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Ecotypic variation of *Imperata cylindrica* populations in Taiwan: I. Morphological and molecular evidences

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Abstract. *Imperata cylindrica* L. Beauv, a common C4 grass, is widely distributed in various habitats in Taiwan. Among 55 populations in various habitats, six sites were selected for the study. These sites are namely, Chuwei (an estuary mangrove forest area with high salinity), Sarlun (in a coastal saline area), Kengting (at Taiwan's southern tip with a drought season in winter), Penghu (an islet about 100 km from the west coast that is droughty in most seasons), and Nankang and Hoshe (both inland control sites with mild weather). Leaves of *I. cylindrica* sampled from these sites were examined under light microscope and scanning electron microscope for anatomic study in order to see their difference. The results showed that the grass leaves collected from the Chuwei site exhibited a clear difference from those of other sites, revealing a hollow structure in the midrib of leaves and villous trichomes on the abaxial leaf surface. The rhizomes of the grass were also collected from each site, brought back to the laboratory, and were transplanted into pots and grown in a greenhouse of the Academia Sinica. The phenotypic characters, such as plant height and leaf width, of the grasses collected from the field and from the greenhouse were compared. The results showed that plants collected from Chuwei, Sarlun, and Penghu were significantly shorter than those of the remaining three sites. The grass leaves collected from both the field and greenhouse were analyzed by means of random amplified polymorphic DNA. Forty 10 base pair primers were employed, and only 31 primers were adequate. Of them, 82 distinguished bands resulted from the RAPD study, showing the difference among populations of the six sites. Furthermore, using an Euclidean distance method, the phylogenetic relationship among the six populations was obtained. The findings revealed that the Nankang and Hoshe populations evolved into an ecotype, which was significantly different from another cluster of the remaining 4 populations. However, populations at Chuwei and Sarlun formed into a unique ecotype although they were also closer to other populations Penghu and Kengting. The phenotypic, morphological, and molecular data are correlated to each other.

Keywords: Cogongrass; Ecotypic variation; *Imperata cylindrica*; Population; RAPD.

Introduction

Cogongrass (*Imperata cylindrica* L. Beauv), an aggressive, rhizomatous, and weedy grass, is a troublesome weed in fields throughout the tropical and subtropical regions of the world. Genus *Imperata* comprises ten species in the world, but only one species is in Taiwan (Hsu, 1975). This grass is widely distributed on the island of Taiwan and its islets, exhibiting its great adaptability to various habitats, particularly in the highly saline coastal areas and drought land. Chou (1989) indicated that *I. cylindrica* was an allelopathic plant, revealing strong aggressiveness against other plants. It has been listed as one of the seven worst weeds of the world (Holm, 1969). Flint and Patterson (1980) found two ecotypes of *Imperata cylindrica* (sun ecotype and shade ecotype) in Iraq according to morphological characteristics such as leaf area, leaf weight, leaf/stem ratio, etc. Al-Juboory and Hassawy (1980) collected this grass from fifteen locations throughout Iraq and compared their morphological development. They found that plant height and density of stands varied more than two-fold; the number of flowering heads varied eleven-fold among different locations; and mature-leaf width varied from 4.7 to 6.9 mm, reflecting the existence of different ecotypes of *I. cylindrica* in Iraq. Matumura et al. (1983) and Matumura and Nakajima (1988) described several biotypes of *I. cylindrica* in Japan based on morphological characters under various environmental stresses, such as drought, salt, etc.

The aforementioned studies emphasized the morphological and phenotypical characters among the ecotypes of *I. cylindrica* under different habitats and stress environments, yet a biochemical or molecular approach to elucidate the mechanism of the ecotype formation received little attention. A newly developed technique, random amplified polymorphic DNA (RAPD), has recently received high recognition as a powerful and efficient tool with applications to ecology, systematics, evolution studies, and especially to population biology (Halward et al., 1992; Cheng, 1994; Tao et al., 1993; Tinker et al., 1993; Visrling et al., 1994; Welsh and

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McClelland, 1990; Wikie et al., 1993; Yang and Quiros, 1993). Van Heusden and Bachmann (1992) employed the RAPD technique to determine the genotypic relationships among ten populations of *Microseris elegans* collected throughout California. RAPD markers were used to detect various cultivars of *Brassica* (Hu and Quiros, 1991) and the genetic structure of *Gliricidia sepium* and *G. rnaculata* (Chalmers et al., 1992). Using the RAPD technique with nine primers for eight populations of *G. sepium* revealed that 60% diversity occurs between the populations, from which several specific markers were found good for population detection. Huff et al. (1993) also employed the RAPD to study populations of buffalo grass and revealed a great genetic difference between two regional ecotypes. The aim of the present study is to determine the ecotypic variation of *I. cylindrica* populations from six representing sites in Taiwan by means of RAPD.

Materials and Methods

Study Sites and Materials

Among the 55 populations of *Imperata cylindrica*, which are widely distributed in Taiwan and studied, six sites representing different environmental regimes and habitats were selected. The populations and geographic locations of the study sites are given in Figure 1. The climatic data of the six selected locations were described by Cheng (1994). The six sites where the *I. cylindrica* plant grows are: Chuwei (an estuary area with a mangrove forest nearby), Sarlun (a highly saline area on the west coast), Kengting (located at the south tip of Taiwan with droughty winters), and Penghu (located in an islet 100 km west of Taiwan proper and receiving salt spray all year). The other two populations, Nankang and Hoshe, are located inland and receive no salt spray. The rhizomes of grasses collected from the six sites were grown in pots in a greenhouse of the Institute of Botany, Academia Sinica, Taipei. The phenotypic characters of more than forty individuals were examined for each population sampled from both field and the greenhouse, and the morphological characters of leaf samples were also examined under a light microscope and a scanning electron microcope. To determine the intra- and inter- population variation of *I. cylindrica*, the leaves of twelve individuals from each population were sampled for analysis. Leaves of *I. cylindrica* sampled were immediately placed in an ice box, brought back to the laboratory, and stored at -70°C until used.

DNA Extraction

Leaves of *I. cylindrica* sampled from fields of the six sites were ground to powder in liquid nitrogen. Approximately 20 grams of leaf powder were mixed with 60 ml of urea extraction buffer containing 0.42 g/ml urea, 50 mM Tris-HCl, pH 8.0, 20 mM EDTA (pH 8.0), 50 mM NaCl, and l% sarcosine (Shure et al., 1983). After gentle mixing, a solvent of an equal volume of phenol:chloroform (1:1, V/V) was added. The slurry was kept at room temperature for 15 min, followed by centrifugation at 10,000 *g* for 10 min. DNA was precipitated by adding a solvent containing an equal volume of isopropanol and 4.4 M NaOAc. The pellet was dissolved in 4 ml TE buffer (10 mM Tris-HCl, pH 8.0, l mM EDTA, pH 8.0), treated with ribonuclease, and then extracted with phenol. The DNA was recovered by ethanol precipitation.

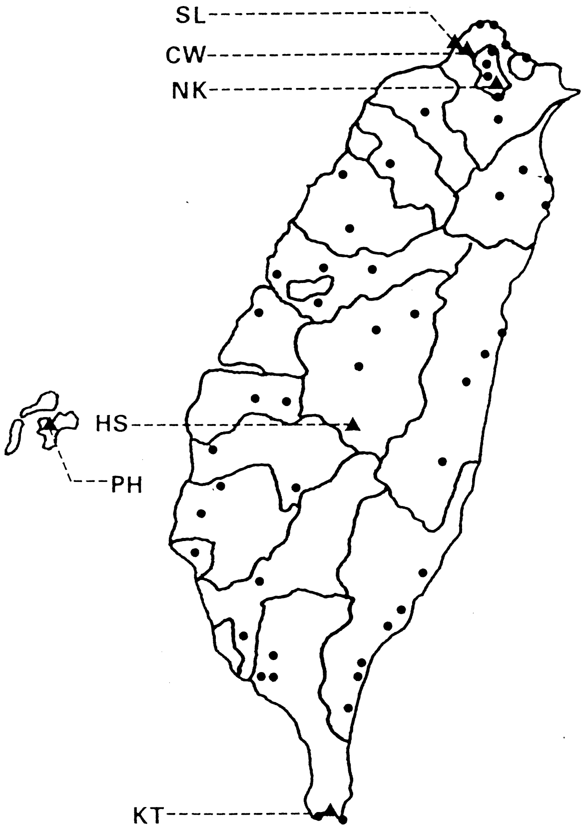

Figure 1. The sampling locations of *Imperata cylindrica* populations in Taiwan. Six sites selected for present study are designated as NK: Nankang, HS: Hoshe, CW: Chuwei, SL: Sarlun, PH: Penghu, and KT: Kengting.

Amplification Conditions

A polymerase chain reaction (PCR) technique was employed to study the genetic diversity of different ecotypes of *I. cylindrica*. The PCR procedures were a modification as described by Williams et al. (1990). The reactions were performed in 25 µl reaction mixture, containing 0.1 mM dNTP, 0.2 µM primer, 2 mM $MgCl_2$, 0.625 unit Taq DNA polymerase (Promega) and 10 ng genomic DNA. Forty arbitrary 10-mer primers (kits K and Q) described by Cheng (1994) were purchased from Operon Technologies, USA. The reaction was performed in a Perkin-Elmer Cetus DNA Thermal Cycler programed for 45 cycles of 1 min at 94°C for denaturing, 1 min at 36°C for annealing, and 2 min at 72°C for extension. After all cycles were completed, the amplifed products were separated by electrophoresis on 2% agarose gels, then stained with ethidium bromide and visualized under long wavelength UV light.

Homology Analysis

The RAPD amplified products separated on agrose gel were transferred to nylon membrane filter (MSI WESTBOARD, MAO1581). The markers after gel separation were excised. The purpose of the analysis was to determine whether the marker on genome of *I. cylindrica* is a single copy sequence or a repetitive sequence. The probe was radiolabelled with α-^{32}P-dCTP (3000 Ci/mmol, Amersham) by a random priming method. Hybridization was performed in 50% formamide, 5X SSC (0.75M NaCl, 0.075M sodium citrate), Denhardts solution (0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.l% BSA), and 0.l% SDS for 16 h at 42°C. Membrane was washed at room temperature in 2X SSC, 0.1% SDS for 20 min twice, and followed by 0.5X SSC, 0.1% SDS at 60°C for 20 min. Autoradiography was done with Kodak XAR-5 film at -70°C.

Statistical Analyses

Data obtained from the measurements of field and greenhouse experiments were analyzed by using a Duncan's multiple range test. The undistinguishable bands revealed from the PCR reaction of 9 primers were ignored. The frequencies of bands present in the zymogram of the remaining 31 primers were statistically analyzed by means of Euclidean distance (Sneath and Sokal, 1973) using an unweighted pair group method with arithmetic mean (UPGMA). The fomula for Euclidean distance is:

$$d_{jk} = \left[\sum_{i=1}^{n} (X_{ij} - X_{ik})^2 \right]^{1/2}$$

n = number of band

X_{ij} = the frequency of I band in j population

X_{ik} = the frequency of I band in k population

The dendrogram of the cluster analysis based on the data of Euclidean distance was obtained.

Results

Morphological Variation Among Populations of I. cylindrica Plants

Phenotypic characteristics of *I. cylindrica* plants growing in the aforementioned six sites and those transplanted to pots growing in the greenhouse of Academia Sinica were compared. The findings of these comparisons are shown in Table 1. There were significant differences in shoot height, leaf length, and leaf width among the various habitats. For example, comparing the shoot length of *I. cylindrica* collected from the field, the length was significantly longer in populations from Nakang, Hoshe, and Kengting, which ranged from 106 cm to 148 cm, than in populations from Penghu, Sarlun, and Chuwei, which ranged from 71 cm to 92 cm. Leaf length of the six populations was also significantly different, ranging from 47 cm to 105.7 cm, and was the longest in the Kengting population. Comparing the leaf width of the six populations showed that the leaves of Nangkang, Hoshe, and Kengting were wider than those of the remaining three populations. It can be concluded that the grasses of the former three populations grew much better than those of the remaining three populations.

On the other hand, the grasses of the six populations grown in the greenhouse six months after transplanting were also compared with the aforementioned characters. The values were generally lower in the transplanted grasses than in the field grasses (Table 1). For example, taking the Chuwei population for comparison and the samples grown in the field with its transplanted grasses grown in the greenhouse, the shoot height was higher in the field (92.4 cm) than in the greenhouse (51.8 cm). This finding indicated that the NK and SL populations reflected reversed order though most of the remaining populations exhibited the same order. On the other hand, regarding the results of leaf length the orders among population did not fully agree with each other, especially for the NK population. This difference could be due to environmental conditions that are more variable in the field than in the greenhouse. To clarify this point, further study is needed.

Comparing the leaf anatomy of *I. cylindrica* between populations of the Chuwei (saline soil) and Nankang (non-saline soil) sites under a microscope showed that the midrib of *I. cylindrica* leaves from Chuwei was hollow in structure where the pith or vascular bundle disappeared, while that of the Nankang populations was normal (Figure 2A and 2B). Under examination by the scanning electron microscope, villous trichomes were found on the abaxial leaf surface of the Chuwei population but not on those of the Nankang or other remaining populations (Figure 3A

Table 1. Comparison of phenotypic characteristics of *Imperata cylindrica* grasses collected from 6 sites and those transplanted to pots grown in the greenhouse of Academia Sinica.

Sampling site	Shoot height, cm		Leaf length, cm		Leaf width, cm	
	Field	Transplanted	Field	Transplanted	Field	Transplanted
Nankang	106.3b*	69.0b	69.2bc	58.0c	1.00c	0.80b
Hoshe	148.2a	106.8a	87.9ab	115.7a	1.44a	0.95a
Kengting	140.6a	102.4a	105.7a	103.1a	1.13b	0.94a
Penghu	71.1e	53.1c	47.0c	66.8bc	0.76e	0.70b
Sarlun	83.9d	66.2b	60.9c	73.9b	0.95c	0.83ab
Chuwei	92.4c	51.8c	53.9c	73.3b	0.84d	0.74b

* Mean in a column followed by the different letters are significantly different at 1% level using Duncan’s multiple range test.

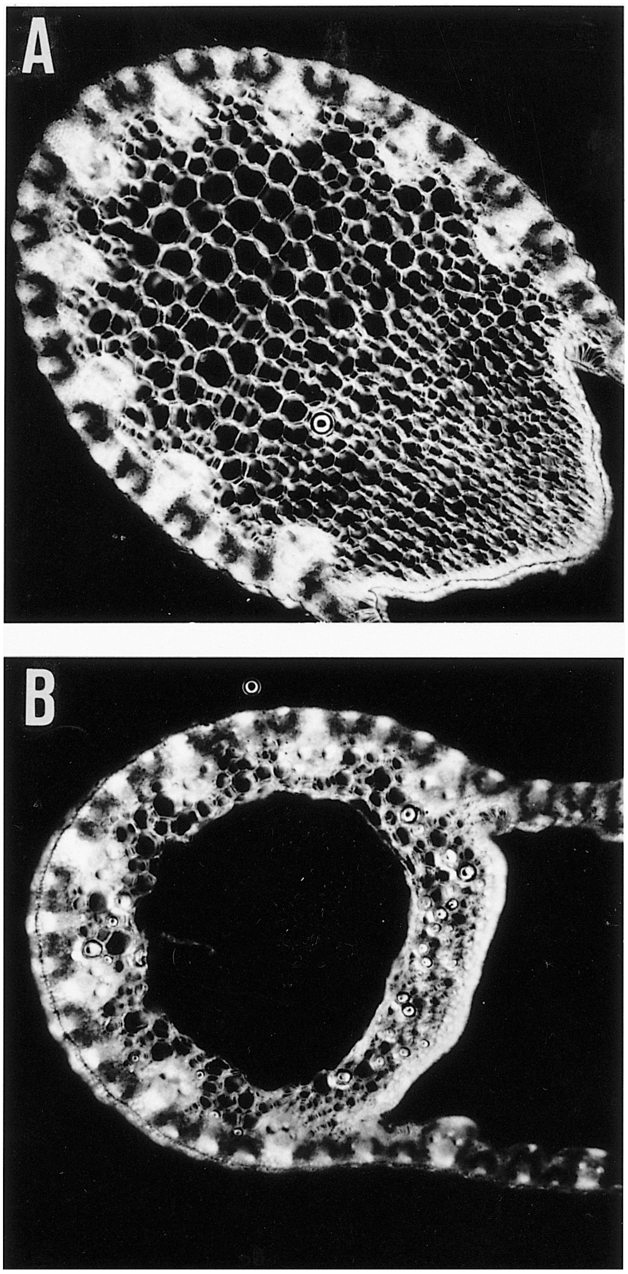

Figure 2. Comparison of microscopic examination of the cross section of *Imperata* leaves (×6.3) collected from A: Nankang as control site; B: the estuary of Chuwei.

and 3B). These two distinct anatomic characters reflected that an adaptive mechanism of the Chuwei population to saline environments had developed.

Screening DNA Markers for Imperata Populations

In order to select DNA markers for characteristics of the *I. cylindrica* population, various primers were used in RAPD analysis (data described by Cheng, 1994). Two markers, 1605 bp and 700 bp, were specific to populations Chuwei (CW) and Sarlun (SL) (Figure 4) but were absent in the other four populations when an Operon primer Q14 (5GGACGCTTCA3') was used. However, a unique marker with 685 bp was clearly present in all six populations when a primer RT8 (5GGAAAAGCCC3') was employed (Figure 5). The DNA sequence of the marker was obtained by us (Cheng, 1994). In addition, several markers, around 550 bp and 300 bp, were also found in all populations (Figure 5).

Phylogenetic Relationships Among Imperata Populations

To elucidate the phylogenetic relationship among populations of *I. cylindrica* collected from the six sites mentioned previously, forty 10-mer primers were used, from which nine primers were not successful and produced irregularly amplified products. The remaining 31 primers resulted in 82 bands of DNA markers for the *Imperata* populations. And the frequency of each band distributed in the 6 sites is given in Table 2. Among the 82 bands, several —namely 1, 14, 22, 24, 31, 49, and 76—were specific to certain populations. For example, band 14 was only present in the SL and CW populations; band 24 was present in a high frequency in the SL and CW, but was rather scarce in the remaining populations and completely

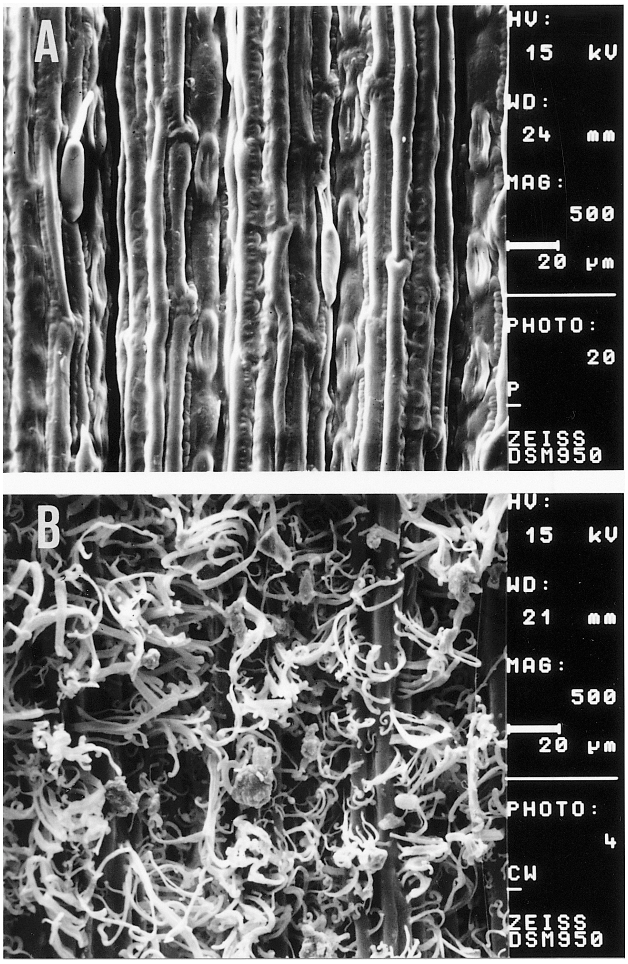

Figure 3. Comparison of scanning electron microscopic examination of abaxial leaf surface of *Imperata* leaves collected from A: Nankang as control site; B: the estuary of Chuwei.

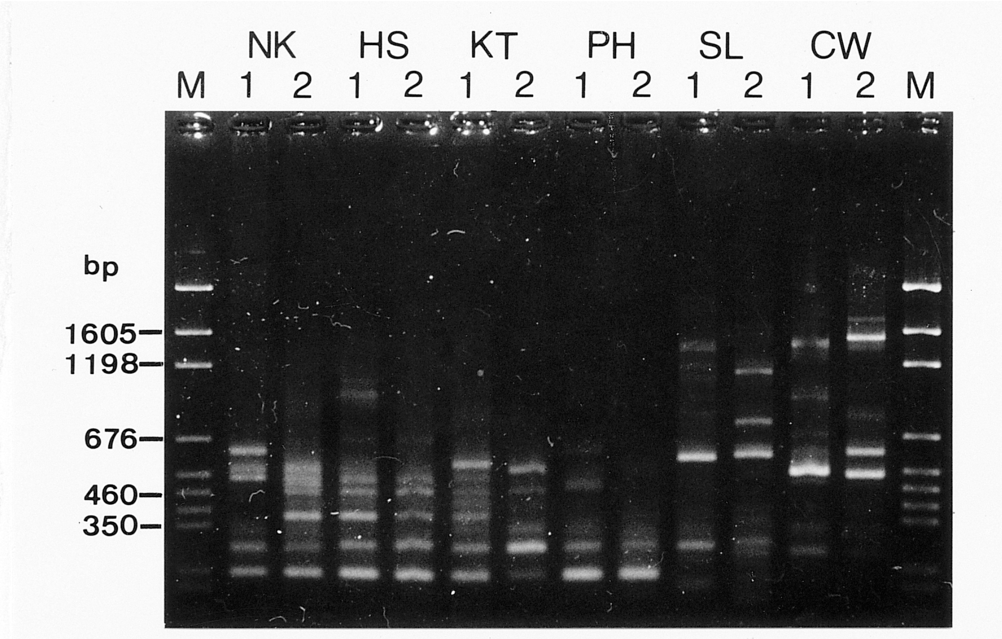

Figure 4. Amplification products obtained from DNA of the six populations of *I. cylindrica* with Operon primer Q14: 5'GGACGCTTCA3'. Two samples representing each population were loaded for agrarose gel electrophoresis. The site abbreviations are NK: Nankang, HS: Hoshe, KT: Kengting, PH: Penghu, SL: Sarlun, and CW: Chuwei.

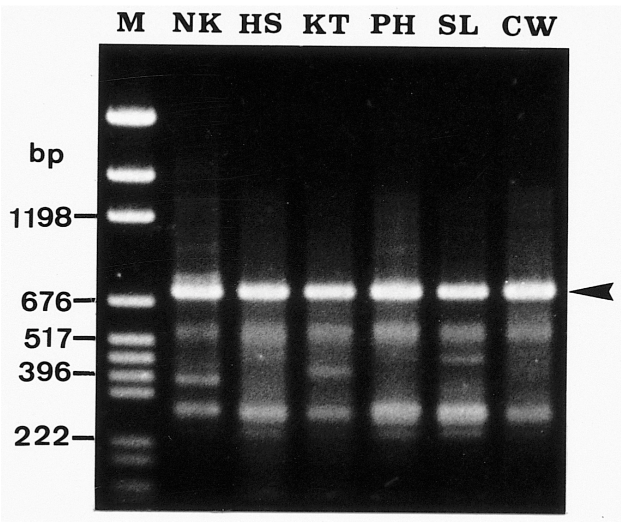

Figure 5. The RAPD profile of genomic DNA from leaves of *I. cylindrica* generated by the primer RT8: 5'GGAAAAGCCC3' reacted with six populations at NK: Nankang, HS: Hoshe, KT: Kengting, PH: Penghu, SL: Sarlun, CW: Chuwei, and M: DNA marker.

missing from the KT population. These findings indicated that some bands were formed only in saline areas, such as CW and SL.

By means of the Euclidean distances (d_{jk}) based on RAPD band-frequency data, a dendrogram of six. *I. cylindrica* populations was constructed. The smaller the values of d_{jk}, the closer the relationship between the two populations. The smallest distance of 0.33 was found between the populations of Chuwei and Sarlun. The longest distance, between the Nankang and Hoshe populations, was 0.49 (Figure 6). Based on the Euclidean distance, two clusters were formed; one cluster included populations Chuwei, Sarlun, Penghu, and Kengting; another cluster involved populations Nankang and Hoshe. The populations Chuwei, Sarlun, Penghu, and Kengting are located in highly saline coastal areas, receiving salt spray year around, while the populations Nankang and Hoshe are located inland where soil salinity is relatively low (Cheng, 1994). It could also be interpreted that genetic adaptation might be evolved. Although much work needs to be done, the aforementioned findings indicate ecogenetic variability among these populations. It is thus concluded that the two ecotypes of *I. cylindrica* are Ecotype I: the Chuwei and Sarlun populations and Ecotype II: the Nankang and Hoshe populations.

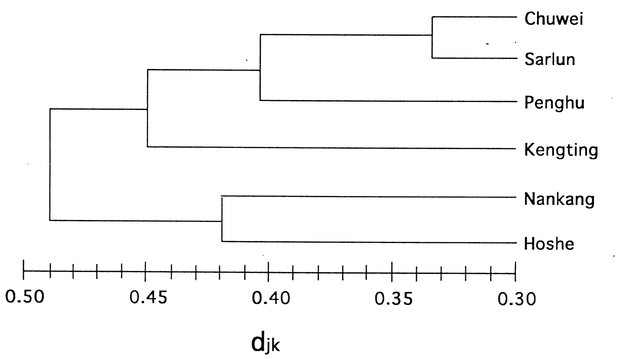

Figure 6. The dendrogram of the six populations of *Imperata cylindrica* based on frequency of bands of RAPD by means of Euclidean distance.

Table 2. The frequency of each band in RAPD profile present in the six populations designated as NK, HS, KT, PH, SL, and CW of *I. cylindrica.*

Band	Population						Band	Population					
No.	NK	HS	KT	PH	SL	CW	No.	NK	HS	KT	PH	SL	CW
1	0.83	0.17	0.50	0.25	0.00	0.00	42	0.92	1.00	0.92	0.50	0.67	0.50
2	0.67	0.17	0.58	0.08	0.00	0.11	43	0.83	1.00	1.00	0.92	1.00	0.92
3	0.17	1.00	0.08	0.17	0.00	0.00	44	0.42	0.58	0.75	1.00	1.00	0.67
4	0.33	0.00	0.17	0.42	0.25	0.22	45	0.83	0.67	1.00	1.00	1.00	0.42
5	0.00	0.09	0.67	0.17	0.33	0.22	46	0.67	0.67	0.58	0.25	0.17	0.08
6	0.25	0.67	0.25	0.00	0.08	0.17	47	0.50	0.25	0.08	1.00	0.92	0.42
7	0.75	0.92	1.00	0.08	0.08	0.33	48	0.92	1.00	1.00	0.75	0.50	0.75
8	0.75	0.67	1.00	0.42	0.50	0.00	49	0.25	0.00	0.00	0.00	0.33	0.83
9	0.17	0.08	0.00	0.08	0.00	0.42	50	0.58	0.92	0.50	0.42	0.17	0.50
10	0.08	0.67	1.00	0.67	0.58	0.33	51	0.58	0.33	0.67	0.17	0.25	0.33
11	0.50	0.50	0.08	0.42	0.08	0.00	52	0.58	0.58	0.58	0.58	0.58	0.58
12	0.08	0.08	0.25	0.67	0.42	0.42	53	0.83	1.00	1.00	1.00	0.25	0.33
13	1.00	1.00	1.00	0.58	1.00	0.67	54	0.83	1.00	1.00	1.00	0.83	0.83
14	0.00	0.00	0.00	0.00	0.17	0.50	55	0.83	0.83	1.00	0.33	0.67	0.75
15	0.67	0.67	0.50	0.08	0.25	0.17	56	0.83	0.42	1.00	1.00	0.42	0.50
16	0.33	0.75	0.33	0.00	0.25	0.33	57	0.17	0.75	0.08	0.42	0.33	0.42
17	0.67	0.00	0.58	0.67	0.58	0.58	58	1.00	1.00	1.00	1.00	0.67	0.83
18	0.50	0.33	0.25	0.17	0.25	0.83	59	0.92	1.00	0.83	0.67	0.33	0.67
19	0.50	0.50	0.25	0.17	0.17	0.17	60	0.75	1.00	1.00	0.83	0.83	0.67
20	0.33	0.58	0.92	0.67	0.33	0.17	61	0.33	0.17	0.67	0.83	0.17	0.08
21	0.83	1.00	0.83	1.00	0.33	0.00	62	0.42	0.00	0.08	0.25	0.25	0.33
22	0.75	1.00	1.00	0.00	0.42	0.17	63	0.92	1.00	1.00	0.50	0.08	0.67
23	1.00	1.00	1.00	1.00	0.08	0.33	64	0.33	0.17	0.00	0.17	0.25	0.58
24	0.33	0.17	0.00	0.33	0.92	1.00	65	0.25	0.25	0.08	0.17	0.17	0.58
25	0.08	1.00	0.92	0.42	0.33	1.00	66	0.67	0.92	0.83	0.33	0.67	0.33
26	0.58	1.00	0.83	0.92	0.42	1.00	67	0.58	0.67	0.33	0.08	0.17	0.25
27	0.50	0.92	0.42	0.58	0.58	0.58	68	0.92	0.83	0.67	0.58	0.50	0.42
28	0.50	0.17	1.00	1.00	0.75	0.33	69	0.75	0.92	0.75	0.33	0.42	0.33
29	0.83	0.83	0.67	0.58	0.67	0.75	70	0.67	0.92	1.00	0.67	0.50	0.25
30	0.33	1.00	0.33	0.08	0.33	0.17	71	0.58	0.92	0.42	0.50	0.25	0.17
31	0.17	0.00	0.00	0.17	0.42	0.25	72	0.58	0.75	0.50	0.42	0.17	0.33
32	1.00	0.92	0.92	0.92	1.00	0.50	73	0.67	0.67	0.17	0.17	0.25	0.17
33	1.00	0.67	0.83	1.00	1.00	0.83	74	0.67	0.50	0.50	0.00	0.17	0.25
34	0.67	0.42	0.25	0.33	0.50	0.25	75	0.50	0.25	0.25	0.17	0.25	0.25
35	0.25	0.42	0.00	0.33	0.17	0.33	76	0.75	0.50	0.33	0.17	0.00	0.00
36	0.58	0.58	0.92	0.58	0.25	0.25	77	0.92	0.75	0.83	0.33	0.08	0.67
37	0.25	1.00	0.17	0.67	0.50	0.75	78	0.17	0.83	0.75	0.50	0.58	0.83
38	0.50	0.83	0.92	0.25	0.67	0.58	79	1.00	0.67	0.83	0.58	0.75	0.75
39	0.83	0.92	1.00	0.42	0.33	0.25	80	0.17	0.50	0.00	0.92	0.08	0.58
40	0.75	0.83	0.58	0.08	0.33	0.50	81	0.75	0.75	0.50	0.25	0.08	0.33
41	0.50	0.50	0.33	0.33	0.08	0.25	82	0.25	0.33	0.83	0.33	0.08	0.08

NK=Nankang, HS=Hoshe, KT=Kengting, PH=Penghu, SL=Sarlun, CW=Chuwei.

Discussion

The phenotype of *I. cylindrica* varied with habitat and environment, being affected by edaphic, temperature, and other climatic factors. For example, temperature markedly affected the growth of this grass. Plants grown at 29/23°C for 87 days were taller and produced more dry weight and leaf area than did plants grown at 23/17°C. In addition, the growth was better with a 16-h photoperiod than with a 12-h photoperiod (Patterson et a1., 1980). Plant height was obviously shorter in the presence of competitors (Wilcut et al., 1988b). It has also been reported that this plant grew better in soil of pH 4.7 than in soil of pH 6.7 (Wilcut et al., 1988a). Besides the aforementioned environmental factors, the density of plant growth might also influence the phenotype. In the present study, morphological characters such as plant height, leaf length, and leaf width among six populations of *I. cylindrica* grown in the field had a 2-fold deviation from plants in the greenhouse. The plants adapted to various habitats possess different growth potential. The characters were still observed when the plants developed under the same growth conditions in the greenhouse. Obviously, there are two stable traits, such as villous trichome and hollow midrib, that are specific to the Chuwei population of *I. cylindrica*, indicating that the plants growing in the mangrove area of Chuwei have developed into an ecotype.

The RAPD technique provides a simpler and more rapid method of DNA finger printing than does the restriction fragment length polymorphism (RFLP) technique. RAPD allows the detection of genomic diversity among organisms using single, short primers of arbitrary sequence in polymerase chain reaction. The degree of genetic relationship can subsequently be determined through analysis of the RAPD data. In the present study, a dendrogram was constructed for six populations of *Imperata* based on the frequencies of presence of the RAPD markers. Some of these markers were present in some populations, but absent in others (Cheng, 1994). These population-specific markers are important in the study of population ecology. Arnold et al. (1991), using genetic markers, demonstrated that there was interspecific gene flow between *Iris fulva* and *I. hexagona* and there was a hybrid of *I. nelsonii* originated from the interaction of *I. fulva* and *I. hexagona*. We further screened two population-specific markers found only in the Chuwei population (Cheng, 1994). More RAPD markers were also needed to determine the structure of phylogenetic relationships within a population and between populations of *I. cylindrica*. The extent of DNA polymorphism present in the species depends on its genetic complexity. Some plant species, such as tomato (Miller and Tanksley, 1990) and soybean (Apuya et al., 1988), exhibit low frequencies of RFLP. However, other plants, such as maize (Smith, 1988) and potato (Gebhardt et al., 1989), reveal a high degree of DNA polymorphism. Hu and Quiros (I991) identified broccoli and cauliflower cultivars with RAPD markers and found that 28% of the markers were monomorphic in both crops. Of them, 12.5% were specific to either crop and the rest were polymorphic in either or both crops. Among twenty-one genotypes, almost 80% of the markers showed polymorphisms (Heun and Helentjaris, 1993). In RAPD analysis of *I. cylindrica*, 80% of the markers were polymorphic in the species (Cheng, 1994). However, there were high similarities between the populations from 0.83 to 0.94 (Cheng, 1994).

The variation of band frequency revealed by the RAPD profile indicated a high level of genetic heterogeneity among the populations of *I. cylindrica* in Taiwan. The six populations were grouped into two clusters according to their genetic relationships. The first cluster included the populations from Chuwei, Sarlun, Penghu, and Kengting, located in various habitats along the coast of the island. The population of Sarlun is found on the seashore; the Chuwei population is located in the estuary of the Tamshui river. The Chuwei and Sarlun populations grow in areas of high salinity. Severe drought or flooding makes them different from the population of Nankang. Salt stress may be thought of as the principal driving force enhancing population divergence. The Penghu and Kengting populations suffer from drought stress. The amount of annual precipitation on the islet of Penghu is relatively low (964 mm) and is concentrated in the summer season. The islet also receives high light intensity and strong wind, resulting in the plants growing short. At Kengting, there is a dry season for five months from November to March; however, suffcient precipitation and moderate weather provide better conditions for plant growth in the rest of the year. Thus, the growth of grass in the Kengting area is not significantly different from that of Hoshe. Nevertheless, the roles of these markers in the adaptation mechanism of *I. cylindrica* to various environmental regimes needs to be further studied.

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