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Genetic variation in the intergenic spacer of ribosomal DNA of *Imperata cylindrica* (L.) Beauv. var. *major* (Cogongrass) populations in Taiwan

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Abstract. Genetic variation in the intergenic spacer (IGS) of ribosomal DNA (rDNA) repetitive sequences in inter- and intra-specific populations of Cogongrass [*Imperata cylindrica* (L.) Beauv. var. *major* (Nees) Hubb] in Taiwan, was studied by employing PCR-amplified RFLP analysis. Each IGS region of 45 individuals from 15 populations was amplified using a pair of complementary primers to conserved regions in the 5'end of 17S and 3'end of 25S rRNA genes. Five IGS length variants were found at 2,730 bp, 2,830 bp, 2,930 bp, 3,030 bp and 3,130 bp. Of them, 2930 bp was the main type of IGS length of Cogongrass in Taiwan. Within an individual Cogongrass, one to two IGS length types were distinguished. Those samples amplified by PCR were digested with 13 restriction enzymes, and 283 bands were revealed. Of them, 248 bands were polymorphic. Clustering analysis was conducted based on data obtained from bands distributed in all samples mentioned, and two major clusters were found. The Chuwei population was distinctly different from the remaining populations. Moreover, the findings of the PCR-amplified RFLP analysis also indicated that the IGS region of rDNA provides a good genetic marker and a potential tool for the study of microevolutionary process.

Keywords: Cogongrass; IGS; *Imperata cylindrica*; PCR-amplified RFLP; rDNA.

Introduction

Imperata cylindrica (Cogongrass), an aggressive rhizomatous grass, is distributed widely in many parts of the world from tropical to subtropical areas (Al-Juboory and Hassawy, 1980). It can be spread both by seeds and rhizomes (Wilcut et al., 1988). The light, thistle-like seeds of Cogongrass are capable of being transported over great distances, and this has probably contributed to increased distribution. The seed production is rapid and well adapted to new environments (Al-Juboory and Hassawy, 1980; Dickens, 1974). The plant exhibits a wide adaptability to different climatic and environmental regimes but is sensitive to salinity, leading to many ecotypes. Basic information on the taxonomy, physiological ecology, and morphology of this grass is fairly extensive. Little was known regarding the molecular ecology of the grass until Cheng and Chou (1997) initiated a series of molecular studies by selecting six Cogongrass populations in Taiwan. They (1997) concluded that the Chuwei (CW) population located at the mouth of the Tamshui River has formed an ecotype distinct from the remaining five populations in other parts of Taiwan.

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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, and from 500 to 40,000 copies per diploid cell in plants. Each repeating unit usually consists of the transcribed region coding for 17S, 5.8S and 25S rRNAs, which are highly conserved among various organisms, and the intergenic spacer (IGS) which is highly variable in length and primary structure among organisms and individuals (Appels et al., 1980; Waldron et al., 1983; Rogers and Bendich, 1987; Taira et al., 1988; Kato et al., 1990; Perry and Palukaitis, 1990; Beech and Strobeck, 1993; Borisjuk et al., 1994). The primary source of rDNA length variation is the number of subrepeats within the IGS (Bhatia et al., 1996; Kaufman et al., 1996). Therefore, sequence comparisons of rRNAs genic regions were used to study phylogenetic relationships among organisms (Schaal and Learn, 1988; Molina et al., 1993) while the IGS region provided a good genetic marker for the study of the microevolutionary process in inter- and intra-specific populations (Schaal and Learn, 1988).

Recently, techniques such as restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) have been widely used for genetic diversity analysis (Torres et al., 1993). However, conventional RFLP techniques involving southern blotting are

laborious, time-consuming, and inadequate for large-scale analysis (Halward et al., 1992; Williams et al., 1990). On the other hand, RAPD techniques provide a powerful tool to detect genetic variation in natural and man-made populations. The technique requires only small amounts of DNA, is simple, and costs less than other techniques (Huff et al., 1993). Nevertheless, it has certain limitations, including its sensitivity to reaction conditions and difficulties surrounding the reproducibility of amplification products (Hadrys et al., 1992; Schierwater and Ender, 1993). Recently, the PCR technique has been increasingly applied to problems in genetics, biomedical sciences, evolutionary biology, and ecology (Arnheim and Erlich, 1992). The technique of PCR-amplified RFLP was initially introduced to detect variations in the chloroplast gene *rpo*C_1, C_2 region among 14 species of *Astragalus* (Fabaceae) (Liston, 1992). After that, several reports attempted to detect genetic variation by PCR-amplified RFLP, but most of them focused on detecting genetic variations of the chloroplast DNA region in plants (Ghareyazie et al., 1995; Tsumura et al., 1995, 1996; Lashermes et al., 1996; Ziegenhagen and Fladung, 1997). In the present study, the technique was employed to detect the variation in IGS of rDNA among 15 populations of Cogongrass in Taiwan.

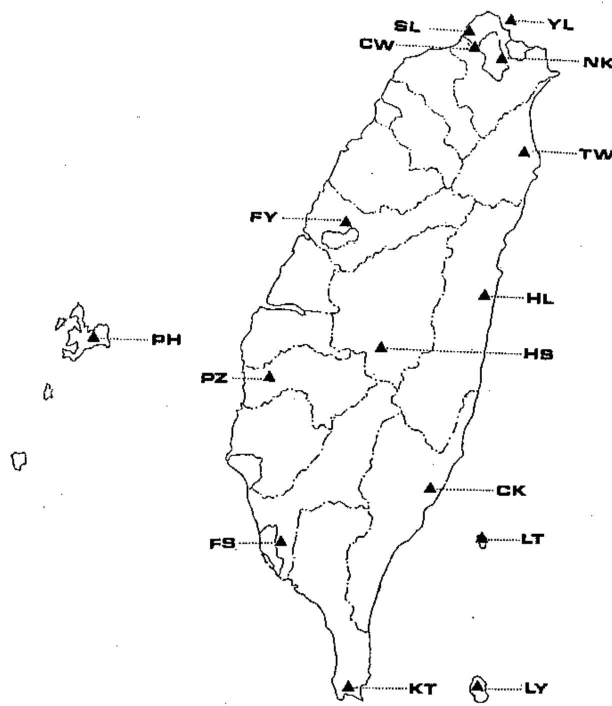

Figure 1. The sampling locations of *I. cylindrica* in Taiwan. The alphabes indicate that the samples were analyzed in the present study. The abbreviations of sampling sites are: FS (Fengshan), PZ (Potzu), FY (Fengyuan), TW (Chuangwei), HL (Hualien), CK (Chengkung), YL (Yehliu), SL (Sarlum), HS (Hoshe), KT (Kengting), PH (Penghu), LT (Lutao), LY (Lanyu), CW (Chuwei) and NK (Nankang).

Materials and Methods

Sites of Sampling

Fifteen sites—located at Fengshan (FS), Potzu (PZ), Fengyuan (FY), Chuangwei (TW), Hualien (HL), Chengkung (CK), Yehliu (YL), Sarlum (SL), Hoshe (HS), Kengting (KT), Penghu (PH), Lutao (LT), Lanyu (LY), Chuwei (CW), and Nankang (NK)—were selected for this study (Figure 1). Characteristics of the habitats, including soil texture, altitude, and weather conditions, were described by Tsai (1994). The sites were chosen to be representative of different habitats and climates in Taiwan.

Plant Materials

Rhizomes of Cogongrass collected from the 15 habitats of Taiwan (Figure 1) were transplanted into pots which were set in the greenhouse of the Institute of Botany, Academia Sinica at Taipei, Taiwan. Environmental conditions—such as the amount of water, soil type, humidity, temperature, and light intensity—were under control.

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). Three grams of fresh leaves were harvested and ground to powder with liquid nitrogen in a mortar and pestle, then transferred into a 30 ml centrifuge tube containing 10 ml 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°C water. We mixed the sample thoroughly and incubated it in a water bath at 60°C, inverting the tube constantly. We then, added 10 ml 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°C. We transferred the supernatant to a new centrifuge tube by filtering through two layers of miracloth, after adding 0.7 volume of 2-propanol and 1/10 volume 4.4 M NH_4OAc. The tube was centrifuged at 10,000 rpm for 10 min at 4°C to collect precipitated DNA. The DNA pellet was resuspended with 5 ml TE (10 mM, Tris-HCl pH 8.0, 1 mM EDTA) and incubated with 50 µg DNase-free RNase (Sigma) for 10 min at 65°C. 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°C. The supernatant was transferred to a new tube. Then, the 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°C, washed with 70% ethanol twice, and dried before being redissolved in 100-150 µl of TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA). Approximate yield amounts were calculated by a spectrophotometer (Beckman DU-20), and the DNA samples were stored at -20°C.

PCR Primers

Oligonucleotides used for PCR priming were designed from conserved regions of the 5'end of the 17S rDNA sequence and the complementary sequence of the 3'end of 25S rDNA from previously described sequences (Takaiwa et al., 1990; Kiss et al., 1989a; Kiss et al., 1989b; Lakshmikumaran and Negi, 1994). Two primer sets for amplifying IGS of rDNA were designated as IG1: 5'-CTACTGGCAGGATCAACCAGG-3' and IG2: 5'-TTGCTGCCACGATCCACTGAG-3'.

PCR Amplification

PCR reaction was performed by using a 50 µl mixture, containing 30 mM Tricine pH 8.4, 2 mM $MgCl_2$, 0.01% BSA, 5 mM 2-mercaptoethanol and 0.1% Thesit, with four dNTPs (0.8 mM each), primers (0.5 µM each), 2.5 units of *Taq* DNA polymerase (Promega) and 32 ng genomic DNA, and 50 µl mineral oil (Ponce and Micol, 1992). Amplification reactions were done in dry-block, two-step thermal cycles. In the first step, the mixture was incubated at 94°C for 5 min, followed by 10 cycles of denaturation at 94°C for 1 min, annealing at 61°C for 1 min and extension at 72°C for 3 min. The second step was conducted as follows: 30 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 3 min, followed by a final extension for 10 min at 72°C. Those reaction samples were detected by agarose gel electrophoresis of 10 µl of the PCR products (0.8%, w/v in TAE), staining by 0.5 µg/ml of ethidium bromide (EtBr), and photographing under the exposure of UV light.

Restriction Enzyme Digestion

DNA fragments amplified by PCR were digested with 13 restriction enzymes, namely *Hae*III, *Taq*I, *Alu*I, *Rsa*I, *Ban*I, *Cfo*I, *Msp*I, *Bst*OI, *Eco*0109I, *Hinf*I, *Sty*I, *Eco*RV and *Sma*I. All restriction enzymes digested were carried out under the conditions recommended by manufacturers (BRL or Promega), using 5-10 units of enzyme per microgram (µg) of DNA, and incubating the reaction for 3 h to overnight.

Gel Electrophoresis

About 5-10 µg of digested DNA mixed with tracking dye [0.25% bromophenol blue, 40% (w/v) sucrose in water] were electrophoresed overnight at 50 V-80 V in 2.5%-5.0% NuSieve 3:1 agarose gels (FMC Bioproducts) with 0.5 µg/ml EtBr. The pattern of bands was examined under UV light.

DNA Labeling and Southern Hybridization

The DNA band on the gels was recovered by glassmilk (BIO 101, 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 Kodac x-omat film. Immunological detection was also done using color solution (containing NBT solution and x-phosphate) on a membrane (Boehringer Mannheim). The aforementioned reaction protocol was recommended by the manufacturers.

DNA Cloning and Sequencing

PCR product of CW_3 DNA samples in Cogongrass 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 (by 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 protocol was recommended by the manufacturers.

Data Analysis

Data of bands obtained from PCR-amplified RFLP of Cogongrass populations were analyzed statistically using similarity coefficients (S), where $S=2N_{AB}/N_A+N_B$, and N_{AB} represents the number of co-migrating fragments (in considering all enzymes). N_A and N_B are the total number of bands for sample A and sample B, respectively (Chapco et al., 1992; Wilde et al., 1992). A dendrogram was constructed based on data of the similarity matrix, using the unweighted pair-group method analysis (UPGMA) (Rohlf et al., 1982).

Results

PCR Amplification

DNA fragments amplified from PCR using primers IG1 and IG2 were completed from 45 samples of Cogongrass. Five PCR products of different lengths were found, 2,800 bp, 2,900 bp, 3,000 bp, 3,100 bp and 3,200 bp, respectively (Figure 2). Both the 5'end and 3'end of the PCR product in the CW_3 sample was partly sequenced to identify the PCR product that was IGS of rDNA (data not shown). Using the PCR product of the CW_3 sample and hybridizing it with the others indicated that the PCR products of all samples fell into IGS regions (data not shown). The PCR product of the CW_3 sample was identified and contained both 48 bp of 25S rRNA gene and 24 bp of 17S rRNA gene. Thus, five different IGS length variants of rDNA of Cogongrass in Taiwan were detected, namely 2,730 bp, 2,830 bp, 2,930 bp, 3,030 bp and 3,130 bp. Among the five IGS lengths, type 2,930 bp was the most abundant with a 75.5% presence among 45 samples of Cogongrass; type 2,730 bp was the second most abundant with 24.4%; type 3,030 bp was third with 22.2%, and types 2,830 bp and 3,130 bp were as low as 4.4%. Furthermore, populations FS, PZ, TW, CK, YL, HS, KT, LY and NK analyzed by PCR possess two types of IGS length with a 31.1% presence among 45 samples of Cogongrass, one of them was minor, and the remaining populations revealed only one type with 68.8% (Table 1). Restriction enzyme *Eco*RI was chosen for rDNA length variation analysis because it cleaved both

25S rRNA gene and 17S rRNA gene once (Taira et al., 1988). Total genomic DNAs of all samples were digested by *Eco*RI. After electrophoresing by 0.8% agarose gel and staining with EtBr, a band of about 8.8 kb appeared (data not shown). Those bands can be hybridized with the IGS of CW_3 sample (Figure 3). The findings confirmed that the rDNA of Cogongrass exhibited many copy numbers in tandem repeat and only one main type of rDNA length within individuals of Cogongrass.

PCR-Amplified RFLP

Each PCR product of 45 samples of Cogongrass was digested with 13 restriction enzymes, namely *Hae*III, *Taq*I, *Alu*I, *Rsa*I, *Ban*I, *Cfo*I, *Msp*I, *Bst*OI, *Eco*0109I, *Hinf*I, *Sty*I, *Eco*RV and *Sma*I, revealing 283 bands in total. Of them, 248 bands were polymorphic, reaching 87.6% (Table 2). Of these restriction enzymes, *Taq*I, *Alu*I, *Rsa*I, *Msp*I, *Eco*0109I, *Sty*I, and *Sma*I revealed powerful polymorphic bands above 90% in the IGS region of rDNA among the 15 Cogongrass populations, while *Eco*RV exhibited rather low polymorphism (Figure 4).

Clustering Analysis

Based on PCR-amplified RFLP of IGS, the similarity was computed between pairs of samples (data not shown). A phylogenetic tree was constructed from similarity by UPGMA. Based on this tree, the genetic variation within the population was greater than that between populations in some individuals of aforementioned populations, namely FS, CK, YL, HS, KT, LY and NK. Furthermore, minor population differentiation was revealed, namely in PZ, FY, TW, HL, SL, PH and LT. Obviously, the aforementioned populations of Cogongrass in Taiwan could be divided into two clusters. The CW population forms one cluster, and the remaining 14 populations revealed another cluster (Figure 5).

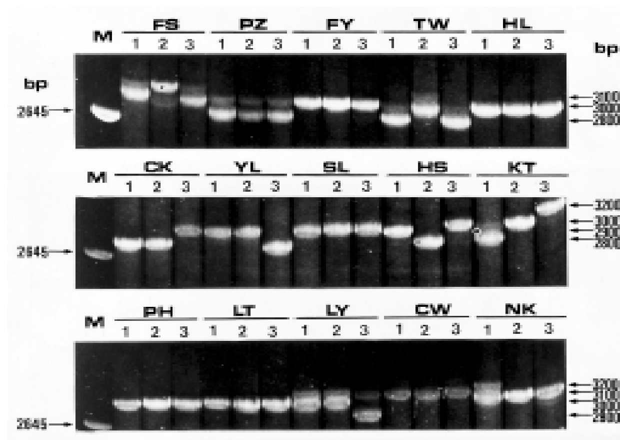

Figure 2. The IGS region of rDNA in 15 populations of *I. cylindrica* in Taiwan. The IGS region was obtained by the PCR amplification of primers IG1 and IG2. The abbreviations of sampling sites see Figure 1. The arabic numbers indicate different samples of each population.

Discussion

Since the 17S-5.8S-25S structural gene region is very conserved in sequence and is approximately 6.0 kb in most grasses, the differences in rDNA repeat sizes are due to variation in the IGS length (Pillay, 1996). However, in some plants, there are two or more types of IGS length among individual members of a plant population while in others the length heterogeneity of IGS cannot be detected (Rogers and Bendich, 1987; Lakshmikumaran and Negi, 1994). Generally, the variability of IGS length is smaller in cultivated species than in wild type species. A reduction in the variability of rDNA spacer may be due to domestication (Cordesse et al., 1990). However, the results of PCR showed the variability of IGS length in Cogongrass, which is wild, to be rather low, most individuals being of only

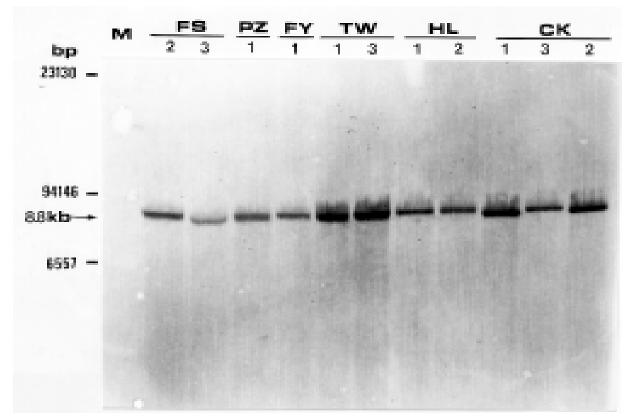

Figure 3. The result of southern hybridization from six populations of *I. cylindricain* Taiwan hybridized by PCR product of CW_3 sample. The abbreviations of sampling sites see Figure 1. The arabic numbers indicate different sample of each population.

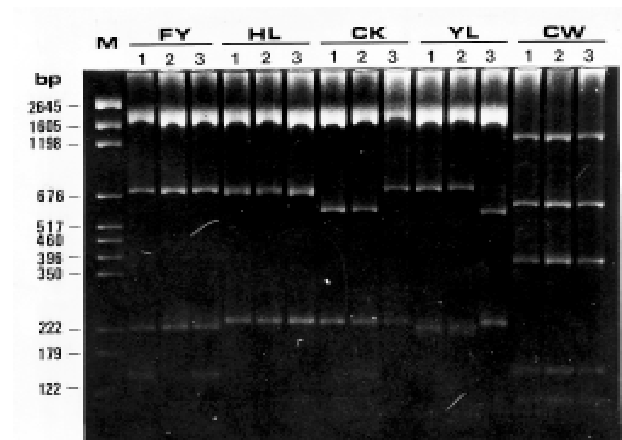

Figure 4. The band pattern of IGS region of rDNA of *I. cylindrica* obtained by using PCR, digested with *Rsa*I restriction enzyme, and separated by 2.5% NuSieve 3:1 agarose gel. The abbreviations of sampling sites see Figure 1. The arabic numbers indicate different samples of each population.

Table 1. A summary of IGS length and number of IGS length variation from 15 populations of *I. cylindrica* based on PCR.

Location of population and (abbreviation)		Length of IGS (bp)				
		2730	2830	2930	3030	3130
Fengshan (FS)	1			+	+*	
	2			+*	+	
	3			+	+*	
Potzu (PZ)	1	+		+*		
	2	+		+*		
	3	+		+*		
Fengyuan (FY)	1			+		
	2			+		
	3			+		
Chuangwei (TW)	1	+		+*		
	2			+	+*	
	3	+		+*		
Hualien (HL)	1			+		
	2			+		
	3			+		
Chengkung (CK)	1	+				
	2	+				
	3			+		
Yehliu (YL)	1			+		
	2			+		
	3	+				
Sarlum (SL)	1			+		
	2			+		
	3			+		
Hoshe (HS)	1		+			
	2	+				
	3			+		
Kengting (KT)	1	+	+*			
	2			+		
	3					+
Penghu (PH)	1			+		
	2			+		
	3			+		
Lutao (LT)	1			+		
	2			+		
	3			+		
Lanyu (LY)	1			+	+*	
	2			+	+*	
	3	+			+*	
Chuwei (CW)	1				+	
	2				+	
	3				+	
Nankang (NK)	1			+		+*
	2			+		
	3			+		
Total	45	11	2	34	10	2
% of total sample		24.4	4.4	75.5	22.2	4.4

+ : Presence of major band.
+*: Presence of minor band.

Table 2. The polymorphic fragment of IGS of rDNA from 15 populations of *I. cylindrica* digested by 13 restriction enzymes.

Enzyme	Recognition site	Restriction fragment number (A)	Polymorphic band	
			Number	% of (A)
*Hae*III	GG↓CC	38	32	84.2
*Taq*I	T↓CGA	11	10	90.9
*Alu*I	AG↓CT	9	9	100
*Rsa*I	GT↓AC	15	15	100
*Ban*I	G↓G(T/C)(A/G)CC	13	11	84.6
*Cfo*I	GCG↓C	32	25	78.1
*Msp*I	C↓CGG	46	42	91.3
*Bst*OI	CC↓(A/T)GG	32	27	84.4
*Eco*0109I	Pu[b]G↓GN[a]CCPy[c]	12	11	91.7
*Hinf*I	G↓ANTC	23	19	82.6
*Sty*I	C↓C(A/T)(A/T)GG	25	24	96.0
*Eco*RV	GAT↓ATC	5	2	40.0
*Sma*I	CCC↓GGG	22	21	95.5
Total		283	248	87.6

[a]N, represent A, G, C or T.
[b]Pu, represent purine.
[c]Py, represent pyrimidine.

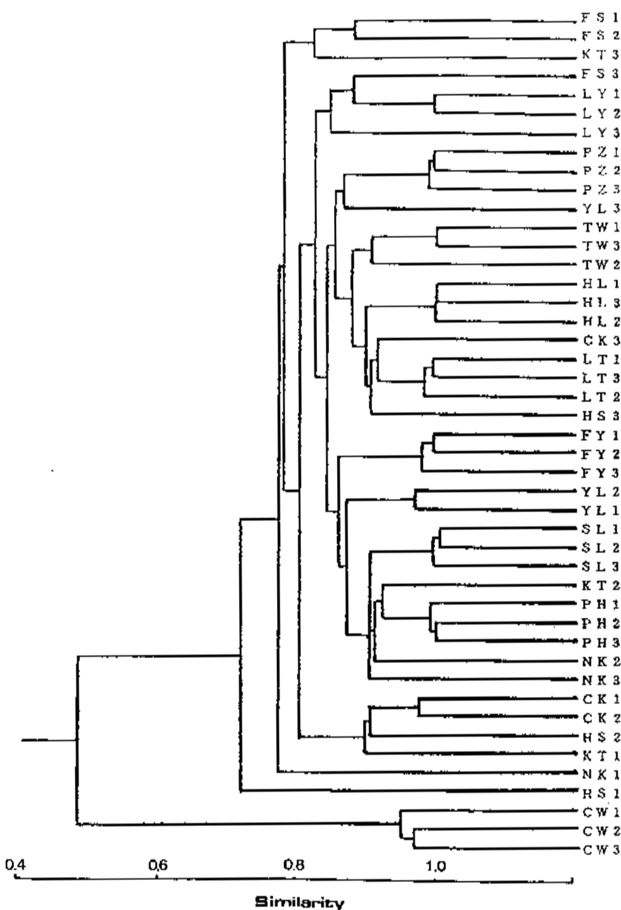

Figure 5. A dendrogram of cluster analysis based on PCR-amplified RFLP of IGS region from 15 populations of *I. cylindrica* in Taiwan. The abbreviations of sampling sites see Figure 1. The arabic numbers indicate different sample of each population.

one type (68.9%) (Figure 2 and Table 1). The variability was also as low as one type when hybridization techniques were used (Figure 3). The variation in rDNA spacer length can reflect both a phylogenetic relationship and a limited ecological introgression (Cordesse et al., 1990). Cogongrass in Taiwan contained five types of IGS length variants, namely 2,730 bp, 2,830 bp, 2,930 bp, 3,030 bp and 3,130 bp. However, no positive correlation appeared between rDNA length variation and geographical distribution. For example, samples collected from YL, SL, NK and CW were located at the coastal area of Taipei county, but the IGS length patterns of the YL, SL and NK populations were different from that of the CW population (Table 1 and Figure 1). The report that the heterogeneous length of IGS varied with the copy number is primarily based on small subrepeats of several plants (Cordesse et al., 1990; Kaufman et al., 1996). The variability of IGS length ranged from 1 kb to 12 kb or higher for different plant species (Rogers and Bendich, 1987), and small subrepeat length varied from 30 bp to 350 bp (Beech and Strobeck, 1993). Therefore, we infer a 100 bp small subrepeat sequence among IGS of rDNA of Cogongrass in Taiwan. A phylogenetic tree of IGS based on PCR-amplified RFLP analysis suggested that the CW population was unique and distinguished from the remaining 14 populations (Figure 5). Among these populations no obvious geographical relationship was exhibited (Figure 1 and Figure 5).

The variability of IGS length that appeared within populations, namely TW, CK, YL, HS, KT, LY and NK, was greater (Table 1). Furthermore, the results of the cluster analysis of PCR-amplified RFLP in IGS of rDNA also showed that the genetic variation within the population was greater than that between population in some individuals of the aforementioned populations, namely FS, CK, YL, HS, KT, LY and NK (Figure 5). In view of the two

points above, we infer that Cogongrass plants among the 15 populations should have a strong gene flow, deriving from the light thistle-like seeds of the grass, which can disperse long distances. In fact, studies with several plants have suggested that genetic variation in the IGS may be adaptive and that the region is under selection (Flavell et al., 1986; Kaufman et al., 1996), or rapid conversion under γ-ray induction (Fukuoka et al., 1994). Flavell et al. (1986) analyzed individuals and populations of *Triticum dicoccoides* from Israel. Patterns of IGS length variation in *T. dicoccoides* rDNA are correlated significantly with both allozymic diversity and environmental factors relating to water availability. In the present study, the IGS sequence of rDNAs among intra- and inter-specific populations of Cogongrass analyzed by PCR-amplified RFLP suggested that the IGS sequence of rDNA in the CW population may be influenced by the stressful environment. The unique genetic variation of the CW population could have evolved from the stressful environment, which might cause some populations to differentiate and adapt uniquely even under high levels of gene flow (Bradshaw, 1972; Sork et al., 1993). In addition to the analysis of IGS sequence based on PCR-amplified RFLP of 15 populations, we detected genetic variation based on the RAPD of bulked DNA (data unpublished). The findings of RAPD analysis confirmed that the CW population was unique and remarkably different from the remaining 14 populations. However, in studying the PCR-amplified RFLP of the internal transcribed spacer (ITS) region among the 45 same samples, we found no population varied significantly among the 15 populations of Cogongrass in Taiwan (data unpublished).

It has been indicated that the maintenance of repetitive DNA sequences including rDNAs is important for plants because they can not move to avoid unfavorable changes in their environment (Rogers and Bendich, 1987). While maintaining a larger rDNA pool is possibly advantageous in times of stress, most wild and cultivated species still maintain a high copy number under nonstressful conditions (Rogers and Bendich, 1987). McClintock (1984) suggested that genome change is a way plants can adapt to environmental stress. Walbot and Cullis (1985) pointed out that flexibility is an important feature of the plant genome. Besides, it seems possible that the genomic instability can be switched on under stress conditions and switched off when the stress is over (Cairns et al., 1988; Fukuoka et al., 1994). Several studies on natural populations of both plants and animals have shown that numerical and sequence divergence among multigene families may be maintained by selection (Govindaraju and Cullis, 1992). Now, we know that the heterogeneity exists in the 5' portion of the IGS of rDNA in many plants (Rogers and Bendich, 1987). However, the origin of the enormous diversity of the IGS sequences can not be explained yet (King et al., 1993). Moreover, many organisms alter their DNA in response to stress (Schaal and Learn, 1988). The amount of rDNA can change under specific environmental conditions, and the observed changes can be transmitted to the progeny (Fukuoka et al., 1994). A new variant rDNA may spread among the structure of tandem repeat rDNAs by the processes of unequal recombination and gene conversion (Beech and Strobeck, 1993; Bhatia et al., 1996). This study has suggested that this variant plays some role in selecting the variants of the IGS sequence in rDNAs. Furthermore, we suggested that the IGS of rDNA family in the CW population of Cogongrass might maintain a few other types of IGS sequence to adapt to the changing environment.

Morphological characters such as leaf length, leaf width, and plant height were measured based on the transplanting of rhizome of Cogongrass from the field to the greenhouse. The Cogongrass growing at the CW site had waxy crystals on its surface and a rib with a hollow structure without ground tissue (Cheng and Chou, 1997). The CW population is located at the mouth of the Tamshui River, where severe environmental pressures are present, such as frequent submergence in high saline water and oxygen deficiency at the roots (Chang, 1996). Therefore, we infer that the development of waxy crystals on the leaf surface is for the purpose of reducing evaporation, and the hollow structure on the rib without the ground tissue has evolved to help the plant adapt to oxygen deficiency.

In conclusion, the present study has shown the genetic variation of IGS length and sequence of rDNA among intra- and inter-specific populations of Cogongrass based on PCR-amplified RFLP. It supported the previous findings of RAPD and morphological characters that the CW population of Cogongrass was distinctly different from the remaining populations and that microevolution was running in the CW population. Moreover, the analysis of IGS of rDNA based on PCR-amplified RFLP seems to be an easy and powerful tool to elucidate population structure and microevolution.

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Literature Cited

Al-Juboory, B.A. and G.S. Hassawy. 1980. Comparative morphological development of Cogongrass (*Imperata cylindrica*) in Iraq. Weed Sci. **28:** 324-326.

Appels, R., W.L. Gerlach, E.S. Dennis, H. Swift, and W.J. Peacock. 1980. Molecular and chromosomal organization of DNA sequence coding for the ribosomal RNAs in cereals. Chromosoma **78:** 293-311.

Arnheim, N. and H. Erlich. 1992. Polymerase chain reaction strategy. Annu. Rev. Biochem. **61:** 131-156.

Beech, R.N. and C. Strobeck. 1993. Structure of the intergenic spacer region from the ribosomal RNA gene family of white spruce (*Picea glauca*). Plant Mol. Biol. **22:** 887-892.

Bhatia, S., M.S. Negi, and M. Lakshmikumaran. 1996. Structural analysis of the spacers of the three diploid *Brassica* species. J. Mol. Evol. **45:** 460-468.

Borisjuk, N., L. Borisjuk, G. Petjuch, and V. Hemleben. 1994. Comparison of nuclear ribosomal RNA genes among *Solanum* species and other Solanaceae. Genome **37:** 271-279.

Bradshaw, A.D. 1972. Some evolutionary consequences of being a plant. Evol. Biol. **5:** 25-47.

Cairns, J., J. Overbaugh, and S. Miller. 1988. The origin of mutants. Nature **335:** 142-145.

Chang, Y.F. 1996. Physio-Ecological Responses of *Imperata cylindrica* from Different Habitats to Saline and Flooding. M.S. Thesis, National Taiwan University, Taipei, pp. 64-70.

Chapco, W., N.W. Ashton, R.K.B. Martel, and N. Antonishyn. 1992. A feasibility study of the use of random amplified polymorphic DNA in the population genetics and systematics of grasshoppers. Genome **35:** 569-574.

Cheng, K.T. and C.H. Chou. 1997. Ecotypic variation of *Imperata cylindrica* populations in Taiwan: I. Morphological and molecular evidences. Bot. Bull. Acad. Sin. **38:** 215-223

Cordesse, F., G. Second, and M. Delseny. 1990. Ribosomal gene spacer length variability in cultivated and wild rice species. Theor. Appl. Genet. **79:** 81-88.

Dickens, R. 1974. Cogongrass in Alabama after sixty years. Weed Sci. **22:** 177-179.

Flavell, R.B., M. O'dell, P. Sharp, E. Nevo, and A. Beiles. 1986. Variation in the intergenic spacer of ribosomal DNA of wild wheat *Triticum dicoccoides*, in Israel. Mol. Biol. Evol. **3:** 547-558.

Fukuoka, H., Y. Kageyama, K. Yamamoto, and G. Takeda. 1994. Rapid conversion of rDNA intergenic spacer of diploid mutants of rice derived from γ-ray irradiated tetraploids. Mol. Gen. Genet. **243:** 166-172.

Ghareyazie, B., N. Huang, G. Second, and J. Bennett. 1995. Classification of rice germplasm. I. Analysis using ALP and PCR-based RFLP. Theor. Appl. Genet. **91:** 218-227.

Govindaraju, D.R. and C.A. Cullis. 1992. Ribosomal DNA variation among populations of a *Pinus rigida* Mill (pitch pine) ecosystem: 1. Distribution of copy numbers. Heredity **69:** 133-140.

Hadrys, H., M. Balick, and B. Schierwater. 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol. Ecol. **1:** 55-63.

Halward, T., T. Stalker, E. LaRue, and G. Kochert. 1992. Use of single-primer DNA amplifications in genetic studies of peanut (*Arachis hypogaea* L.). Plant Mol. Biol. **18:** 315-325.

Huff, D.R., R. Peakall, and P.E. Smouse. 1993. RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloe dactyloides* (Nutt.) Engelm]. Theor. Appl. Genet. **86:** 927-934.

Kato, A., T. Nakajima, J. Yamashita, K. Yakura, and S. Tanifuji. 1990. The structure of the large spacer region of the rDNA in *Vicia faba* and *Pisum sativum*. Plant Mol. Biol. **14:** 983-993.

Kaufman, B., T.R. Rocheford, R.J. Lambert, and A.R. Hallauer. 1996. Change in ribosomal DNA spacer length composition in maize recurrent selection populations. 2. Analysis of BS10, BS11, RBS10 and RSSC. Theor. Appl. Genet. **92:** 680-687.

King, K., R.A. Torres, U. Zentgraf, and V. Hemleben. 1993. Molecular evolution of the intergenic spacer in the nuclear ribosomal RNA genes of Curcurbitaceae. J. Mol. Evol. **36:** 144-152.

Kiss, T., M. Kis, and F. Solymosy. 1989a. Nucleotide sequence of a 25S rRNA gene from tomato. Nucleic Acids Res. **17:** 796.

Kiss, T., A. Szkukalek, and F. Solymosy. 1989b. Nucleotide sequence of a 17S (18S) rRNA gene from tomato. Nucleic Acids Res. **17:** 2127.

Lakshmikumaran, M. and M.S. Negi. 1994. Structural analysis of two length variants of the rDNA intergenic spacer from *Eruca sativa*. Plant Mol. Biol. **24:** 915-927.

Lashermes, P., J. Cros, M.C. Combes, P. Trouslot, F. Anthony, S. Hamon, and A. Charrier. 1996. Inheritance and restriction fragment length polymorphism of chloroplast DNA in the genus *Coffea* L. Theor. Appl. Genet. **93:** 626-632.

Liston, A. 1992. Variation in the chloroplast genes *rpo*C1 and *rpo*C2 of the genes *Astragalus* (Fabaceae): Evidence from restriction site mapping of a PCR-amplified Fragment. Amer. J. Bot. **79:** 953-961.

McClintock, B. 1984. The significance of responses of the genome to challenge. Science **226:** 792-764.

Molina, F.I., S.C. Jong, and J.L. Huffman. 1993. PCR amplification of the 3'external transcribed and intergenic spacers of the ribosomal DNA repeat unit in three species of *Saccharomyces*. FEMS Microbiol. Lett. **108:** 259-264.

Perry, K.L. and P. Palukaitis. 1990. Transcription of tomato ribosomal DNA and the organization of the intergenic spacer. Mol. Gen. Genet. **221:** 102-112.

Pillay, M. 1996. Genomic organization of ribosomal RNA genes in *Bromus* (Poaceae). Genome **39:** 198-205.

Ponce, M.R. and J.L. Micol. 1992. PCR amplification of long DNA fragments. Nucleic Acids Res. **20:** 623.

Rogers, S.O. and A.J. Bendich. 1987. Ribosomal RNA genes in plants: variability in copy number and in the intergenic spacer. Plant Mol. Biol. **9:** 509-520.

Rohlf, F.J., J. Kishpaugh, and D. Kirk. 1982. Numerical Taxonomy System of Multivariate Statistical Programs. State Univ. New York, Stony Brook, New York.

Schaal, B.A. and G.H. Learn. 1988. Ribosomal DNA variation within and among plant populations. Ann. Missouri. Bot. Gard. **75:** 1207-1216.

Schierwater, B. and A. Ender. 1993. Different thermostable DNA polymerase may amplify different RAPD products. Nucliec Acids Res. **21:** 4647-4648.

Shure, M., S. Wessler, and N. Fedoroff. 1983. Molecular identification and isolation of the waxy locus in maize. Cell **35:** 225-233.

Sork, V.L., K.A. Stowe, and C. Hochwender. 1993. Evidence for local adaptation in closely adjacent subpopulations of northern red oak (*Quercus rubra L.*) expressed as resistance to leaf herbivores. Amer. Nat. **142:** 928-936.

Taira, T., A. Kato, and S. Tanifuji. 1988. Difference between two major size classes of carrot rDNA repeating units is due to reiteration of sequences of about 460 bp in the large spacer. Mol. Gen. Genet. **213:** 170-174.

Takaiwa, F., S. Kikuchi, and K. Oono. 1990. The complete nucleotide sequence of the intergenic spacer between 25S and 17S rDNAs in rice. Plant Mol. Biol. **15:** 933-935.

Torres, A.M., N.F. Weeden, and A. Martin. 1993. Linkage among

isozyme, RFLP and RAPD markers in *Vicia faba*. Theor. Appl. Genet. **85:** 937-945.

Tsai, C.C. 1994. A Study of the Population Differentiation of *Imperata cylindrica* in Taiwan. M.S. Thesis, National Taiwan University, Taipei.

Tsumura, Y., K. Yoshimura, N. Tomaru, and K. Ohba. 1995. Molecular phylogeny of conifers using RFLP analysis of PCR-amplified specific chloroplast genes. Theor. Appl. Genet. **91:** 1222-1236.

Tsumura, Y., T. Kawahara, and R. Wickneswari. 1996. Molecular phylogeny of Dipterocarpaceae in Southeast Asia using RFLP of PCR-amplified chloroplast genes. Theor. Appl. Genet. **93:** 22-29.

Walbot, V. and C.A. Cullis. 1985. Rapid genomic change in higher plants. Annual Rev. Pl. Physiol. **36:** 367-396.

Waldron, J., P. Dunsmuir, and J. Bedbrook. 1983. Characterization of the rDNA repeat units in the *Mitchell petunia* genome. Plant Mol. Biol. **2:** 57-65.

Wilcut, J.W., R.R. Dute, B. Truelove, and D.E. Davis. 1988. Factors limiting the distribution of Cogongrass, *Imperata cylindrica*, and Torpedograss, *Panicum repens*. Weed Sci. **36:** 577-582.

Wilde, J., R. Waugh, and W. Powell. 1992. Genetic fingerprinting of *Theobroma* clones using randomly amplified polymorphic DNA markers. Theor. Appl. Genet. **83:** 871-877.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18:** 6531-6535.

Ziegenhagen, B. and M. Fladung. 1997. Variation in the *psb*C gene region of gymnosperms and angiosperms as detected by a single restriction site polymorphism. Theor. Appl. Genet. **94:** 1065-1071.

台灣地區白茅族群核糖體核酸基因間隔區之遺傳變異

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利用 PCR-amplified RFLP 分析核糖體 (rDNA) 核酸基因間隔區 (intergenic spacer, IGS)，以探討台灣白茅族群之遺傳變異。在 17S rRNA 基因區的 3' 端與 25S rRNA 基因區的 5' 端各設計一條引子，利用 PCR 技術將來自 15 族群的 45 個白茅樣本之 IGS 進行複製，共可發現 5 種不同長度的 IGS 區域，分別為 2730 bp, 2830 bp, 2930 bp, 3030 bp 和 3130 bp， 其中以 2930 bp 為白茅 IGS 的最主要型式。另外在單一白茅個體中亦可發現 1 至 2 種不同長度的 IGS。上述 PCR 產物再經 13 種限制酵素切割，共可記錄 283 條帶，其中有 248 條帶具多型性。經群叢分析後，可將台灣地區白茅分成二群，一群為竹圍族群；另一群為其它地區白茅族群。由上述研究可知，IGS 經 PCR-amplified RFLP 的分析，不僅可獲取不少遺傳標誌，在微演化的研究上亦有幫助。

關鍵詞：基因間隔區；核糖體核酸；白茅；PCR-amplified RFLP。