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Population study of *Miscanthus* IV. Growth performance of *M. floridulus* and *M. transmorrisonensis* and their acclimation to temperatures and water stresses

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Abstract. *Miscanthus transmorrisonensis* is distributed in many mountainous areas at elevation above 2200 m in Taiwan. A study site exhibiting a unique dominant vegetation of *M. transmorrisonensis* in Tartarchia Anpu, which is located at 2600 m in elevation in Nantou, Taiwan, was used for field experiments. The floristic composition was recorded after clearing. Some 12 plant species including *M. transmorrisonensis* were found 18 months after the clearing; the percent coverage of weeds and *M. transmorrisonensis* was significantly increased up to 18 months but was drastically decreased thereafter. The growth performances of *M. floridulus* and *M. transmorrisonensis* differed according to results of reciprocal transplanting experiments. Six months after transplanting, *M. transmorrisonensis* plants had grown well at a Hoshe site but *M. floridulus* grew so poorly that it could hardly survive two winters in Tartarchia. The effects of temperature and water stress on the two *Miscanthus* species were studied by employing polyacrylamide gel electrophoresis to analyze two isozymes, namely peroxidase and esterase. The patterns of isozymes present in leaves of *M. transmorrisonensis* treated variously were different six months later. The response of the *M. transmorrisonensis* seedlings to drought stress was significantly different from that of water-flooded ones.

Key words: Acclimation; Esterase; Growth performance; *Miscanthus floridulus*; *M. transmorrisonensis*; Peroxidase; Population; Water stress.

Introduction

In recent years, isozyme composition has received increasing attention by geneticists studying the genes involved in enzyme synthesis, by biochemists and physiologists looking at the physico-chemical properties of isoenzymes as a means to understand the regulation of cell metabolism, and by evolutionary ecologists studying the function of isoenzymes in enhancing the biochemical adaptability of the organism and protecting 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 have been carried out and a tremendous wealth of relevant information has 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 *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 analysis of isozymes can play a significant role in clarifying the taxonomic and phylogenetic position of plants.

Miscanthus floridulus (Labill.) Warb., a dominant endemic grass, is ubiquitously distributed in areas below 2000 m in elevation in Taiwan, and possesses an allelopathic potential that permits it to form relatively pure stands in fields (Chou and Chung, 1974). On the other hand, *M. transmorrisonensis* is distributed in mountainous areas above 2200 m. Chou *et al.* (1987, 1988) reported that four major clusters of ecotypic populations were found among 27 populations of *M. floridulus* and unique ecotypes were also present in various habitats in Taiwan. It was shown that *Miscanthus* exhibited a wide heterogeneity, resulting in a wide adaptability to different environmental regimes, such as those of high salinity, polluted soil, and severely dry land. However, the adaptive mechanism is due presumably to isoenzyme polymorphism that has not been fully investigated. In seeking to understand the mechanism of adaptation of *M. transmorrisonensis* to the areas of high elevation in Taiwan, temperature and water availability were thought to be principal factors involved. By using polyacrylamide gel electrophoresis, isozymes present in *M. transmorrisonensis* were analyzed for elucidating the adaptive mechanism by which the plants adapt to such environmental regimes.

Study Site and Field Experiments

A study site chosen for most field experiments in the present study is located at 2600 m in elevation in Tartarchia Anpu of Yushan National Park, Nantou County, Taiwan. Another site selected for only field transplantation experiments and production of sampling material is located at 1200 m in elevation in the farm of the Hoshe Forest Experimental Station of the National Taiwan University. According to climatic data (Central Weather Bureau, 1988-1990), the weather patterns of these two sites are very much different. During summer periods of 1988-1990, the temperature in Hoshe ranged from 21° to 24°C (Fig. 1). Because no weather station at Tartarchia Anput has yet been

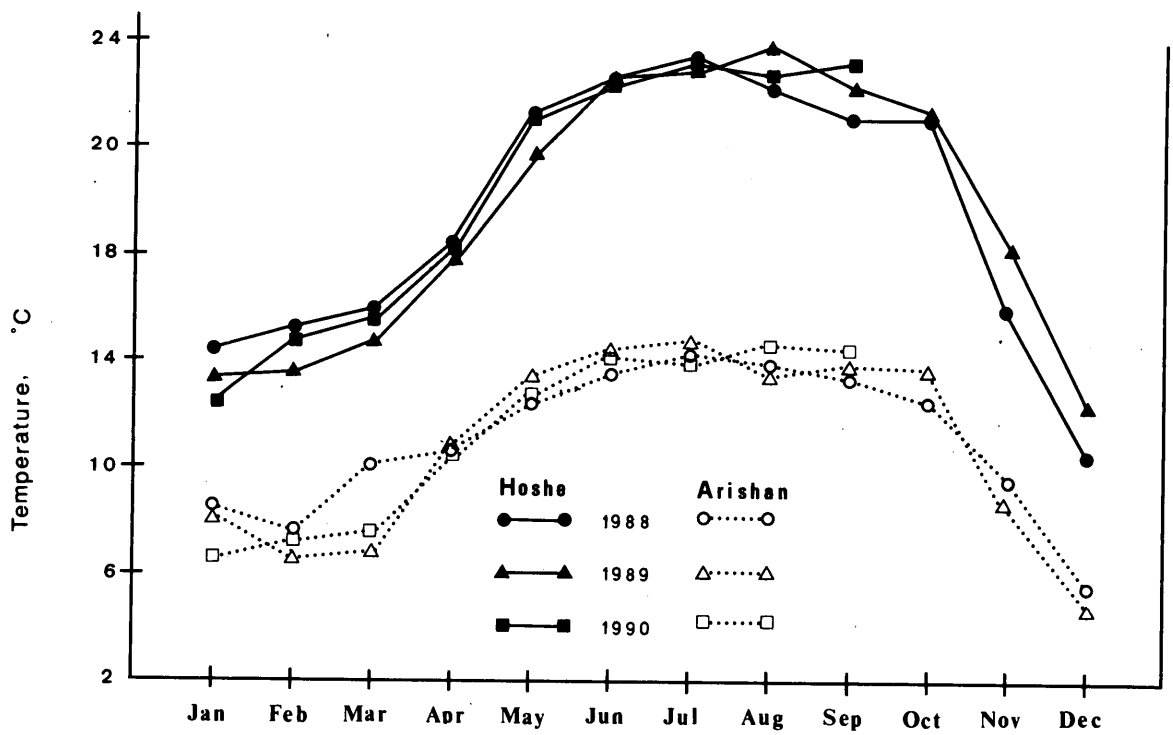

Fig. 1. The average monthly temperature of Hoshe (1200 m in elevation) and Arishan (2600 m) during 1988-1990.

established, the data for this area are represented by those of the Arlishan weather station, about 20 km away from the Tartarchia but located at the same elevation. The temperature is thus about 6°C lower in the area of Tartarchia than in Hoshe. In winter, the temperature at the Hoshe site ranged from 10° to 14°C, while that at the Tartarchia ranged from 4.5° to 8°C (Fig. 1). The amount of precipitation was also significantly different between the two sites, being higher in Tartarchia than in Hoshe (Fig. 2). The vegetation composition of the Tartarchia study site is simple and unique, a relatively pure stand of *Miscanthus transmorrisonensis*, while that of the Hoshe possesses a mixed conifer-hardwood forest with a dominant grassland vegetation of *M. floridulus*.

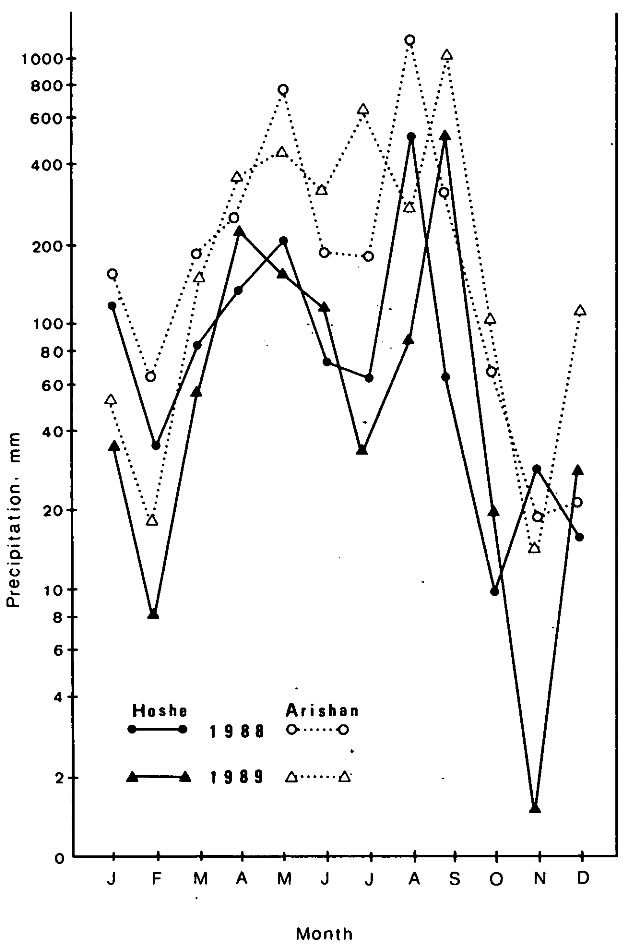

Fig. 2. The amount of monthly precipitation in Hoshe and Arishan during 1988-1989.

Clearing Experiment Location

Four quadrats, 2 × 2 m for each, in the Tartarchia Anpu grassland area were selected for a ground clearing experiment in order to learn the vegetation succession in the area.

Reciprocal transplanting of ***M. floridulus*** *and* ***M. transmorrisonensis***

For *M. floridulus*, 20 defoliated plants with roots were transplanted from the farm of the Hoshe Forest Experimental Station (HFES) to Tartarchia Anpu on May 3, 1988 and January 18 and May 12, 1989. Reciprocally, 14 defoliated *M. transmorrisonensis* plants with roots were transplanted from Tartarchia Anpu to the farm of the HFES on the same dates. Six months or longer after transplanting, the numbers of tillers, plant heights (including length to leaf top and length to leaf collar), and numbers of leaves of both species were recorded.

Phytotron and Greenhouse Experiments

Growth of ***M. transmorrisonensis*** *Seedlings in Phytotron and Greenhouse*

Seedlings of *M. transmorrisonensis* grown in pots with 3 replications were placed in different temperatures, namely (1) 15° to 20°C, (2) 20° to 25°C, and (3) 25° to 30°C under Phytotron conditions at the National Taiwan University. When the length of seedlings reached about 50 cm, leaves of the plants were harvested for peroxidase and esterase analyses.

A second set of *M. transmorrisonensis* seedlings grown in a greenhouse of the Institute of Botany, Academia Sinica, Taipei was also employed for experiments involving either water-flooded or drought conditions. When seedlings reached about 50 cm tall they were harvested for peroxidase and esterase analyses.

Experimental Setting of Water Flooding and Drought Treatments

Three seedlings of *Miscanthus transmorrisonensis*, about 15 cm tall, were transplanted to an 1/ 5000 acre Wagner's pot and regularly irrigated with tap water for one month. The seedlings grew very well up to 50 cm tall, then were received water flooded continously as a flooded treatment, while the other seedlings were stopped to receive water as a drought treatment. Each treatments were set at least for three replications. At appropriate times, 2, 3, 5, and 6 months after treatments, seedlings were harvested for further isozyme analyses.

Materials and Methods

Materials

Leaves of both *Miscanthus* species were sampled from the study sites and immediately stored in an ice box to keep the temperature below 5°C. The samples were brought back to a laboratory of Academia Sinica, Taipei for polyacrylamide gel electrophoresis (PAGE) (Chou and Chang, 1988; Chou *et al*., 1987). The chemicals and solvents for electrophoresis and chromatography were purchased from either the Sigma Corp. (USA) or Merck Ltd. (West 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*. (1984). Peroxidase and esterase isozymes were selected for the study. After the analysis by electrophoresis, the gel was dried and a permanent sheet zymogram was obtained. With this, the R_f value of each band present in the zymograms was calculated. At the present study we were unable to measure the density of each isozyme band, thus the occurrence of each band was represented by average of percent frequency of present in population or treatment. At least 10 replications for each population or treatment were emplyoed for analysis.

Statistical Analysis

Data of experimental findings were subjected to statistical analysis by a Student-*t* test and one-way analysis of variation in order to find the significant levels of difference between treatments (Gomez and Gomez, 1976).

Results

Seedling Emergence on Cleared Plots

In Tartarchia Anpu, seedlings emerged in soil of the cleared plots several months after clearing; the botanical composition varied with subsequent measured times. The number of species generally increased up to 18 months after clearing, then decreased (Table

Table 1. *Comparison of percent coverage and number of species in quadrats after a surface clearing in Tartarchia grassland*

The data were obtained by means of 4 replications.

MACs[1]	Coverage, % Weed	*Miscanthus transmorrisonensis*	Number of species
12	8.46	2.77	4
18	82.31	21.80	13
20	55.08	9.77	9

[1]MACs: Month after clearing of the quadrats.

Table 2. *Floristic composition on the quadrats after an experimental clearing*

Species		Months after clearing 12	18	20
Agrostis clavata Trin. subsp. *matsumurae* (Hack. ex Honda) Takeoka	(剪股穎)		+	
Baeothryon subcapitatum (Thwaites) T. Koyama	(玉山針藺)		+	+
Carex satsumensis Franch. Sav	(油 苔)		+	+
Cyperus spp.	(莎草科)		+	+
Gaultheria itoana Hayata	(高山白珠樹)		+	+
Gentiana atkinsonii Burk. var. *formosana* (Hay.) Yamamoto	(台灣龍膽)		+	
Hypericum nagasawai Hayata	(玉山金絲桃)		+	
Lycopodium cernuum Linn.	(過山龍)		+	+
Haloragis micrantha (Thunb.) R. Br.	(小二仙草)		+	+
Miscanthus transmorrisonesis	(高山芒)	+	+	+
Scleria terrestris (L.) Fassett	(陸生珍珠茅)		+	
Smilacina formosana Hayata	(台灣鹿藥)		+	
Yushania nittakayamensis	(玉山矢竹)		+	+

1). At the 18th month after clearing, the percent coverage by *M. transmorrisonensis* and weeds had greatly increased, up to 22% and 82%, respectively, but both then decreased significantly (Table 1). This reflected the fact that these plants can not survive long enough to come through a severe winter. Furthermore, the botanical composition of the cleared quadrats is given in Table 2, showing that most species are annual and can not live through a severe winter. Eighteen months after clearing, the species in the quadrat were as follows: *Agrostis clavata, Baeothryon subcapitatum, Carex satsumensis, Cyperus* spp., *Gaultheria itoana, Gentiana atkinsonii* var. *formosana, Hypericum nagasawai, Lycopodium cernuum, Haloragis micrantha, Miscanthus transmorrisonensis, Scleria terrestris, Smilacina formosana*, and *Yushania nittakayamensis*.

*Reciprocal Transplanting of **M. floridulus** and **M. transmorrisonensis***

To understand why the habitats of the two species of *M. floridulus* and *M. transmorrisonensis* have a sharp boundary at 2000 to 2200 m in elevation, defoliated plants with roots of both species were reciprocally transplanted in Hoshe and Tartarchia Anpu, as already noted. The results of transplanting experiments showed that *M. floridulus* can survive in Tartarchia from spring to winter but leaves are senescent during the winter to spring. The newly developed tillers and leaves were possibly generated from the energy-rich rhizomes. The growth performance of transplanted plants, expressed by the number of tillers, length of leaves to top, length of leaves to collar, and number of leaves, drastically decreased following of transplanting (Table 3). All transplanted *M. floridulus* plants died within 18 months after transplanting, indicating that these plants could not survive the severe winter.

On the other hand, the growth of *M. transmorrisonensis* transplanted to the Hoshe was much better than that of *M. floridulus*. The number of tillers, length of leaves, and number of leaves had generally decreased a year after transplanting (Table 4), with no indication of injury or wilt indicating that *M. transmorrisonensis* plants could adapt to warm weather. Furthermore, the transplanted *M. transmorrisonensis* plants grown in the farm of the Academia Sinica were healthy.

The above findings showed that *M. floridulus* can survive a severe winter but no longer than 2 years in Tartarchia; however, *M. transmorrisonensis* grows healthily and survives in areas of low altitude, such as Hoshe, or even at locations at much lower elevation, such as Taipei. The findings, therefore, demonstrate the difference of adaptability of these two species.

*Survivial and Growth Performance of **M. transmorrisonensis** in a Greenhouse*

One thousand seeds of *M. transmorrisonensis* were sown in a 43 × 34 cm tray filled with soil and kept in a greenhouse at the Academia Sinica, Taipei. Results of this experiment are given in Table 5. The numbers of plants surviving was counted 2, 3, 5 and 6 months after sowing. The rate of plant survival per 1000 seeds after this time ranged from 8.00 to 8.50, but not statistically significantly. The average length of seedlings signifi-

Table 3. *Growth performance of **M. floridulus** transplanted from Hoshe (1100 m) to Tartarchia (2600 m)*
The data are the average of 20 replications.

	Transplanted on							
	May 3, 1988			January 18, 1989			May 12, 1989	
	Months after transplanting							
	6	12	18	4	10	12	6	8
Number of tillers per cutting[1]	1.84	1.67	0	2.75	2.14	0	2.00	0
Length of leaves to top, cm	56.05	23.95	0	24.10	56.05	0	56.34	0
Length of leaves to collar, cm	17.16	6.03	0	7.60	--	0	--	0
Number of leaves per cutting	9.62	2.17	0	3.25	14.17	0	10.69	0

[1]The basal area of each cutting was about 0.05 m^2.

Table 4. *Growth performance of* ***M. transmorrisonensis*** *transplanted from Tartarchia anpu (2600 m) to Hoshe (1100 m)*

The data are the average of 10 replications.

	Transplanted on			
	Jan 18, 1989		May 3, 1988	
	Months after transplanting			
	10	12	6	8
Number of tillers per cutting	27.30	21.20	67.00	35.00
Length of leaves to top, cm	93.70	30.81	49.80	43.50
Length of leaves to collar, cm	58.00	10.18	45.75	11.35
Number of leaves per cutting	134.80	--	331.50	68.00
Dry weight (gram) per cutting	28.23	--	45.40	--

Table 5. *Survival and growth performance of* ***Miscanthus transmorrisonensis*** *seedlings after sowing 1000 seeds per pot*

	Months after sowing			
	2	3	5	6
Number of survivors per 1000 seeds	8.25	8.00	8.50	8.00
Length of seedling (cm)	2.96	7.98	12.53	19.09
Number of leaves per plant	2.08	3.15	3.77	6.57

Table 6. *Comparison of peroxidase isozyme variation between transplanted and nontransplanted plants of* ***M. transmorrisonensis***

The plants were transplanted from Tartarchia to Hoshe.

Band No.	R_f	Nontransplanted	Transplanted
1	0.067	--	0.08[1]
2	0.109	0.30	0.50
3	0.137	0.20	0.42
4	0.259	0.85	0.75
5	0.295	0.70	0.33
6	0.327	0.65	0.33
7	0.386	0.20	--
8	0.477	0.10	--
9	0.534	0.10	--
10	0.567	0.90	0.25
11	0.618	0.10	--
12	0.626	0.95	1.00
13	0.678	0.90	1.00
14	0.700	--	0.17
15	0.721	0.50	1.00
16	0.760	0.25	0.33
17	0.809	0.20	--

	Statistical analysis (Student t-test)	
Pairing method	df=9	$t_{0.05}=2.262$
	$t=0.2723$	$t_{0.01}=3.250$
Unpairing method	df=25	$t_{0.05}=2.060$
	$t=1.01$	$t_{0.01}=2.787$

[1]The data are expressed by the average frequency of the isozyme bands.

cantly increased with time, and the number and length of leaves per seedlings also increased with time after sowing. The findings indicate a low survival rate of the plant under the greenhouse conditions.

Comparison of Isozyme Variation Between Nontransplanted and Transplanted ***M. transmorrisonensis***

In order to evaluate the adaptability of *M. transmorrisonensis*, the defoliated *Miscanthus* plants with root were transplanted from Tartarchia Anpu to Hoshe as noted. The distribution of peroxidase isozymes in leaves of *Miscanthus transmorrisonensis* plants is given in Table 6, showing that 17 bands of peroxidase isozymes were found. However, five bands, 7, 8, 9, 11, and 17, were obviously missing in the record from the transplanted plants. Based on the Stduent t-test, the zymogram patterns between the nontransplanted and transplanted plants were not statistically different at 5 % level (Table 6).

On the other hand, the isozyme pattern of esterases as affected by transplanting is given in Table 7. Nineteen bands of esterase were found for the population of Tartarchia Anpu, but 4 bands, 1, 11, 13, and 19, were obviously missing for the Hoshe population, indicating that the isozyme pattern can be affected by such a transplanting. This was also confirmed by statistical analy-

Table 7. *Comparison of esterase isozyme variation between non-transplanted and transplanted plants of* ***M. transmorrisonensis***

The plants were transplanted from Tartarchia to Hoshe.

Band No.	R_f	Non-transplanted	Transplanted
1	0.281	0.40[1]	--
2	0.317	0.45	0.92
3	0.354	0.60	0.92
4	0.399	0.65	0.83
5	0.442	0.45	0.67
6	0.485	0.50	0.58
7	0.520	0.25	0.25
8	0.552	0.25	0.17
9	0.625	0.55	0.58
10	0.706	0.40	0.58
11	0.743	0.40	--
12	0.770	0.25	0.58
13	0.800	0.35	--
14	0.831	0.45	0.67
15	0.865	0.75	0.83
16	0.905	0.95	0.92
17	0.939	0.90	0.92
18	0.958	0.80	0.92
19	0.981	0.50	--

	Statistical analysis (Student t-test)	
Pairing method	df=14	$t_{0.05}=2.145$
	$t=-3.55^{**}$	$t_{0.01}=3.250$
Unpairing method	df=32	$t_{0.05}=2.037$
	$t=2.25^{*}$	$t_{0.01}=2.74$

[1]See Table 6.

Table 8. *Effects of temperature on the variation of peroxidase isozymes present in leaves of* ***M. transmorrisonensis***

Band No.	R_f	CK[1]	Temperature treatment: 15° to 20°C				20° to 25°C				25° to 30°C			
			Months after treatments											
			3	6	9	12	3	6	9	12	3	6	9	12
1	0.109	+[2]	−	−	−	−	−	−	−	−	−	−	−	−
2	0.137	+	+	+	+	+	+	+	+	+	+	+	+	+
3	0.259	+	−	+	+	−	+	+	+	+	−	−	+	+
4	0.295	+	−	+	+	−	+	+	+	−	+	−	+	−
5	0.337	+	+	+	+	+	−	+	+	−	+	+	+	+
6	0.386	+	−	−	−	−	−	−	−	−	−	−	−	−
7	0.477	+	−	−	−	−	−	−	−	−	−	−	−	−
8	0.567	+	+	+	+	+	+	+	+	+	+	+	+	+
9	0.596	+	−	−	+	−	−	−	+	+	−	−	+	−
10	0.618	+	−	−	−	−	−	−	−	−	−	−	−	−
11	0.626	+	+	+	+	+	+	+	+	+	+	+	+	+
12	0.678	+	−	+	+	+	+	+	+	+	+	+	+	+
13	0.721	+	−	+	−	+	+	−	−	+	+	+	+	+
14	0.761	+	−	−	−	−	−	−	−	−	−	−	−	−
15	0.801	+	−	−	−	−	−	−	−	−	−	−	−	−

[1]CK: Leaves collected from the Tartarchia site.

[2]+: Presence of isozyme band; −: Absence of isozyme band.

sis of Student t-test, showing there are significant at 1% level based on pairing method and at 5% level based on unpairing method (Table 7).

Response of Isozymes of ***Miscanthus*** *Seedlings to Temperature Change*

The effects of a series of temperatures on isozyme variation of *Miscanthus transmorrisonensis* were determined. Compared to the *M. transmorrisonensis* grown in Tartarchia Anpu, plants grown under various temperature regimes gave zymograms that lacked five bands of peroxidase isozymes (Table 8). For peroxidase, bands 1 and 14 are missing for nontransplanted plants and bands 7, 8, 9. 11, 17 are missing for transplanted. For esterase, bands 1, 11, 13, and 19 were missing for transplanted and none missing for nontransplanted; thus there appears to be more difference for peroxidase than for esterase.

Regarding the effects of temperature regimes on esterase, the isozyme pattern was significantly different at the period three months after temperature treatment. The difference was pronounced for the treatment at temperature regimes from 15°C to 20°C and from 20°C to 25°C (Table 9). It was particularly noticeable in bands 1, 5, 6, 7, 8, and 9. The reason of these different isozymes responses to temperature effect is yet not clear and requires further study on a molecular basis.

Response of ***Miscanthus transmorrisonensis*** *to Water Stresses*

In order to evaluate the reponse of *M. transmorrisonensis* to water stresses, seedlings of *Miscanthus transmorrisonensis* grown in a tray were subjected to

Table 9. *Effects of temperature on the variation of esterase isozymes present in leaves of **M. transmorrisonensis***

Band No.	R_f	CK[1]	15° to 20°C				20° to 25°C				25° to 30°C			
			Months after treatments											
			3	6	9	12	3	6	9	12	3	6	9	12
1	0.281	+[2]	−	+	+	+	−	−	−	+	−	−	−	+
2	0.317	+	−	+	+	+	+	+	+	+	+	+	+	+
3	0.354	+	+	+	−	+	+	+	+	+	+	+	+	+
4	0.399	+	+	+	−	+	+	+	−	+	+	+	+	+
5	0.442	+	−	+	−	−	+	+	−	+	−	+	+	+
6	0.485	+	−	+	−	−	−	+	−	−	−	+	+	+
7	0.520	+	−	−	−	−	−	−	−	−	−	−	−	−
8	0.552	+	−	−	−	−	−	−	−	−	−	−	−	−
9	0.865	+	−	+	+	+	−	+	+	+	−	+	+	+
10	0.905	+	+	+	+	+	+	+	+	+	+	+	+	+
11	0.939	+	+	+	−	−	+	+	−	−	+	+	−	−
12	0.958	+	+	+	+	+	−	+	+	+	+	+	+	+
13	0.981	+	+	+	+	+	+	+	+	−	+	+	+	+

[1]CK: Leaves collected from the Tartarchia site.

[2]+: Presence of isozyme band; −: Absence of isozyme band.

Table 10. *Effects of water flooding and drought on the variation of peroxidase isozymes present in leaves of **M. transmorrisonensis***

Band No	R_f	Control		Flooding		Drought	
		Months after treatment					
		1	3	1	3	1	3
1	0.109	+	+	+	+	−	−
2	0.139	+	+	−	+	−	−
3	0.259	+	+	+	+	+	−
4	0.295	+	+	+	+	+	+
5	0.327	+	+	+	+	+	+
6	0.477	−	−	−	−	−	+
7	0.554	−	+	−	−	−	−
8	0.567	+	+	+	+	+	+
9	0.626	+	+	+	+	+	+
10	0.678	+	+	+	+	+	+
11	0.721	+	+	+	+	+	+
12	0.761	+	+	−	+	+	+

Table 11. *Effects of water flooding and drought on the variation of esterase isozymes present in leaves of **M. transmorrisonensis***

Band No	R_f	Control		Flooding		Drought	
		Months after treatment					
		1	3	1	3	1	3
1	0.281	+	+	+	+	+	+
2	0.317	+	+	+	+	+	−
3	0.354	+	+	+	+	−	+
4	0.399	+	+	+	+	+	+
5	0.442	+	+	+	+	−	+
6	0.485	−	−	−	−	−	−
7	0.520	+	+	−	+	−	−
8	0.625	+	+	−	+	−	−
9	0.770	−	−	−	−	+	+
10	0.831	+	+	−	+	−	−
11	0.865	+	+	−	−	−	+
12	0.905	+	+	−	+	−	+
13	0.939	+	+	−	+	−	+
14	0.958	+	+	+	+	+	+
15	0.981	+	+	+	+	+	+

such stresses and then analyzed for the isozymes. For peroxidase, there was no significant difference between flooding and drought treatments (Table 10). However, three bands, 1, 2, and 7, were missing after the drought treatment, while little change in the zymogram was found after the flooding treatment. It was reasonable that the zymogram patterns were different because of the water treatments were much different.

For esterase, the isozyme pattern was not significantly affected by water flooding; bands 8, 10, 11, 12, and 13 were missing in the first month after treatment but these bands, except band 11, reappeared three months after treatment. For the drought treatment, pronounced change of missing bands 7, 8, and 10 was shown three months after treatments (Table 11). However, band 2 appeared in the first month after drought treatment but missing at 3 months after treatment. In overall, the observed effects of water stress on the transplanted experiments do not indicate that such stress has been significantly involved in acclimation of *M. transmorrisonensis*. In the field, the water is sufficient for *M. transmorrisonensis*; however, if the plants are transplanted to a water deficient area the plants would not be able to grow so well as those in normal situation.

Discussion

In a series population study of *Miscanthus floridulus*, we reported that the ecotypes of *Miscanthus* were found in various industrial areas in Taoyuan county, west of the Taiwan plain, and in mountainous areas along the Shenmonlingtao of Nantou county (Chou *et al.*, 1987; Chou and Chang, 1988). The coastal area of Taoyuan county has been notorious for industrial pollution for nearly twenty years, and vegetation, 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* flourishes, indicating that an adaptive mechanism has developed. Chou and his coworkers showed that the isozyme patterns for peroxidase and esterase present in leaves of *Miscanthus* could explain the reason for such adaptation.

Chou (1989) further reported that the population divergence of *Miscanthus floridulus* was significant between *Miscanthus* populations in island Taiwan and those of some islets, Pescadero, Green, and Orchid. It is very likely that the differentiation of *Miscanthus* is due to a long isolation of the islets from the island proper. Furthermore, Chou and Chen (1990) found that the populations of *Miscanthus floridulus* in Green and Orchid are discontinuous rather than continuous although the environmental gradient of the populations is continuous. The mechanism producing discontinuity needs to be further studied.

Chou and Ueng (1990) proposed an evolutionary trend of *Miscanthus* populations that was greatly influenced by geographic location. They concluded that *M. sinensis* could be an original species of *Miscanthus* taxa and *M. transmorrisonensis* the most recent evolved species. The present work showed that transplanted *M. floridulus* plants failed to survive in Tartarchia Anpu two to three years after transplanting. However, *M. transmorrisonensis* plants grow very well in Hoshe after transplanting, confirming that *M. transmorrisonensis* could be evolved from *M. floridulus*. Such a speciation is obviously due to change in temperature, indicating that ecotypes of *Miscanthus* had been developed during the evolutionary stages. Consequently, the ecotypes of *M. sinensis* gradually evolved to *M. floridulus, M. flavidus, M. sinensisis* var. *formosanus*, and finally to *M. transmorrisonensis*. Furthermore, the effect of altitude on the isozyme patterns of *Miscanthus* is certainly related to temperature. The present experiments on the effects of temperatures on the variation of isozymes present in *M. transmorrisonensis* under Phytotron and field conditions help clarify the role of temperature in the acclimation of *Miscanthus* populations.

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芒草族群研究 IV. 五節芒與高山芒之生長表現及其對溫度與水分壓力之馴化反應

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高山芒(*Miscanthus transmorrisonensis*)係廣布於台灣山區海拔2,200公尺以上的優勢植物，而五節芒(*M. floridulus*)卻是海拔 2,200 公尺以下的優勢植物。兩者很難在不同環境下成爲優勢種。爲探究兩種芒草對不同環境壓力之適應機制，本研究遂採兩者交替種植。將五節芒從和社地區(1100 公尺)移植到塔塔力鞍部(2700 公尺)，及將高山芒從塔塔加鞍部移植到和社地區，在移植 6, 12, 18 月後，分別觀察兩者之生長情形，並採其葉子經聚丙烯醯胺電泳法(polyacrylamide gel electrophoresis)分析葉中酯酶(esterase)及過氧化酶(peroxidase)同功酶分布。研究結果指出高山芒移植到低海拔地區，其植物生長良好。然而，五節芒被移植到塔塔加鞍部後卻生長不良，且不易過冬。翌年春天，五節芒可利用貯存於根部的營養而萌芽生葉，但再隔一年冬年，五節芒顯有凋萎現象，此顯示五節芒不能生長於高海拔之主因。分析兩者葉中之同功酶分布則顯示，經移植後，兩者在過氧化酶及酯酶上均有顯著差異。進一步，將高山芒幼苗移植於不同溫度的人工氣候室以觀察及分析高山芒對溫度之反應，則顯現高山芒對溫度有顯著敏感的反應。尤有進者，高山芒幼苗在乾旱及浸水之處理後，則該幼苗對上述環境壓力有明顯的反應。