

POPULATION STUDY OF *MISCANTHUS FLORIDULUS*
(LABILL.) WARB.

I. Variation of Peroxidase and Esterase in
27 Populations in Taiwan^{1,2}

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Abstract

Miscanthus floridulus, the most dominant endemic grass, is widely distributed in many areas from low elevation to high elevation. Twenty seven populations of *Miscanthus* concentrated in the west and east coast and the central parts of Taiwan were chosen for this study. *Miscanthus* leaves sampled from various habitats were analyzed for peroxidase and esterase by means of polyacrylamide gel electrophoresis. Results of isozymes analyses showed that 29 bands and 31 bands are respectively found in esterase and peroxidase and the isozyme patterns are shown in some difference among populations. By using statistical equations, such as Euclidean distance, average distance, and a simple matching coefficient, the data were computed and dendrograms of populations obtained. This study concludes that the patterns of isozymes of peroxidase and esterase can be grouped into 4 major clusters among 27 populations. The populations of coastal areas of Taoyuan and Hualien form a cluster; populations from Hoshe, Tapinting, Yushan areas can group into a second cluster; populations of Chingjing and Tayulin areas can form a third cluster; and the population of Taoyuan industrial area can unite into a fourth cluster that is noticeably different from the former three clusters. Possible mechanisms of the development of ecotypic variation of *Miscanthus* populations in various habitats were discussed.

Key words: Population; *Miscanthus floridulus*; isoenzyme; ecotype; peroxidase; esterase.

Introduction

In recent years, isoenzyme study has increasingly received attention by

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genetics to study the genes involved in enzyme synthesis, by biochemists and physiologist to look upon the different physico-chemical properties of isoenzymes as means to study the regulation of cell metabolism, and by evolutionary ecologists to study the existence of isoenzymes to enhance the biochemical adaptability of organism and to protect it against loss of function occasioned by mutation or environmental stresses (Shannon, 1968; Gottlieb, 1982; Schmitz and Kowallik, 1986). Isoenzyme studies in a variety of plants were carried out and tremendous growth of information appeared in literatures (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 isozyme as parameter and found that the isozyme patterns were significantly different among five grass populations where the soils were polluted by copper factory established in different year and from area without copper pollution. Chou *et al.* (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 play a significant role in clarifying the taxonomic and phylogenetic position of plants studied.

Miscanthus floridulus (Labill.) Warb., a dominant endemic grass, is widely distributed in areas of roadsides, hillsides, riverbed, and abandoned fields from low elevation to high elevation in Taiwan. The grass exhibits a strong allelopathic interaction against its associated herbaceous plants sharing the same habitat (Chou and Chung, 1974). Hsu (1986) found that the ubiquitous distribution of *Miscanthus floridulus* is due in part to the behavior of seed germination insensitive to temperature. Weng (personal communication) showed that the compensation point for carbon dioxide of this grass varied with habitats, but the phenotypic characters among different populations are hard to distinguish. It is thus thought that the grass should possess a wide heterogeneity, resulting in a wide adaptability to different environmental regimes, such as high salinity, polluted soil, and severe dry land. This adaptation is presumable due to the regulation of isoenzyme polymorphism. The genetic study of polymorphism in *Miscanthus floridulus* is beyond present capacity, thus at the present we aim to analyze the variation of isoenzymes in order to characterize the ecotypic variation of *Miscanthus floridulus* populations in various habitats in the Island proper.

Materials and Methods

Materials

Fresh leaves of *Miscanthus floridulus* were collected from areas of the east and west coast and the central part of Taiwan. Twenty seven populations were

chosen for the study. Most populations studied were centered in the Taoyuan and Nantou counties. In the west coast of Taoyuan county, several populations were exposed to heavy industrial pollution. The description of phenotypic characters of *Miscanthus floridulus* of 27 populations and list of characters of sites studied are given in Table 1. Leaves of *Miscanthus* were sampled at the DBH (depth of breast high), and were immediately placed in an ice box. The samples were brought back to laboratory, then stored in a freezer till use. For each population, at least, 30 plants collected from various sites were sampled for analysis. The chemicals and solvents for electrophoresis were purchased either from the Sigma Corp. (USA) or from Merck Ltd. (West Germany).

Electrophoresis Analysis

A vertical gel electrophoresis (M & S Slab Electrophoresis, model SG-80) was employed and techniques for electrophoresis of *Miscanthus* leaves were described by Chou *et al.* (1984a). Peroxidase and esterase isozymes were selected for the study. After the analysis of electrophoresis, the gel was dried and a permanent sheet of zymogram was obtained. Accordingly, the Rf value of each band present in the zymograms was calculated and the frequency of each band in each population was obtained.

Statistical Analyses

In order to understand the ecotypic variation of the *Miscanthus* in 27 populations, three mathematic equations, such as a simple matching coefficient (*Ssm*), Euclidean distance (d_{jk}) hereafter we called it as *population distance*, and average distance (D_{jk}) were used (Sneath and Sokal, 1973). Regardless of the number of sampling in each population, each isozyme band found in samples was regarded as a present band (+), while no isozyme band in samples of each population was designated as a missing band (-). These data were then analyzed by a formula of similarity coefficient, $Ssm = m/m + u$ was employed, where *m* is the number of matches, and *u* the number of mismatches. The data of *Ssm* were then set in a simple matrix table using each population as an operational populational unit (OPU's). The second formula, Euclidean distance, d_{jk} between OPU's *j* and *k* is

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

Where X_{ij} is the average frequency of *i* band in the zymogram of *j* population and X_{ik} is the average frequency of *i* band in the zymogram of *k* population. The third formula called *average distance*, D_{jk} , used in this study was a modification of the former one. The formula is thus given as below:

$$D_{jk} = [d_{jk}^2 / n]^{1/2}$$

Table 1. Description of sampling locations, their designations, soil texture, and phenotypic characters of *Miscanthus floridulus* in 27 populations in Taiwan

Population number	Sampling location	Designation	Soil texture	Phenotypic characters of <i>Miscanthus floridulus</i> (mean)		
				Plant height (cm)	Leaf length (cm)	Leaf blade width (cm)
01	Tayulin, Nantou	Tayulin	Clay loam	140	85	1.5
02	Lishan, Nantou	Lishan	Sandy	100	72	1.0
03	Chulu, Taitung	Taitung C	Sandy	120	85	2.0
04	Tayuan industrial A, Taoyuan	Taoyuan I _a	Sandy	150	80	1.0
05	Tayuan industrial B, Taoyuan	Taoyuan I _b	Sandy	200	75	1.5
06	Tayuan industrial C, Taoyuan	Taoyuan I _c	Sandy	200	105	1.5
07	Yean Liao, Hualien	Hualien	Sandy	170	110	2.5
08	Suita, Taoyuan	Taoyuan W	Loam	100	75	1.5
09	Haihu, Taoyuan	Taoyuan H _i	Sandy loam	120	75	1.0
10	Linco EP plant, Taoyuan	Linco	Sandy loam	135	95	3.5
11	Livestock farm, Taitung	Taitung L	Sandy	150	80	1.5
12	Sugarcane field, Taitung	Taitung S	Sandy	160	80	1.5
13	Shahu A, Taoyuan	Taoyuan H _a	Sandy loam	150	105	1.5
14	Shahu B, Taoyuan	Taoyuan H _b	Sandy loam	150	110	1.5
15	Imin village, Taoyuan	Taoyuan I	Sandy loam	100	80	1.0
16	Hoshe Tapinting A, Nantou	Tapinting A	Loam	130	80	1.8
17	Hoshe Tapinting B, Nantou	Tapinting B	Loam	150	80	1.8
18	Hoshe Tapinting C, Nantou	Tapinting C	Loam	140	75	1.7
19	Suili Minghu, Nantou	Minghu	Sandy loam	170	90	2.5
20	Chingjing farm, Nantou	Chingjing F	Clay loam	210	100	3.7
21	Chingjing Tatung, Nantou	Chingjing T	Clay loam	200	110	3.0
22	Yushan Lingtao A, Nantou	Yushan A	Sandy loam	245	95	4.7
23	Yushan Lingtao B, Nantou	Yushan B	Sandy loam	200	104	4.4
24	Hoshe Tungfu A, Nantou	Hoshe A	Clay loam	210	112	3.5
25	Hoshe Tungfu B, Nantou	Hoshe B	Clay loam	205	100	3.5
26	Hoshe Study Site, Nantou	Hoshe C	Loam	150	80	1.0
27	Hoshe Widewel, Nantou	Hoshe D	Sandy loam	150	80	1.0

By using these equations the data were computed to obtain a matrix for each isozyme and clustering analysis between populations was obtained by an unweighted pair-group method using simple arithmetic average described by Sneath and Sokal (1973); thus, a dendrogram was obtained.

Results

Distribution and Frequency of Peroxidase Isozymes in Miscanthus Populations

The zymogram of peroxidase present in 27 populations of *Miscanthus floridulus* is given in Fig. 1, where each band found in samples is the presence of band in population regardless of the frequency of band present. And the Rf value and frequency of each band present in the peroxidase of *Miscanthus* is shown in Table 2. In Fig. 1, bands 15, 17, 19, and 21 presented in all populations, and the frequency of the bands were also high in many populations. Band 5 was abundant in all

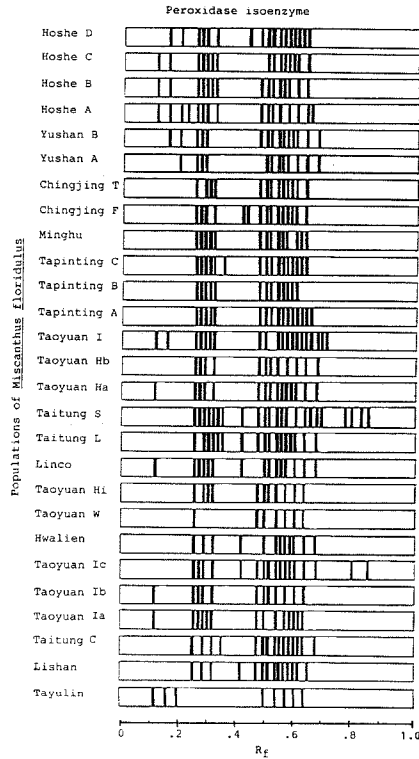


Fig. 1. The zymogram patterns of peroxidase in leaves of *Miscanthus floridulus* from 27 populations in Taiwan. The designation of each population see Table 1.

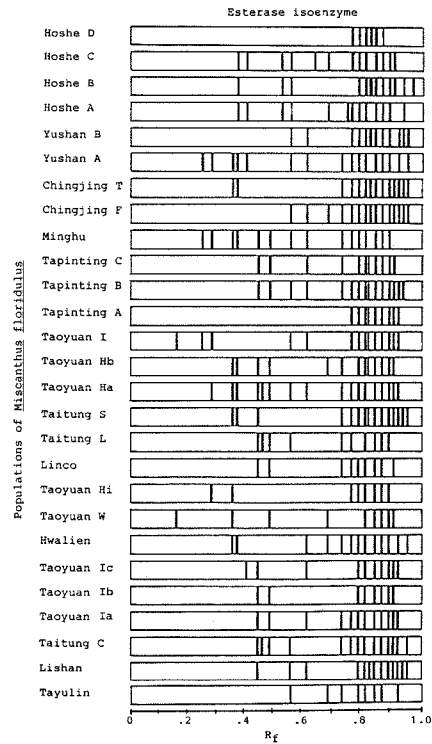


Fig. 2. The zymogram patterns of esterase in leaves of 27 populations of *M. floridulus* from 27 populations in Taiwan. The designation of each population see Table 1.

Table 2. *The frequency of each band in peroxidase isozymes*

Band No.	R_f	Operational											
		01	02	03	04	05	06	07	08	09	10	11	12
01	0.12	0.800			0.250	0.100					0.053		
02	0.16	0.300											
03	0.20	0.100											
04	0.22												
05	0.25		0.842	0.553	0.600	0.900	1.000	1.000	1.000	0.133	0.474	0.600	0.433
06	0.27				0.400	0.100	0.350				0.263		0.133
07	0.28		0.368	0.400	0.750	0.450	0.700	0.350		0.400	0.737	0.667	0.300
08	0.30				0.100					0.400	0.158	0.067	0.067
09	0.32		0.526	0.800	0.550	0.350	0.350	0.350		0.867	0.579	0.933	0.667
10	0.33											0.067	0.067
11	0.35			0.067								0.133	0.067
12	0.41		0.158				0.100	0.250			0.105	0.067	0.100
13	0.43												
14	0.47		0.474	0.867	0.300	0.800	0.550		0.500	0.067		0.267	0.125
15	0.49	1.000	0.684	0.333	0.900	0.300	0.400	0.850	0.900	0.800	0.842	0.667	0.567
16	0.51		0.368	0.667		0.150	0.050			0.133	0.421	0.200	0.500
17	0.53	1.000	0.737	0.667	1.000	0.650	0.950	0.950	1.000	0.867	0.842	0.867	0.833
18	0.55		0.158	0.400			0.050	0.050			0.263	0.133	0.433
19	0.56	0.900	0.684	0.600	0.850	0.600	0.950	0.800	0.500	0.867	0.684	0.733	0.600
20	0.58		0.105	0.333	0.300		0.050	0.100				0.067	
21	0.60	0.700	0.421	0.333	0.550	0.450	0.650	0.850	0.500	0.867	0.474	0.467	0.733
22	0.61		0.105	0.067	0.150								
23	0.63	0.600		0.200	0.150	0.100	0.450	0.500	0.500	0.600	0.158	0.200	0.200
24	0.64		0.105										0.133
25	0.67			0.067			0.050	0.200			0.053	0.133	0.333
26	0.68												0.067
27	0.70												
28	0.76												0.133
29	0.79						0.200						0.467
30	0.82												0.100
31	0.84						0.200						0.500

present in 27 populations of Miscanthus floridulus in Taiwan

population unit																										
13	14	15	16	17	18	19	20	21	22	23	24	25	26	27												
0.233		0.133										0.425	0.325	0.533												
		0.100									0.100	0.125	0.100	0.400	0.400											
										0.033	0.067	0.575		0.133												
											0.225															
0.900	0.900	0.667	0.633	0.567	0.433	0.600	0.833	0.567	0.367	0.333	0.950	0.675	0.367	0.700												
0.100	0.033	0.300	0.133	0.167	0.233	0.500	0.167		0.367	0.333	0.550	0.225	0.100	0.167												
0.467	0.467	0.700	0.300	0.567	0.667	0.633	0.800	0.433	1.000	0.633	0.775	0.700	0.700	0.733												
		0.100	0.167	0.133	0.200	0.033		0.033			0.075	0.133														
0.400	0.533	0.367	0.267	0.433	0.233	0.133	0.633	0.100			0.300	0.225	0.133	0.333												
					0.033																					
								0.033																		
								0.033																		0.333
0.300	0.233	0.600	0.233	0.167	0.200	0.300	0.033	0.267		0.300	0.100	0.225		0.333												
0.400	0.833	0.567	0.767	0.733	0.933	1.000	1.000	0.833	1.000	0.933	1.000	0.950	0.100	1.000												
0.300	0.233		0.500	0.767	0.667	0.733	0.133	0.167	0.433	0.400	0.600	0.750	0.500	0.167												
0.933	0.833	1.000	0.967	0.700	0.833	0.933	1.000	0.933	0.467	0.700	0.925	0.950	0.933	0.567												
0.133		0.167	0.033	0.467	0.367	0.067	0.067	0.167	0.667	0.400		0.050	0.100	0.067												
0.800	0.733	0.833	0.833	0.200	0.500	0.800	0.633	0.700	0.767	0.500	0.975	0.725	0.367	0.533												
0.300		0.100	0.033	0.033	0.100		0.033	0.033		0.100		0.100	0.167													
0.567	0.433	0.867	0.633	0.100	0.267	0.600	0.033	0.533	0.633	0.400	0.825	0.450	0.100	0.333												
		0.067	0.033		0.033	0.100								0.033												
0.333	0.333	0.800	0.300		0.033	0.100	0.033	0.067	0.400	0.133	0.175	0.050	0.067	0.200												
		0.033	0.033													0.050										
0.067	0.033	0.367													0.100	0.067										
		0.033																								
		0.033																								

populations, except in the Tayulin population. Band 7 was found in all populations except populations Tayulin and Taoyuan W. Band 23 was present in all populations except populations of Tapinting, and Lishan. Bands 1, 2, and 3 were missing from populations of Tapinting, Chingjing, Lisan, Minghu, Hwalien, and Taitung, and some populations in Taoyuan industrial area. The frequency of band 8 was relatively as low as 0.1, except the population Taoyuan Hi. Bands 10, 11, 12, and 13 were randomly distributed in most populations, but the first three bands were all present in the Taitung populations. Bands 18 and 20 were abundant in population of central part of Taiwan. Band 22 was occasionally distributed in all populations; however, this band was missing from the Taoyuan industrial area. The remaining bands of peroxidase isozyme were particularly found in the population of Taitung S. In conclusion, the patterns of peroxidase isozyme varied with 27 populations of *Miscanthus floridulus*.

Distribution and Frequency of Esterase Isozymes in Miscanthus Populations

The zymogram of esterase in the 27 populations of *Miscanthus floridulus* is given in Fig. 2, where each band found in samples is the presence of band in population regardless of the frequency of band present, and the frequency of each band present in the zymogram is shown in Table 3. There were bands 18, 19, 22, 23, and 24 commonly present in all populations and the frequency of each band in the population is exceedingly high, while some bands were scatteredly distributed in the populations studied. For example, bands 1 to 6 were mostly present in several coastal populations, while band 7 was missing from the populations of Taoyuan industrial area, Chingjing, and Yushan A and B. Band 8 was present in few populations, namely, Lishan, Taitung C and L, and Taoyuan Ha. Band 9 was irregularly found in all populations, while bands 10, 13 and 16 were only present in the populations at Hoshe area. Noticeably, band 20 was found in the populations of central Taiwan, while bands 27 and 28 are mostly missing in the populations of Taoyuan industrial area. From the above findings, it was obvious that some bands of esterase isoenzyme are present in the population of polluted area, while other bands are only present in areas without pollution or in high elevation land. The irregular distribution of isozyme patterns in *Miscanthus* populations is difficult to understand the population clustering or divergence; thus further treatment of statistical analysis is necessary.

The Variation of Population Distance Based on Isozymes in Miscanthus

The major objective of this study was aimed to find out the ecotypes of *Miscanthus* in different habitats in Taiwan. The first mathematic equation, d_{jk} , was used to answer the Euclidean or called *population distance* among 27 populations of *Miscanthus*, and the data are given in a matrix of Table 4, in which the

lower the value of the *population distance* between two populations is the closer the populations found. The distance between populations Hoshe and Minghu is 0.511, which is the lowest one, next to this are the populations Yushan B and Tapinting C ($d_{jk}=0.514$); the following populations are between populations Chingjing T and Tapinting A ($d_{jk}=0.552$); between populations Lishan and Taoyuan Hb; then between populations Linco and Taitung L. Populations between Taoyuan I and Taoyuan Ic are also close together. However, populations of Tayuling, Hoshe A, C, and Yushan A are quite far from that of former ones. A clustering analysis among populations was then obtained and derived to a dendrogram of Fig. 3. Accordingly, 5 major clusters are grouped; the first cluster consists of 5 populations, namely, Minghu, Tapinting B, C, and Yushan B and Hoshe B; the second one consists of Tapinting A, Chingjing T, Lishan, Taoyuan Ha, Hb, and Hualien; the third one includes populations Chingjing F, Linco, Taitung L, and Taoyuan Ia; the fourth one includes Taoyuan I, Ic, Ib, and Taitung C. Populations such as Taoyuan W, Taoyuan Hi, and Taitung S are close to the fourth cluster. Among these clusters, the second cluster is close to the third, and the first is then linked

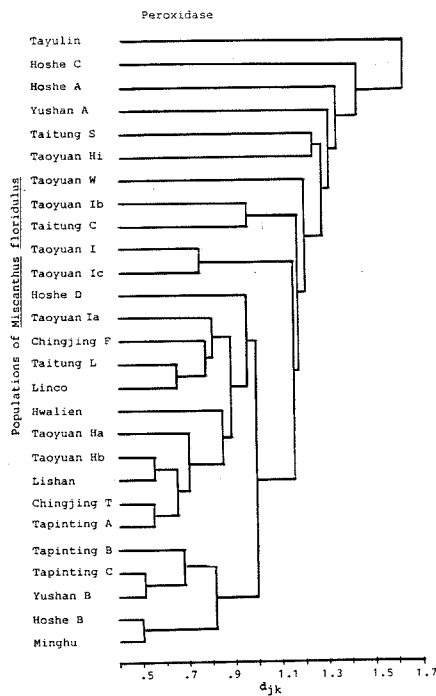


Fig. 3. The dendrogram of 27 populations of *M. floridulus* in Taiwan based on the Euclidean *distance* (hereafter we called it as *population distance*) of peroxidase.

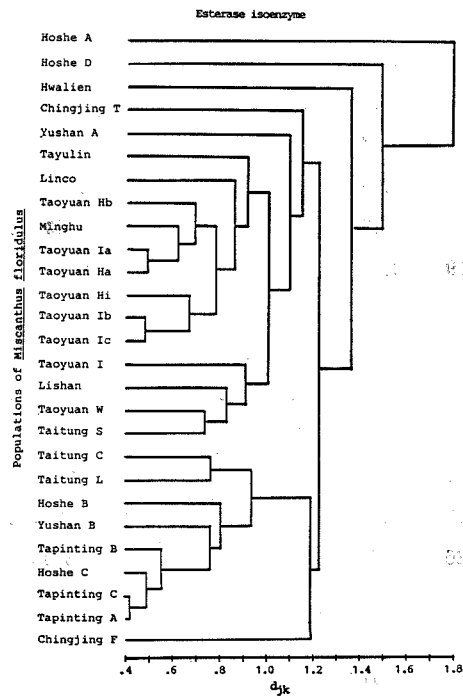


Fig. 4. The dendrogram of 27 populations of *M. floridulus* in Taiwan based on the Euclidean *distance* of esterase.

to the second and third ones. The fourth and fifth clusters are far from the above mentioned three clusters. However, the remaining five populations are not linked to population mentioned and are quite far from them. From these findings, it is obvious that some populations grown in polluted soil are quite a distance from that grown in the inland area. In addition, the patterns of clustering analysis based on the formula of *average distance* is exactly the same as that based on the Euclidean *distance* (or called *population distance*), although the data were different from each other (Table 5).

Regarding the variation of esterase isozymes in *Miscanthus* populations, the data expressed by the Euclidean *distance* are shown in Table 6, which derives into a dendrogram of Fig. 4. The *population distance* below 0.6 was found in populations Tapinting A, B, C, and Hoshe C, which formed a cluster. The second cluster involves populations Taoyuan Ia, Ib, Ic, Ha, Hb, Hi, and Minghu. Populations Taoyuan W, Taitung S, Lishan, and Taoyuan I, which form a third cluster. Populations Chingjing T, Hwalien, and Hoshe A, and D reveal a high value of *population distance*. Most of the clustering analysis revealed by esterase is correlated to that of peroxidase (Fig. 3). On the other hand, by using the equation of *average distance*, D_{jk} , the data of esterase isozyme are given in Table 7, from which the pattern of clustering derived is also exactly identical to that of Fig. 4.

Furthermore, when the data of peroxidase and esterase are combined to run for the *population distance* and *average distance* analyses, the data are given in matrixes of Tables 8 and 9, respectively. Then a dendrogram of clustering analysis based on Table 8 is shown in Fig. 5, which concludes three major clusters. Populations Tapinting A, B, C, Yushan A, B, Hoshe B, C, and Chingjing F are grouped into a cluster; populations Taoyuan Ia, Ib, Ic, Ha, Hb, Linco, Minghu, and Taitung L form another cluster; and the remaining populations are shown to be the third cluster. It is clear that the populations from Taoyuan county and the coastal industrial area are entirely different from those of Nantou county and inland area.

The Variation of Similarity Among Populations of Miscanthus

By using a simple matching coefficient, a matrix of similarity based on peroxidase of isozymes among 27 populations is given in Table 10, which derives into a dendrogram of Fig. 6. The conclusion of this analysis is that most populations are similar due to their high similarity coefficient. Taking the similarity coefficient above 0.8, there are five major clusters found. Populations Tapinting A, B, C, Minghu and Chingjing T from a distinguished cluster; populations from Taoyuan area, except Taoyuan W and Taoyuan I, group into a second cluster; populations from Yushan A, B, Hoshe B, C, D, and Chingjing F belong to the third cluster; populations from the coastal area of Taitung and Hwalien become the fourth cluster; and the remaining populations exhibits rather lower similarity (Fig. 6).

The findings of similarity is just negatively correlated to that of *population distance* and *average distance*. However, the patterns of clustering analysis are most agreeable.

On the other hand, the simple matching coefficients based on the data of esterase present in *Miscanthus* population are shown in Table 11, indicating that most populations reveal high similarity coefficient ($S_{sm} > 0.80$). Based on the data a dendrogram derived is given in Fig. 7, but the pattern of clustering are slightly different from that of peroxidase.

Furthermore, when the data of similarity were computed from both peroxidase and esterase, a matrix is thus shown in Table 12, which develops a dendrogram of Fig. 8. The findings of this similarity study conclude that some populations are grouping into a cluster, for example, populations Tapinting A, B, and C form a cluster; populations Hoshu, A, B, and C form an another one, populations Taitung C and L form a third cluster; and populations from Taoyuan county and its vicinity are grouping into a fourth cluster; however, populations Lishan, Chingjing, Taitung L and Hualien are scattering in the clusters mentioned.

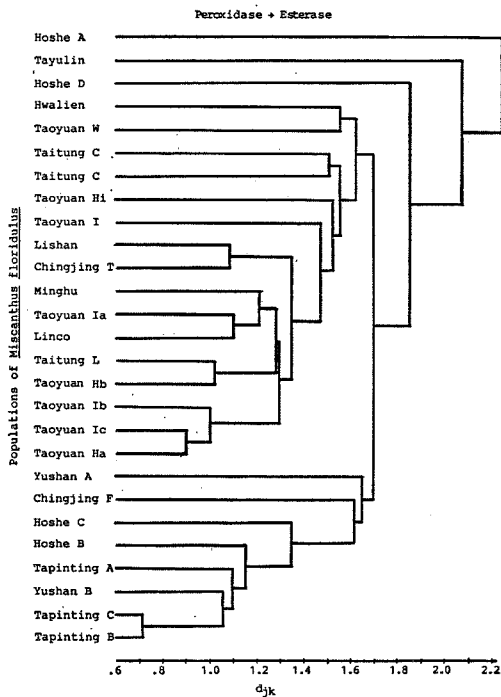


Fig. 5. The dendrogram of 27 populations of *M. floridulus* in Taiwan based on the Euclidean *distance* of peroxidase and esterase.

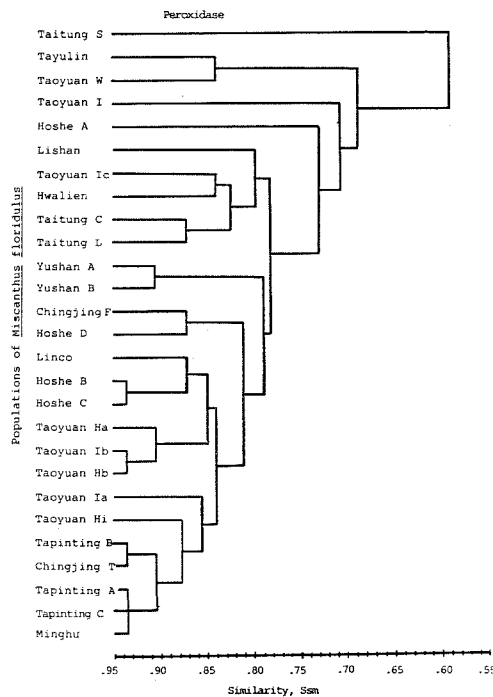


Fig. 6. The dendrogram of 27 populations of *M. floridulus* in Taiwan based on the simple matching coefficient of peroxidase.

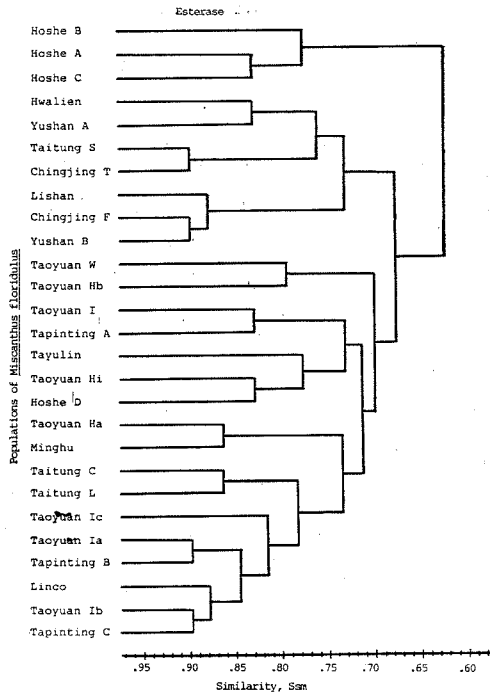


Fig. 7. The dendrogram of 27 populations of *M. floridulus* in Taiwan based on the simple matching coefficient of esterase.

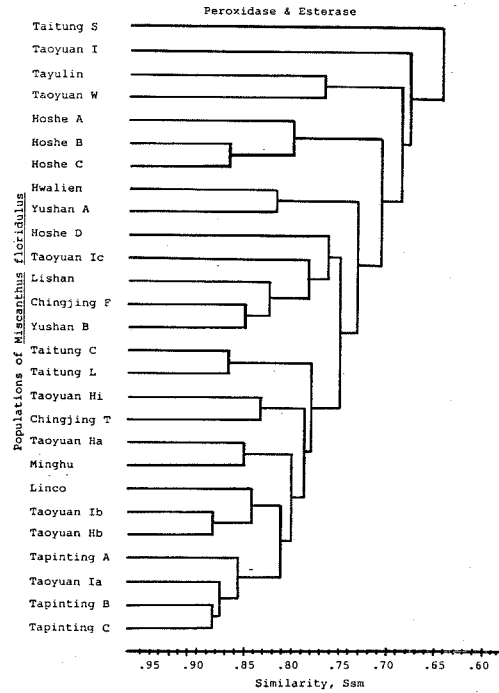


Fig. 8. The dendrogram of 27 populations of *M. floridulus* in Taiwan based on the simple matching coefficient of peroxidase and esterase.

From the above findings, it is concluded that *Miscanthus floridulus* grown in Taiwan is very heterogenous and the variation of population is likely due to the variation of some isozymes present. The isozyme patterns of peroxidase and esterase exhibit four eotypic variations in various habitats in several coastal and inland areas of the Taiwan proper.

Discussion

In the course of population study of *Miscanthus floridulus*, we faced several difficult problems, such as sampling size, location, and season due to their wide and diverse distribution. Regarding the sampling size at the present study we decided to use at least 30 samples from various *Miscanthus* plants for each location where was situated in a radius of about 5 km². Of course, within this radius, it is possible to find some phenotypic variations of *Miscanthus* plants; however, we attempted to randomly sample the leaves of the plants in order to minimize the experimental variation. Accordingly, the variation of band frequency was found

in each isozyme. For example, in Taoyuan County the variation was great. The sampling sites at Linco and Taoyuan Ha, and Hba populations are very close to that of industrial area designated as Taoyuan Ia, Ib, and Ic, where it develops into different isozyme patterns due to ecological effects. The coastal area of Taoyuan County has been notorious for the industrial pollution for nearly twenty years, and vegetations, such as *Casuaria glauca*, *Pandanus odoratissimus*, and *Ipomoea* spp., which are salt tolerant species, were severely injured by air pollutants (Chang and Tang, 1975). Nevertheless, *Miscanthus floridulus* exists healthily, indicating that adaptive mechanism has been developed. Our present data show that the isozyme patterns based on peroxidase and esterase present in leaves of *Miscanthus* could explain the reason for such adaptation. Secondly, the geographic distribution of *Miscanthus floridulus* may not be so important as far as the ecotypic variation is concerned, because we found populations Linco and Taitung L are forming a cluster based on population distance of peroxidase (Fig. 3). These two populations are situated in the east and west coast of the Island. However, 3 populations of Tapinting and 2 populations of Yushan were found close intraspecifically, but these 5 populations are far from the former populations in coastal area, indicating the difference would be ascribable to the air pollutants, such as sulfur dioxide and others, instead of geographic reason.

One may also question on the effect of climate on the isozyme patterns of *Miscanthus*. The effect of seasons on the variation of isozyme patterns were unlikely significant (Chou, unpublished data). However, the effect of temperature based on altitude on the isozyme variation is significant, that finding will be reported in a subsequent paper shortly. Additionally, the divergence between populations of *Miscanthus* is also great (Chou *et al.*, unpublished data), this investigation is in progress.

It is quite interesting to note that the wide distribution and heterogeneous characters of *Miscanthus floridulus* are found in Taiwan. We lack information concerning the successional mechanism of *Miscanthus*, perhaps this vegetation may be already there for many centuries. However, according to a 15-year observation by the senior author, the *Miscanthus* is often found as a pioneer species in the primary and secondary successions. Inasmuch as seeds of *Miscanthus* are light and small, seed dispersal by wind is easy, thus the distribution becomes ubiquitous in Taiwan. Furthermore, the seed germination is insensitive to temperature (Hsu, 1986), and the compensation point for carbon dioxide is quite low that belongs to C₄ plant. (Weng, personal communication). Chou and Chung (1974) also pointed out that the *Miscanthus* possesses a high allelopathic potential against its associated weeds grown nearby. It is no doubt that this ubiquitous distribution of *Miscanthus floridulus* becomes the most important grass in Taiwan. Although the genetic variability in *Miscanthus* is not known to us and has not been studied, the

authors at the present time can only conclude that the populations of *Miscanthus floridulus* can be grouped into several ecotypes based on the isozyme studies as mentioned. Particularly, the populations in the industrial pollution area of the west Taoyuan county are obviously different from those of inland and non-polluted area.

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臺灣五節芒之族羣研究

I. 過氧化酶及脂酶的生態變異

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臺灣五節芒族羣分布廣大，遍布本省海邊、山麓、荒郊、河床及高山地。本研究選擇桃園、中壢、南投、臺東、花蓮及南港等地為採樣地，共27個族羣。每一族羣中取至少30個點以上之五節芒葉，取後立即置放於小冰箱中（5°C 左右），帶同研究室冰凍冷藏。嗣以聚丙烯醯胺膠電泳法以分析過氧化酶及酯酶。再將每個同功酶之出現頻率算出族羣距離、平均距離及相似度係數。由此數據劃出族羣間之羣叢（clustering）型。綜合各項分析結果，上述27個族羣可歸納出四個主要羣叢即：一、桃園、花蓮及臺東濱海地區羣叢；二、桃園工業區羣叢；三、和社大坪頂羣叢；四、梨山、清境地區羣叢。各族羣間之歧異度係數相當高，此顯示族羣間之變異性頗大，此亦可知其遺傳上之異質性（heterogeneity）大，此乃導致生態種之形成，亦致使五節芒適應不同氣候及土壤環境的主要機制之一。