enzyme digestion. In this study, *Eco*RV, *Cfo*I, *Ban*I, *Hae*III, *Msp*I, *Hinf*I, *Bst*OI, *Sau*96I and *Sty*I revealed the variant ITS types (ITS1, 5.8S rRNA gene and ITS2) of rDNAs, in which *Sty*I was the most effective. The different types of ITS sequences (shown in the parenthesis) of rDNAs from 45 individuals of Cogongrass in Taiwan could be obtained after digestion with restriction enzymes, namely *Eco*RV (6 types), *Cfo*I (8 types), *Ban*I (2 types), *Hae*III (6 types), *Msp*I (8 types), *Hinf*I (3 types), *Bst*OI (4 types), *Sau*96I (3 types) and *Sty*I (6 types). Only one type DNA pattern was revealed based on *Eco*109I analysis. Based on the digestion with *Sty*I, up to the four different types of the ITS sequences of rDNAs was revealed within an individual of samples F1, M1 and M3 (Table 1). The efficiency of heterogeneity of ITS sequences, including ITS1, 5.8S rRNA gene and ITS2

region within an individual, was obtained from the digestion of restriction enzymes. The data of heterogeneity are given in the parenthesis followed by enzymes, namely *Eco*RV (60.0%), *Cfo*I (28.9%), *Ban*I (2.2%), *Hae*III (8.9%), *Msp*I (31.1%), *Hinf*I (13.3%), *Bst*OI (15.5%), *Sau*96I (0%), *Sty*I (82.2%) and Eco109I (0%), respectively. All samples showed different types of ITS sequences within an individual. More specifically, the heterogeneity of 5.8S rDNAs sequences based on *Eco*RV, *Cfo*I, *Ban*I, *Hae*III, *Hinf*I, *Bst*OI and *Sty*I detection was 22.2, 35.6, 6.7, 11.1, 8.9, 22.2 and 6.7%, respectively. Furthermore, of the 45 individuals, 20 individuals showed the heterogeneity of 5.8S rDNAs sequences within an individual. It is interesting to note that 5.8S rDNAs sequences are quite heterogeneous in Cogongrass in Taiwan.

DISCUSSION

Based on the PCR-amplified RFLP analysis, the genetic variation of the ITS of rDNAs from Cogongrass in Taiwan was found. No length variation of PCR products within an individual from Cogongrass in Taiwan was revealed on gel separation. Sang *et al.* (1994) obtained the sequence of PCR products in 33 species of genus *Paeonia* and found different patterns of nucleotide addition in 14 diploid and tetraploid based on PCR-amplified sequencing. Wendel *et al.* (1995) also used Southern Blotting analysis and found the restriction site variation of the internal transcribed spacer of rDNA within a laboratory allopolyploid cotton. Suh *et al.* (1993) reported two types of ITS sequences in clones within an individual of the Winteraceae. We firstly reported the variant sequences of the ITS of rDNAs within an individual based on PCR-amplified RFLP for the plant of *Imperata cylindrica* in Taiwan.

ITS sequences of the rDNAs from individuals and populations of Cogongrass in Taiwan were very heterogeneous, suggesting that gene conversion or unequal crossing-over might not cause homogenization of the parental types of ITS in a hybrid. It is also suggested that only one parental ITS type might be revealed in the hybrid (Wendel *et al.*, 1995; Hillis *et al.*, 1991). However, Sang *et al.* (1995) pointed out that concerted evolution could not homogenize the parental ITS sequences in the five hybrids of *Paeonia*. The sequence heterogeneity of 5S rRNA gene had exhibited within an individual plant of *Eruca sativa* (Singh *et al.*, 1993). The vegetative reproduction may be a reason for maintenance of parental ITS sequences in the hybrids (Baldwin *et al.*, 1995), and the long generation time may be another reason to slow the rate of nucleotide substitution (Baldwin *et al.*, 1995; Suh *et al.*, 1993). In this study, Cogongrass is a wild plant which reproduces seeds annually, therefore, it should have a gene conversion and unequal crossing-over during meiosis. However, variable ITS sequences within an individual among those populations of Cogongrass in Taiwan were revealed. It is possible that the light thistle-like seeds are capable of being transported over great distances (Dickens, 1974), resulting in high gene flow and new hybrids between populations constantly.

Generally, sequences of 17S, 5.8S, 25S and 5S rRNA are very conserved among organisms. It is amazing that the various types of 5.8S rRNA genes of rDNAs were found in the Cogongrass in Taiwan based on PCR-amplified RFLP analysis and the sequence heterogeneity of 5.8S rRNA genes within an individual plant of the grass was also detected. We, thus, proposed that normal and variant 5.8S rRNA genes could coexist in an individual but they would not affect the survivorship.

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台灣地區白茅核糖體DNA內轉錄間隔區異質性之研究

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摘　　要

利用聚合酶連鎖反應可將45株生長於台灣地區之白茅的核糖體DNA(rDNA)內轉錄間隔區 (internal transcribed spacer, ITS) 複製出來，其長度除一樣本為702 bp外，其餘樣本皆為692 bp。再利用10種限制酶進一步偵測不同白茅樣本之ITS序列，發現台灣白茅族群之ITS區域 (含ITS1，5.8S rRNA基因及ITS2) 之序列異質性相當高，甚致個體內出現不同類型的 ITS 序列之情形亦相當普遍。就整個台灣白茅族群而言，在ITS1及ITS2序列方面，共偵測到8種不同類型之序列。在5.8S rRNA基因方面，共偵測到 5 種不同類型。因此，由本研究發現台灣地區白茅族群之 ITS 區域具有很高之異質度。

關鍵詞：白茅，內轉錄間隔區，核糖體DNA，異質度。

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