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Phylogenetic relationship among species of *Miscanthus* populations in Taiwan

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Abstract. In order to understand the phylogenetic relationship among species of *Miscanthus* population in Taiwan, eight taxa of *Miscanthus* plants distributed widely in Taiwan were employed. Leaves of *Miscanthus* plants which were transplanted from fields and grown in a farm of the Taiwan Sugarcane Research Institute at Pintung, Taiwan, were collected for analysis for four isozymes, using polyacrylamide gel electrophoresis to study the phylogenetic relationship among eight taxa of *Miscanthus* populations. These taxa are *M. floridulus* (designated as Mf), *M. flavidus* (Mfa), *M. sinensis* (Ms), *M. transmorrisonensis* (Mt), *M. sinensis* var. *formosanus* (Msf), hybrid of Ms x Msf (designated as Ms-Msf), hybrid of Mf x Ms x Msf (Mf-Ms-Msf), and hybrid of Mf x Ms (Mf-Ms). The frequencies of isozyme bands present in each taxa were obtained, and data were computed by two statistical equations, such as a simple matching coefficient and Euclidean distance. On the basis of the similarity coefficients (Ssm) of the four isozymes, it is concluded that the taxa of Ms, Msf, Ms-Msf, and Ms-Mf-Msf are genetically close, with high Ssm of 0.738, while those of Mfa and Mt forming a second cluster are far from the former one. Taxa of *M. floridulus* and several hybrids lie between these two clusters. Furthermore, based on the Euclidean distance, the phylogenetic relationship among the represented 8 taxa is by a series as follows: Ms, Ms-Msf, Ms-Mf, Msf, Ms-Mf-Msf, Mf, Mfa, and Mt, indicating that *M. sinensis* is the most primitive taxon. This evolved to *M. sinensis* var. *formosanus, M. floridulus, M. flavidus*, and then to *M. transmorrisonensis* through many years of adaptation.

Key words: Esterase; Indolphenol oxidase: Isozymes; *Miscanthus*; Peroxidase; Phylogensis; Polyphenol oxidase.

Introduction

In recent years, isoenzyme study has received increasing attention by geneticists to study the genes involved in enzyme synthesis, by biochemists and physiologists to look at the physico-chemical properties of isoenzymes as a means to understand the regulation of cell metabolism, and by evolutionary ecologists to study the fuction of isoenzymes to enhance the biochemical adaptability of the organism and to protect it against loss of function by mutation or environmental stresses (Shannon, 1968; Gottlieb, 1982; Schmitz and Kowallik, 1986). Isoenzyme studies in a variety of plants were carried out and a tremendous wealth of information appeared in the literature (Nei, 1965, 1973; Johnson, 1977; Kiang and Wu, 1979; Loukas *et al*., 1983; Silander, 1984). For example, Wu and Bradshaw (1972) studied the copper tolerance of *Agrostis tenuis* and *festuca ovina* by using esterase isozymes as a parameter and found that the isozyme patterns were significantly different among five grass populations where the soils were polluted by copper from a factory and from those of an area without copper pollution. Chou and his co-workers (1984a, 1984b, 1985, 1986) employed different isozymes to study the phylogenetic relationship among bamboo species, and Hsiao (1980) also used isozymes to study the chemotaxonomic relationship among taxa of

Chamecyparis. They concluded that isozymes can play a significant role in clarifying the taxonomic and phylogenetic position of plants.

Miscanthus floridulus (Labill.) Warb. as well as *M. flavidus Honda, M. sinensis* Anderss, and *M. sinensis* var. *formosanus Hack* are widely distributed in Taiwan, particularly in roadsides, river bed, abandoned fields, and hillsides of areas below 2200 m in elevation. On the other hand, *M. transmorrisonensis* Hay. occurs in areas above 2400 m (Chou and Chang, 1988). The populations of these species exhibited ecotypical variation not only in Taiwan proper but also in Green and Orchid islets about 50 kilometer off Taiwan proper (Chou and Chang, 1988; Chou and Chen, 1990). However, the phylogenetic relationship among eight taxa of *Miscanthus* populations is not known. It is the aim of the present study to elucidate this relationship.

Materials and Methods

Material

Defoliated plants with roots of eight taxa from 104 populations of *Miscanthus*, namely *M. floridulus* (hereafter called Mf), *M. flavidus* (Mfa), *M. sinensis* (Ms), *M. sinensis* var. *formosanus* (Msf), *M. transmorrisonensis* (Mt), hybrid of Ms x Msf (Ms-Msf), hybrid of Mf x Ms (Mf-Ms), and hybrid of Mf x Ms x Msf (Mf-Ms-Msf) collected from many populations in various habitats in Taiwan were transplanted into cement pots and grown in an experimental farm of the Taiwan Sugarcane Research Institute at Wandan, Pintung, Taiwan. For each taxon, *Miscanthus floridulus* was collected from 12 populations in various locations, *M. sinensis* from 15, *M. flavidus* from only one, *M. sinensis* var. *formosanus* from three, hybrid of Ms-Msf from 14, hybrid of Mf-Ms from 39 and hybrid of Mf-Ms-Msf from 10 populations. The taxonomic identification of these taxa were based on the work done by specialists of the Taiwan Sugarcane Research Institute. Prior to collection of the samples for the present study, the plants had grown there for at least five years. The leaves collected were immediately stored in a hand-carried ice box to keep temperature below 5°C. The samples were brought back to the laboratory at Academia Sinica, Taipei, for analyses by polyacrylamide gel electrophoresis (PAGE) (Chou *et al*., 1987). The chemicals and solvents for electrophoresis were purchased either from the Sigma Corp., or from Merck Ltd., Germany.

Electrophoresis Analysis

A vertical gel electrophoresis apparatus (M & S Slab Electrophoresis, model SG-80) was employed, and techniques for electrophoresis of *Miscanthus* leaves were as described by Chou *et al*. (1984a). Many isozymes were employed to analyze the isozyme patterns of *Miscanthus* leaves and found that peroxidase, esterase, indophenol oxidase, and polyphenol oxidase isozymes were quite stable, thus these four isozymes were chosen for the study. After the analysis by electrophoresis, the gel was dried and a permanent record (zymogram) was obtained, and the R_f value of each band present in the zymograms was calculated and the frequency of each band in each population was obtained.

Statistical Analysis

In order to understand the phylogenetic relationship and ecotypical variation of other eight *Miscanthus* taxa of 104 populations in Taiwan, two mathematical quantities, a simple matching coefficient (Ssm) and an Euclidean distance (d_{jk}), were used (Sneath and Sokal, 1973). The formula for similarity coefficient, $Ssm = m/(m+n)$ was used, where m is the number of matches and n the number of mismatches. The data of Ssm were then set in a simple matrix table, each taxa on being used as an operation taxonomic unit. The second quantity, the taxonomic distance, d_{jk}, between these units is defined as

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

where X_{ij} is the average frequency of i band in a zymogram of j population and X_{ik} is the average frequency of i band in the zymogram of k population. By employing this equation the data were used to obtain a matrix for each isozyme, and clustering analysis between taxa was based on an unweighted pair-group method using the simple arithmetic average described by Sneath and Sokal (1973); Thus the phylogenetic relationship among taxa of *Miscanthus* was obtained.

Results

*Distribution and Frequency of Peroxidase Isozymes in **Miscanthus** Populations*

The distribution and frequency of peroxidase isozyme in 8 taxa of *Miscanthus* populations are given in

Table 1. *The frequency of each band in peroxidase isozymes present in 8 taxa of* ***Miscanthus*** *plants grown in pots at Wandan, Taiwan*

Band No.	R_f	Mf[1]	Ms	Mfa	Taxa Mt	Msf	Ms-Msf	Mf-Ms-Msf	Mf-Ms
1	0.042	0.50	0.27	-	-	0.5	0.500	-	0.28
2	0.105	0.58	0.13	1.00	-	0.6	0.214	0.70	0.23
3	0.130	0.50	0.33	1.00	-	0.2	0.268	0.60	0.46
4	0.154	0.33	0.20	-	1.00	-	0.429	0.50	0.31
5	0.177	-	0.066	-	-	-	-	-	0.03
6	0.207	0.33	0.533	1.00	0.66	1.00	0.643	0.60	0.54
7	0.238	0.83	0.400	1.00	1.00	0.60	0.500	0.80	0.44
8	0.277	0.50	0.533	1.00	0.30	0.40	0.790	0.20	0.64
9	0.304	-	-	-	0.30	-	0.070	-	0.05
10	0.380	-	-	-	-	-	-	-	0.03
11	0.408	0.08	0.13	1.00	-	0.20	-	0.20	0.15
12	0.423	-	0.13	-	-	0.20	-	0.20	0.08
13	0.453	-	0.13	-	-	-	0.070	-	-
14	0.485	-	-	-	-	-	-	-	0.05
15	0.500	0.166	0.47	-	0.30	-	0.710	0.40	0.28
16	0.525	1.00	0.47	1.00	1.00	0.10	0.360	0.60	0.62
17	0.542	0.75	0.13	-	-	0.10	0.070	0.50	0.15
18	0.565	1.00	0.93	1.00	1.00	1.00	0.860	0.80	0.95
19	0.585	-	0.13	-	-	0.10	0.140	0.60	0.10
20	0.604	1.00	1.00	1.00	1.00	0.90	0.930	0.50	0.41
21	0.615	-	-	-	-	0.10	-	-	-
22	0.623	-	-	-	-	-	-	-	0.13
23	0.652	0.917	0.93	1.00	1.00	0.90	0.786	0.70	0.56
24	0.660	-	-	-	-	0.10	-	0.10	0.03
25	0.685	0.833	0.33	1.00	1.00	0.90	0.400	0.80	0.13
26	0.723	0.917	-	-	0.66	0.10	0.070	-	0.21
27	0.750	-	0.13	-	1.00	-	0.360	0.10	0.26
28	0.773	1.00	1.00	1.00	1.00	-	0.666	0.90	0.51
29	0.815	1.00	1.00	1.00	1.00	1.00	0.790	1.00	0.36
30	0.850	1.00	1.00	1.00	1.00	1.00	0.860	1.00	0.49
31	0.888	-	0.33	-	0.66	1.00	0.500	-	-

[1]The abbreviations of *Miscanthus* taxa are: Mf = *M. floridulus*; Ms = *M. sinensis*; Msf = *M. sinensis* var. *formosanus*; Mt = *M. transmorrisonensis*; Mfa = *M. flavidus*; Mf-Ms = *M. floridulus* x *M. sinensis; Ms-Msf* = *M. sinensis* x *M. sinensis* var. *formosanus*; Mf-Ms-Msf = *M. floridulus* x *M. sinensis* x *M. sinensis* var. *formosanus*.

Table 1. Some of peroxidase bands were observed for the taxa studied. Of them, bands 6, 7, 8, 16, 18, 20, 23, 25, 29, and 30 are shown by all taxa; some of them are predominant, with the high frequency reaching 1.0, such as bands 7, 16, 18, 20, 23, 28, and 29. Unfortunately, only one population of *M. flavidus* was found in the present study, so that we could obtain samples from only one pot. Rather similarly, *M. transmorrisonensis* samples were collected from only three populations. That is the reason why the frequency of each isozyme band for *M. flavidus* illogically reaches 1.00. In particular, some bands, namely 12, 13, and 27, shown by *M. sinensis* and its hybrids are missing from the remaining taxa. Nevertheless, the peroxidase was very stable during the course of study.

Distribution and Frequency of Esterase Isozymes in ***Miscanthus*** *Populations*

The distribution and frequency of esterase isozymes present in 8 taxa of *Miscanthus* populations are

Table 2. *The frequency of each band in esterase isozymes present in 8 taxa of* ***Miscanthus*** *plants grown in pots at Wandan, Taiwan*

Band No.	R_f	Taxa							
		Mf	Ms	Mfa	Mt	Msf	Ms-Msf	Mf-Ms-Msf	Mf-Ms
1	0.11	0.666	-	-	-	-	0.14	-	0.04
2	0.22	0.25	-	-	-	-	-	-	-
3	0.26	0.58	-	1.0	0.33	-	-	-	-
4	0.27	-	-	1.0	0.66	-	-	0.10	0.16
5	0.29	-	-	-	-	-	-	-	0.08
6	0.30	-	0.06	1.0	0.66	0.10	0.43	0.50	0.40
7	0.33	0.58	0.06	1.0	0.66	0.50	0.43	0.60	0.40
8	0.35	-	-	-	-	-	0.21	0.10	0.20
9	0.36	0.58	-	-	1.00	0.30	0.21	0.50	0.32
10	0.38	-	-	-	-	-	-	-	0.04
11	0.40	0.50	-	-	1.00	0.10	0.14	0.60	0.36
12	0.42	-	-	-	-	-	0.07	-	-
13	0.43	0.15	-	-	0.33	0.20	-	-	-
14	0.46	-	-	-	-	0.20	-	-	-
15	0.48	0.15	-	-	-	-	-	0.10	-
16	0.56	0.08	-	-	-	-	-	-	0.04
17	0.58	0.08	-	-	-	-	-	-	-
18	0.62	-	-	-	-	-	0.21	0.10	-
19	0.65	-	-	-	0.33	-	-	0.30	-
20	0.68	0.25	0.20	-	0.33	0.20	0.29	0.50	-
21	0.71	0.17	0.53	-	0.33	-	0.21	0.10	0.12
22	0.72	-	-	-	0.33	0.20	0.21	0.10	0.20
23	0.74	1.00	0.93	-	0.66	0.40	0.21	0.90	0.76
24	0.76	1.00	1.00	1.0	1.00	0.80	1.00	1.00	0.56
25	0.77	-	-	-	1.00	-	0.07	0.00	0.08
26	0.79	1.00	1.00	1.0	-	1.00	1.00	0.90	1.00
27	0.81	0.25	-	-	-	0.40	-	0.00	0.28
28	0.82	0.16	0.73	-	1.00	-	0.29	0.30	0.16
29	0.87	0.08	-	-	-	-	0.64	0.30	0.12
30	0.91	0.08	0.20	-	-	0.30	-	-	0.24
31	-	-	-	-	-	0.30	0.21	0.70	-

The abbreviations of *Miscanthus* taxa see Table 1.

given in Table 2. Some 31 esterase bands are present in *Miscanthus*. Of those, bands 7 and 24 are predominant in all taxa. Other bands, such as 6, 9, 11, 20, 21, 23, 26, 28, and 29 were found for all taxa except *M. flavidus*. It was shown that only six bands were present for *M. flavidus* based on only one population. Moreover, it was noticeable that 9 bands were seen for *M. sinensis*, and many bands were missing from the zymogram. However, in these hybrid taxa, some bands missing for *M. sinensis* were present for the remaining taxa, suggesting that intraspecific hybridization between species occurred.

Distribution and Frequency of Indophenol Oxidase Isozymes in ***Miscanthus*** *Populations*

The distribution and frequency of indophenol oxidase isozymes are shown in Table 3. Twenty-one bands were found for the eight taxa of *Miscanthus*. Of them, bands 5, 8, 10, 20, and 21 were present for all taxa, while bands 6 and 16 were only found in the hybrid Mf-Ms-Msf. In addition, band 1 was shown only for *M. flavidus*. Few bands, such as bands 2, 12, and 19, were found for *M. sinensis* and its hybrides. Excepting bands 18, 19, 20, and 21, the percent frequency of the

Table 3. *The frequency of each band in indophenol oxidase isozymes present in 8 taxa of* ***Miscanthus*** *plants grown in pots at Wandan, Taiwan*

Band No.	R_f	Taxa							
		Mf	Ms	Mfa	Mt	Msf	Ms-Msf	Mf-Ms-Msf	Mf-Ms
1	0.28	-	-	1.0	-	-	-	-	-
2	0.32	-	0.06	-	-	-	-	-	0.03
3	0.36	-	-	-	-	-	-	-	0.03
4	0.39	0.33	-	1.0	0.38	-	-	-	0.05
5	0.42	0.92	0.86	1.0	0.66	0.2	0.71	0.2	0.60
6	0.44	-	-	-	-	-	-	0.1	0.05
7	0.45	0.25	-	-	0.66	-	-	-	0.03
8	0.47	0.58	0.86	1.0	0.66	0.90	0.71	0.1	0.59
9	0.48	0.25	-	-	-	-	-	-	0.08
10	0.49	0.42	0.33	1.0	0.66	0.40	0.57	0.1	0.38
11	0.50	0.08	-	-	-	-	-	0.1	0.03
12	0.53	-	0.13	-	-	-	-	0.1	0.03
13	0.54	0.25	-	-	-	-	-	-	0.08
14	0.55	-	0.53	-	0.66	0.60	0.50	0.2	0.64
15	0.56	0.17	0.06	-	0.66	-	-	0.1	0.05
16	0.60	-	-	-	-	-	-	0.3	-
17	0.63	-	-	-	-	-	-	-	0.08
18	0.68	0.17	0.13	1.0	1.00	0.20	-	1.0	0.36
19	0.72	-	0.20	1.0	0.66	-	-	0.9	0.46
20	0.78	0.25	0.60	1.0	1.00	0.90	0.21	0.7	0.33
21	0.85	1.00	0.80	1.0	0.66	0.60	0.86	1.0	1.00

The abbreviations of taxa see Table 1.

Table 4. *The frequency of each band in polyphenol oxidase isozymes present in 8 taxa of* ***Miscanthus*** *plants grown in pots at Wandan, Taiwan*

Band No.	R_f	Taxa							
		Mf	Ms	Mfa	Mt	Msf	Ms-Msf	Mf-Ms-Msf	Mf-Ms
1	0.04	-	-	-	-	-	-	-	0.31
2	0.07	-	0.47	-	-	0.2	0.36	0.3	0.49
3	0.10	0.92	0.53	-	-	0.9	0.64	0.9	0.90
4	0.13	-	0.26	-	-	-	0.36	0.1	0.38
5	0.15	-	-	-	-	-	-	-	0.03
6	0.17	-	0.20	-	0.66	-	0.14	0.2	0.15
7	0.19	0.08	0.33	-	0.66	0.40	0.64	0.5	0.49
8	0.21	0.25	0.40	-	0.33	-	0.43	0.4	0.46
9	0.23	0.67	0.66	1.00	0.33	0.40	0.64	0.7	0.67
10	0.25	0.25	-	-	-	-	0.07	0.1	-
11	0.28	-	-	-	-	-	-	-	0.05
12	0.34	0.08	0.13	1.0	-	-	-	0.1	0.10
13	0.38	0.08	0.07	1.0	-	0.20	-	-	0.10
14	0.42	0.25	-	-	0.33	-	0.14	-	0.08
15	0.46	1.00	0.80	1.0	1.00	1.00	0.93	0.9	0.77
16	0.48	0.33	0.07	-	-	0.10	0.07	0.1	0.10
17	0.50	0.83	0.80	1.0	1.00	0.90	0.86	1.0	0.77
18	0.51	0.16	-	-	-	0.10	-	-	0.05
19	0.53	0.50	0.47	1.0	1.00	0.90	0.64	0.9	0.51
20	0.55	0.08	-	-	-	-	-	-	0.05
21	0.57	0.33	0.27	1.0	1.00	0.80	0.21	0.3	0.15
22	0.08	-	-	-	-	-	-	-	
23	0.61	0.25	0.13	-	-	0.40	-	-	-
24	0.63	0.08	-	-	-	-	-	-	-
25	0.65	0.08	-	-	-	-	-	-	-

The abbreviations of taxa see Table 1.

other indophenol oxidase bands of the hybrid Mf-Ms-Msf was very low, indicating that occurrence of these bands could be a result of hybridization of the taxa mentioned.

*Distribution and Frequency of Polyphenol Oxidase Isozymes in **Miscanthus** Populations*

The distribution and frequency of polyphenol oxidase isozymes in eight taxa of *Miscanthus* populations are presented in Table 4. Twenty-five isozyme bands were found. Of them, five bands, namely bands 9, 15, 17, 19, and 21, were found predominantly. The isozymes bands presented by the hybrid Mf-Ms were mostly found in their parental taxa of *M. floridulus* and *M. sinensis*. As hybrid Mf-Ms, the isozyme pattern of Mf-Ms-Msf was a result of natural hybridization.

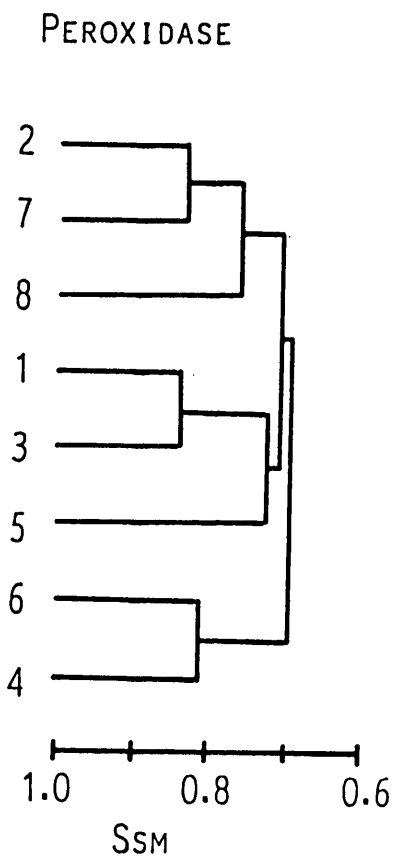

Fig. 1. The dendrogram of 8 taxa of *Miscanthus* based on the presence of peroxidase isozymes and computed by a formula of similarity coefficient (Ssm). Results shown in Figs. 2 to 5 are based on the the same plant material and the same computation. The designation of numbers shown in Fig. 1-10 is the same given as follows: 1 = *M. floridulus*; 2 = *M. sinensis*; 3 = *M. flavidus*; 4 = *M. transmorrisonensis*; 5 = *M. sinensis* var. *formosanus*; 6 = *M. sinensis* x *M. sinesis* var. *formosanus*; 7 = *M. floridulus* x *M. sinesis* x *M. sinensis* var. *formosanus*; 8 = *M. floridulus* x *M. sinensis*.

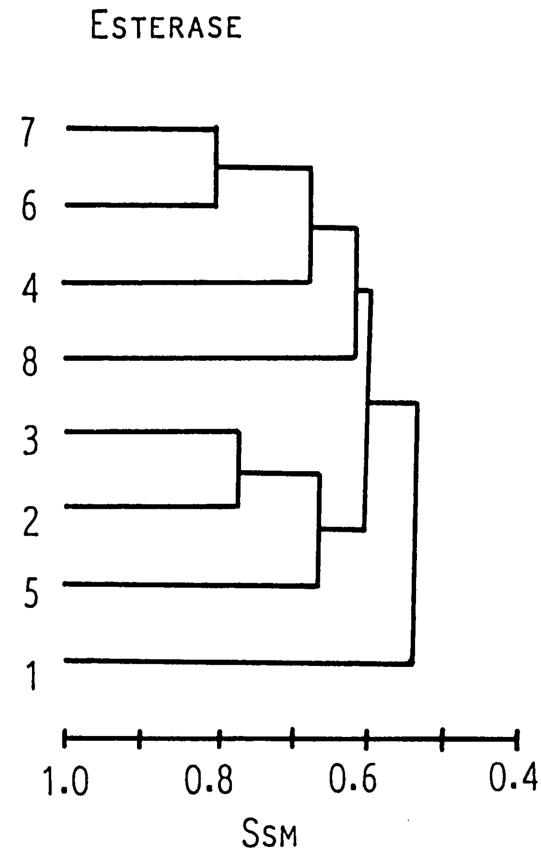

Fig. 2. The dendrogram of 8 taxa of *Miscanthus* based on the presence of esterase isozymes.

*Phylogenetic Relationships Among Taxa of **Miscanthus** Populations Derived from Simple Matching Coefficient*

The data for isozyme analyses were computed by simple matching coefficients based on the peroxidase data for the eight taxa of *Miscanthus*, and a dendrogram was thus derived (Fig. 1). The dendrogram shows that the similarity coefficient (Ssm) between taxa of *M. sinensis* and hybrid Mf-Ms-Msf is 0.839. The similarity coefficient between *M. floridulus* and *M. flavidus* also high. Moreover, similarity coefficient between taxa of *M. transmorrisonensis* and hybrid of Ms-Msf was high at 0.806. These three clusters indicated that their genetic relationship was relatively close. However, *M. sinensis* var. *formosanus* proved to be close to *M. floridulus* and *M. flavidus*, and the hybrid Ms-Mf was close to the taxa of Mf-Ms-Msf (Fig. 1).

According to the data of esterase analysis, 8 taxa of *Miscanthus* can be grouped into two major clusters (Fig. 2). *M. sinensis* and *M. sinensis* var. *formosanus* were very close together with Ssm 0.806. *M. sinensis* and *M. flavidus* form a second cluster with Ssm 0.774, while *M. transmorrisonensis* and the hybrid Mf-Ms are close to the first cluster, and *M. sinensis* var. *formosanus* is close to the second cluster, i. e., *M. sinensis* and *M. flavidus*. Nevertheless, *M. floridulus* is remarkably far from the other taxa.

Based on the indophenol oxidase data, the dendrogram of the taxa is given in Fig. 3, showing that the similarity coefficients between taxa is generally high, above 0.706. The similarity between taxa of Msf and Ms-Msf is extremely high with Ssm 0.967, while that between Mfa and Mt is 0.876. These two pairs then are

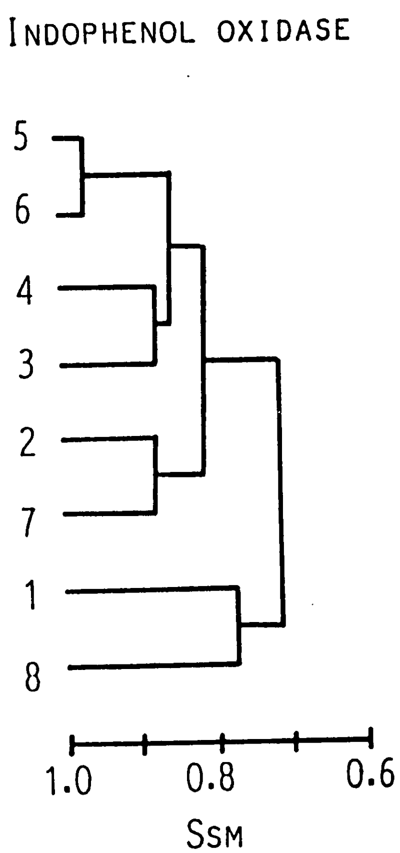

Fig. 3. The dendrogram of 8 taxa of *Miscanthus* based on the presence of indophenol oxidase isozymes.

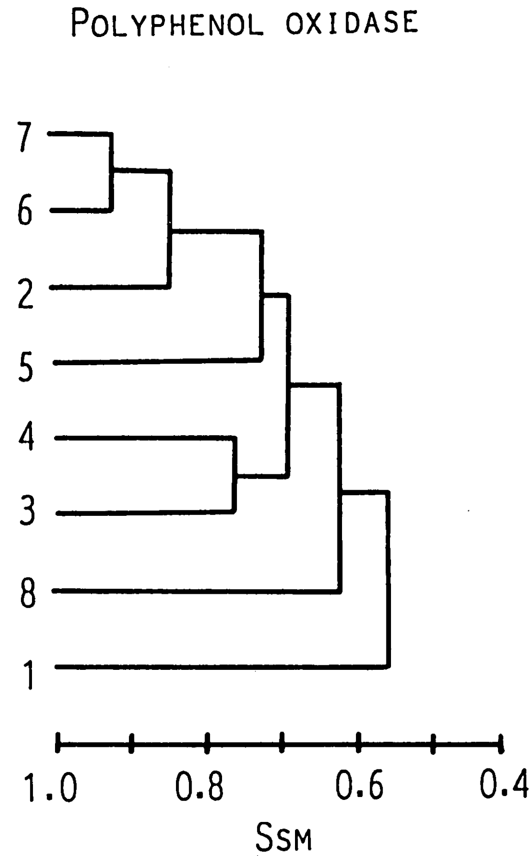

Fig. 4. The dendrogram of 8 taxa of *Miscanthus* based on the presence of polyphenol oxidase isozymes.

also quite close at Ssm 0.850. On the other hand, the taxa between Ms and Mf-Ms-Msf exhibited a high similarity of Ssm, 0.867. Finally, these five taxa are close, at Ssm 0.808. Furthermore, a cluster totally independent from the above taxa was found between *M. floridulus* and Ms-Mf, with Ssm at 0.767.

Similarly, a dendrogram derived from the simple matching coefficients of polyphenol oxidase data is shown in Fig. 4. The similarity pattern between taxa is fairly similar to that shown in Fig. 3; however, the coefficients are lower.

Computing the combined data of these four isozymes by using the similarity analyses showed that two major clusters were present (Fig. 5). Taxa of Ms, Msf, Ms-Msf, and Mf-Ms-Msf form a cluster at Ssm 0.738, while taxa of Mfa and Mt form another cluster at Ssm 0.744. Surprisingly, *Miscanthus floridulus* and Ms-Mf are quite far from the above-mentioned taxa.

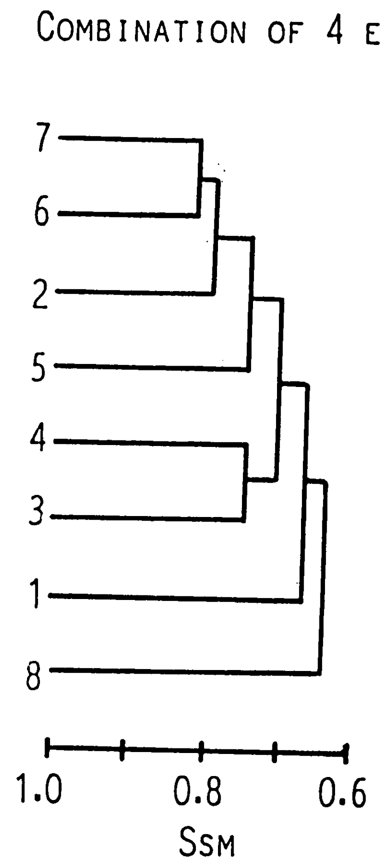

Fig. 5. The dendrogram of 8 taxa of *Miscanthus* based on the combined presence of all 4 isozymes.

Phylogenetic Relationship Among Taxa of ***Miscanthus*** *Populations Derived from Euclidean Distance*

The simple matching coefficient was obtained for a pair from matching and mismatching data for two taxa, but such data on isozyme bands might not be enough for quantitating their similarity or dissimilarity of the taxa. Thus, the frequency of isozyme bands from the population studied was obtained. By computing the frequency of each band by the formula for Euclidean distance, the phylogenetic distance could be expressed. The lower the value of the distance, the closer the relationship between two taxa.

Based on the peroxidase data, the dendrogram of eight taxa of *Miscanthus* populations is given in Fig. 6. The phylogenetic relationship among taxa is thus expressed by the following order of increasing distance: Ms, Ms-Msf, Mf-Ms, Mf-Ms-Msf, Mf, Msf, Mfa, and Mt. This indicates that Ms (*M. sinensis*) and its hybrids are genetically mostly closer than its hybrid with Mf (*M. floridulus*). In addition, *M. transmorrisonensis* and *M. flavidus* are genetically far from *M. floridulus* and *M. sinensis*.

Applying the same formula the esterase analyses gave the following order of increasing distance: Msf, Ms-Mf, Ms-Msf, Ms, Mf, Mfa, and Mt (Fig. 7). Again, the taxa of *M. sinensis* and its hybrid are genetically close together, while *M. transmorrisonensis* and *M. flavidus* are phylogenetically far from other taxa.

Regarding the data based on indophenol oxidase, the phylogenetic relationship is given by the following order of increasing distance: Ms, Msf, Mf-Ms, Msf, Mf,

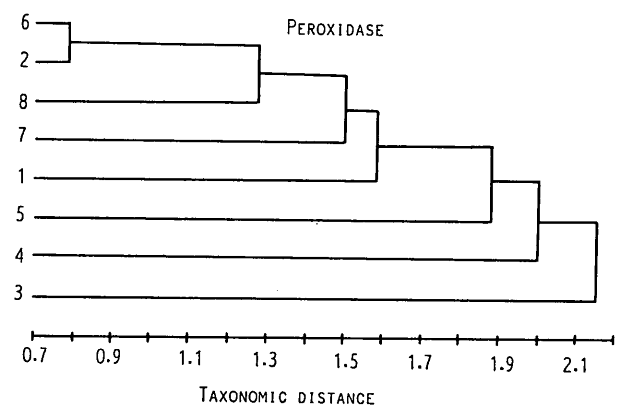

Fig. 6. The dendrogram of 8 taxa of *Miscanthus* based on the frequency of peroxidase isozymes and computed by a formula of taxonomic distance (d_{jk}). Results shown in Figs. 7 to 10 are based on the same plant material material and the same computation.

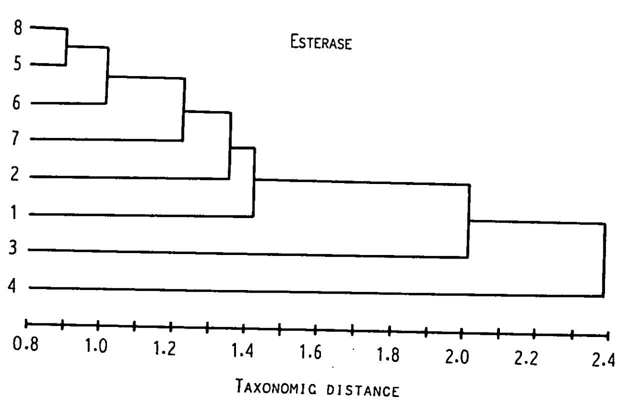

Fig. 7. The dendrogram of 8 taxa of *Miscanthus* based on the frequency of esterase isozymes.

Mf-Ms-Msf, Mfa, and Mt (Fig. 8). The pattern of the dendrogram is very similar to that of peroxidase and esterase.

Furthermore, based on the polyphenol oxidase, the phylogenetic relationship among taxa was obtained (Fig. 9). The order of relationship among taxa derived by using the same treatment mentioned is as follows: Ms, Ms-Msf, Ms-Mf, Mf-Ms-Msf, Msf, Mf, Mfa and Mt. The pattern is also similar to that of the aforementioned case. Finally, combining results for the four enzymes and using the Euclidean distance to elucidate the phylogenetic relationships among the taxa gives the dendrogram in Fig. 10, showing that the order of the distance is as follows: Ms, Ms-Msf, Mf-Ms, Msf, Mf-Ms-Msf, Mf, Mfa, and Mt. In conclusion, *Miscanthus sinensis* is the most primitive taxon. This then evolved to the other taxa in the sequence *M. floridulus*,

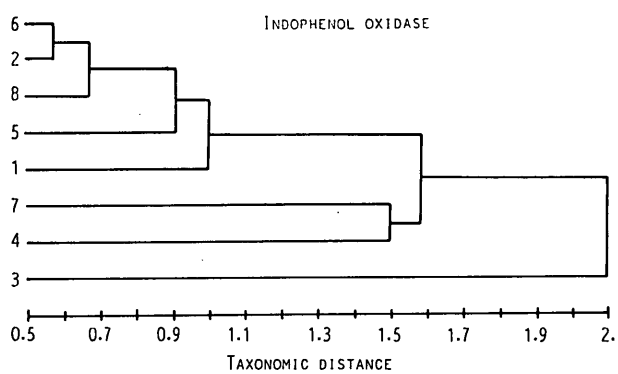

Fig. 8. The dendrogram of 8 taxa of *Miscanthus* based on the frequency of indolphenol oxidase isozymes.

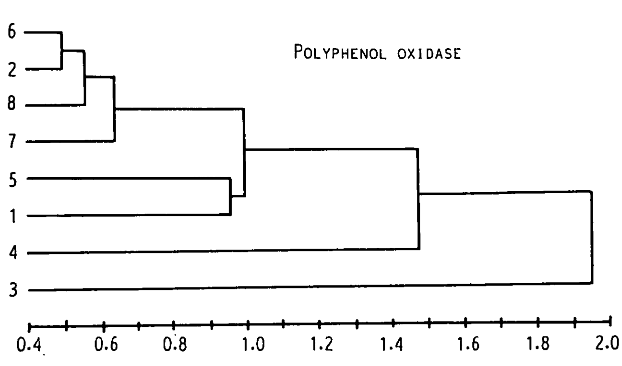

Fig. 9. The dendrogram of 8 taxa of *Miscanthus* based on the frequency of polyphenol oxidase isozymes.

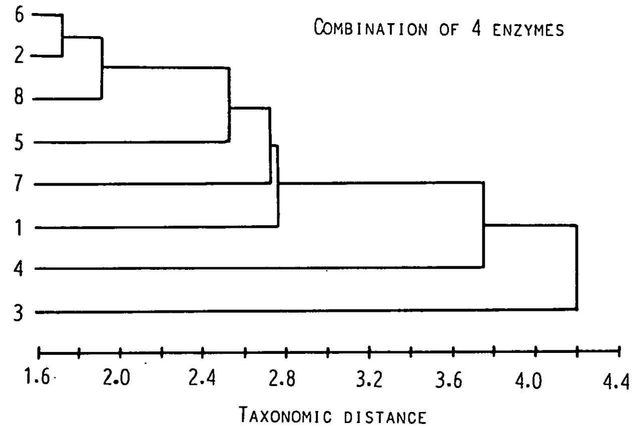

Fig. 10. The dendrogram of 8 taxa of *Miscanthus* based on the frequency of 4 combination of isozymes.

M. sinesis var. *formosanus*, *M. flavidus*, then to *M. transmorrisonensis*. The hybrids of these, Ms-Msf, Msf-Ms-Msf, and Mf-Ms, are indeed derived from the interspecific hybridization of *M. sinenesis* and *M. floridulus*. It is further noted that *M. transmorrisonensis* is the most advanced species evolved from *M. floridulus*. *M. floridulus* migrated to the higher altitude above 2200 m and became well adapted to the local environment of extremely low temeprature in winter, resulting in morphological change after many centuries through natural selection.

Discussion

In a series population study of *Miscanthus*, we have reported that the variability and heterogeneity of *Miscanthus* is obviously found in many habitats in Taiwan, resulting in formation of ecotypes (Chou *et al.*, 1987; Chou and Chang, 1988; Chou, 1989). In the coastal area of Taoyuan county, there are many *Miscanthus* populations that have adapted to heavy pollution, high salinity, and severe drought. For example, a unique population cluster was found in the vicinity of Tayuan Industrial Park, Taoyuan, where the air has been severely polluted for several decades. Vegetations, such as *Casuaria glauca*, *Pandanus odoratissimus*, and *Ipomoea* spp., which are salt-tolerant species, was severely injured by air pollutants, but *M. floridulus* was not damaged thereby (Chang *et al.*, 1987). Chou *et al.* (1987) further showed that the isozyme patterns based on peroxidase and esterase present in leaves of *Miscanthus* could explain such adaptation. In addition, the geo-

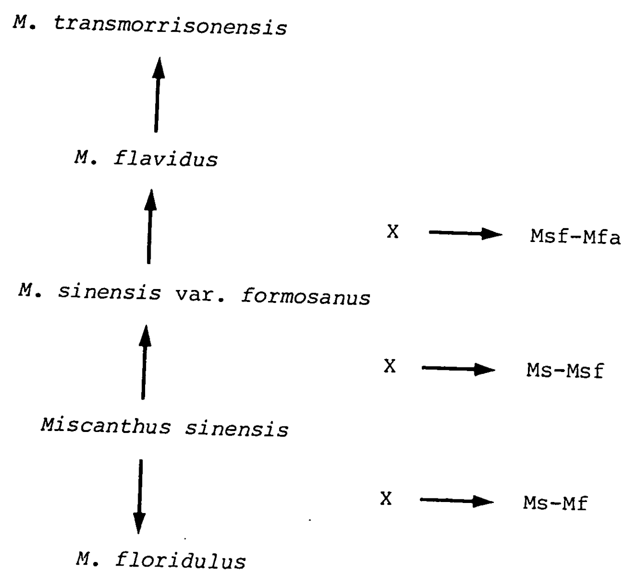

Fig. 11. A proposed evolutionary trend of *Miscanthus* taxa in Taiwan.

graphic distribution of *Miscanthus floridulus* may not be so important as far as the ecotypic variation is concerned, because we found that populations Linco and Taitung L are forming a cluster according to Eucladean distance of peroxidase isozymes (in Fig. 3 of Chou *et al.*, 1987). These two populations are located separating in the east and the west coasts of the Island. However, 3 populations of Tapinting and 2 populations of Yushan were found close intraspecifically.

Chou (1989) reported that the population divergence of *Miscanthus floridulus* was significantly different among *Miscanthus* populations in island Taiwan and islets of Pescadero, Green, and Orchid. This probably is atributable to the isolation of the island without intraspecific hybridization. However, the geographic effect on *Miscanthus floridulus* differentiation was also obvious, resulting in preservation of genetic characters. For example, the zymogram pattern of Yushan population (2750 m in elevation) was quite similar to that of islet Green (Chou, 1989). In recent work (Chou and Chen, 1990), we found that the overall similarity coefficient of 20 populations based on peroxidase and esterase analyses was quite high (Ssm > 0.60), probably because of the long isolation of islets. Thus, we proposed that the origin of *Miscanthus floridulus* in populations of Yushan and islets Green and Orchid could possibly be the same.

Nevertheless, the present findings suggest that the *Miscanthus* populations in Taiwan originated in *Miscanthus sinensis* and evolved to *M. floridulus* and *M. sinensis* var. *formosanus*, which then further evolved to *M. flavidus* and then to *M. transmorrisonensis* (Fig. 11). The hybrids, Ms-Mf, Ms-Msf, and Msf-Mfa, developed from a hybridization of these species. *M. sinensis* is practically a dominant species in China and Japan. However, after a long period of adaptation and evolutionary processes *M. sinensis* may have differentiated into *M. floridulus* and *M. sinensis* var. *formosanus*. The original species has adapted well to the local environment and become widely distributed in Taiwan. On the other hand, *M. sinensis* var. *formosanus* occurs in only a few places in Taiwan, the major populations being in Chisingshan and Nantou area. It is interesting to note that the population of *M. transmorrisonensis* occurs mostly in Nantou county at elevation above 2400 m but the species has been found at lower altitude of Chisingshan mountains (Chou, unpublished data). The differentiation of *Miscanthus* through evolutionary process practically has resulted in a speciation of these plants.

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台灣芒屬植物族群的親緣關係

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為了解台灣芒草(*Miscanthus*)植物的適應與族群的親緣關係。104 個芒草植物族群由台灣各地移植於台灣糖業研究所萬丹實驗場的大型盆栽內。移植數年後，取其鮮葉置冰箱(5°C)中。所採種屬族群數不一，其種屬命名係依據糖研所鑑定者：即中國芒(*M. sinensis*)，台灣芒(*M. sinensis* var. *formosanus*)，五節芒(*M. floridulus*)，黃金芒(*M. flavidus*)，高山芒(*M. transmorrisonensis*)，及三雜交種。以 polyacrylamide gel electrophoresis 法分析上述芒草葉中的四種同功酶如過氧化酶，酯酶，多酚氧化酶，及吲哚酚氧化酶，得同功酶圖譜，再以相似度(Similarity)及尤克利氏距離(Euclidean distance)公式算出各種及雜種間族群距離以了解其親緣關係。其結論指出在八個芒草種之族群親緣關係中，距離越短者，其親緣度最高。因此歸納得出中國芒可能是起源者，依秩則為台灣芒，五節芒，黃金芒，爾後形成高山芒。此演化趨勢有待進一步以分子遺傳學的方法來釐清。