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A BIOCHEMICAL ASPECT OF PHYLOGENETIC STUDY OF BAMBUSACEAE IN TAIWAN

IV. The Genera *Arundinaria*, *Pseudosasa*, *Semiarundinaria*, *Shibataea*, *Sinobambusa*, and *Yushania*[1,2]

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Abstract

A biochemical approach based on phenolics and isozymes of peroxidase and esterase was conducted to study the phylogenetic relationship among ten species, namely *Arundinaria hindsii*, *A. graminea*, *A. simonii*, *Pseudosasa japonica*, *P. usawai*, *Semiarundinaria fastuosa*, *Shibataea kumasasa*, *Sinobambusa kunishii*, *S. tootsik*, and *Yushania niitakayamensis*. By means of paper chromatography, 30 spots of phenolics including flavonoids were found in the genus *Arundinaria*, 35 spots in the genus *Pseudosasa*, 23 spots in the genus *Semiarundinaria*, 31 spots in the genus *Sinobambusa*, and only 11 spots in the *Yushania*. By using acrylamide gel electrophoresis, there were 21 bands of peroxidase isozymes present in *Y. niitakayamensis*, and less than 9 bands in the remaining species, whereas there were 25 bands of esterase isozymes present. The phenolics and isozymes data were computed by a simple matching coefficient and unweighted pair-group with simple average to obtain phenograms. Based on all characters studied we concluded that three major clusters were found in this study: three species of *Arundinaria* and *Se. fastuosa* belong to one cluster, genera *Pseudosasa*, *Shibataea*, and *Sinobambusa* belong to the second cluster, whereas *Y. niitakayamensis* is apparently phylogenetically far from the former two clusters.

Key words: phylogenetic study; Bambusaceae; *Arundinaria; Pseudosasa; Semiarundinaria; Shibataea; Sinobambusa; Yushania;* isozymes; phenolics.

Introduction

Bamboo plants are one of the major forest in the oriental countries and widely planted on many hillsides of Taiwan. The taxonomic research of bamboos has extensively been taken by scientists (Hayata, 1916; Nakai, 1925; Sasaki, 1933, McClure, 1957; Lin, 1961, 1978; Kiang, 1974; Liu, 1962; Kiang, 1974). In which most

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of taxonomic work was primarily based on a description of morphological characters; however, a biochemical appraoch based on plant secondary metabolites, such as phenolic compounds and flavonoids, and isozymes were introduced into the field of plant systematics, leading to a better understanding of the phylogenesis of plant kingdom (Chu *et al.*, 1972; Hsiao, 1973, 1980, 1811: Kiang and Wu, 1979; Chou *et al.*, 1984a, 1983b, 1985) Regarding the phenolic comounds in bamboos, we reported previously that the phenolics including flavonoids are variously distributed in 14 bamboo species (Chou and Hou, 1981), and some of the phenolics play a significant role in the regulation of understories in bamboo plantation (Chou and Yang, 1982). Because the important roles of phenolics and isozymes in plant metabolism that may lead to a preferential development of phylogenesis. we thus conducted a series of studies based on these biochemical characters to elucidate the taxonomic and evolutional significance of Bambusaceae. In the past three years we have done the phylogenetic study of the genera *Arthostylidium*, *Chimonobambusa*, *Dendrocalamus*, *Phyllostachys*, and *Bambusa* (Chou and Hwang, 1985; Chou *et al.*, 1984a and 1984b). Continued to this study, we present here the fourth report on the preliminary identification of phenolic compounds and the isozymes distribution in genera *Arundinaria*, *Pseudosasa*, *Semiarundinaria*, *Shibataea*, *Sinobambusa*, and *Yushania* from which we may clarify some ambiguous taxonomic position of taxa and their phylogenetic relationship among the taxa studied.

Materials and Methods

Materials

Leaves of 10 species, namely *Arundinaria hindsii* Munro, *A. graminea* (Bean) Makino, *A. simonii* (Carr.) A. & C. Riv. *Pseudosasa japonica* (Sieb. & Zuce.) Makino, *P. usawai* (Hayata) Makino & Nemota, *Semiarundinaria fastuosa* (Mitford) Makino, *Shibataea kumasasa* (Zoll.) Makino, *Sinobambusa kunishii* (Hayata) Nakai, *S. tootsik* (Makino) Makino, and *Yushania niitakayamensis* (Hayata) Keng f., were collected in the summer and winter of 1983 in the Chitou Forest Experiment Station of National Taiwan University, and Taipei Botanical Garden of Taiwan Forestry Research Institute. The fresh leaves collected were immediately placed in an ice-box and brought back to laboratory for isozyme study, and dried leaves ground into powder for the extraction of phenolic compounds including flavonoids.

Extraction of Flavonoids from Bamboo Leaves

To 200 grams of leaves of each aforementioned taxa, 2 liters of reagent grade methanol were added and allowed to stand overnight. The methanolic extract was obtained by filtration through Whatman No. 42 filter paper, and the residue was added with 2 liters of methanol and treated by the same way. The subsequent extracts were obtained by the same way and all combined. After the methanolic

extraction, the residues were re-extracted with 80% methanol, 50% methanol till with distilled water. The entire extraction fractions were combined and concentrated to syrup-like solution by a rotatory evaporator *in vacuo*. The concentrated extract was subsequently re-extracted with hexane, chloroform, and ethyl acetate followed the techniques described by Newman *et al.* (1979); and the re-extract soluble in each aforementioned fraction was respectively designated as *Hexane fraction*, *Chloroform fraction*, and *Ethyl acetate fraction*, and the insoluble fraction was called "Water fraction". The extracts of each fractionation were saved for chromatographic analyses.

Isolation of Flavonoids by Chromatography

The syrup-like extract of each fractionation mentioned was first run by paper chromatography in order to see the pattern of phenolics distribution, and the appropriate fraction was chosen for further large scale isolation of compound by column chromatography. About 150 g of polyvinylpolypyrrolidone powder (Sigma Chemical Co., USA) was soaked with 1500 ml methanol or distilled water. The preparation of polyvinylpolypyrrolidone-methanol gel was followed by the techniques described by Newman *et al.* (1979). The techniques and solvent systems used for the column chromatography were described by Mabry *et al.* (1979). After several times of rechromatography, the isolated compounds became relatively pure and were finally passed through a Sephadex LH-20 column and eluted with spectroscopic grade methanol. The clean eluate was concentrated to a small volume and placed in a vial in a refrigerator to allow crystallization.

Isozymes Analyses of Bamboo Leaves

A vertical gel electrophoresis (M & Slab Electrophoresis, Model SG-80) was employed and techniques for electrophoresis of bamboo leaves were described by Chou *et al.* (1984a), which is a modification of Brewer (1970). Isozymes of peroxidase and esterase were investigated. The zymogram of each isozyme gel was kept in permanency by coating a cellophane after the gel was dried.

Simple Matching Coefficient and Clustering Analysis

The phenolic compounds including flavonoids and zymogram patterns of peroxidase and esterase studied were used as the characters to determine the similarity between taxa, using a formula $Ssm=m/(m+u)$, where m is the number of matches or agreements, u is the number of mismatches, and n is equal to $m+u$. The data of Ssm were then set in a simple matrix table using each species as an operational taxonomic unit (OTU). The clustering analysis between species was obtained by an unweighted pair-group method using simple arithmetric average described by Sneath and Sokal (1973).

Results

Distribution of Phenolic Compounds in genera ***Arundinaria, Pseudosasa, Semiarundinaria, Shibataea, Sinobambusa,*** *and* ***Yushania.***

By means of two dimensional paper chromatography, the chromatogram of phenolic compounds distributed in bamboo leaves of plants studied is shown in Fig. 1. Thirty spots of phenolic compounds including flavonoids were found in the methanolic extracts of leaves of the genus *Arundinaria,* 35 spots in *Pseudosasa,* 23 spots in *Semiarundinaria,* 31 spots in *Shibataea,* 28 spots in *Sinobambusa,* and only 11 spots in *Yushania.* The characteristics of each spot are given in Table 1, showing that the phenolics are differentially distributed in the genera studied, particularly there are less number of phenolics in the genus *Yushania.* Based on chromatograms shown in Table 1, spots 3, 4, 5, 6, 7, 14, 15, 19, 20, 25, 26, 34, and 47 are common to all species studied except *Yushania.* The pattern of phenolic compounds in leaves of *Yushania* is very much different from that of the remaining genera studied.

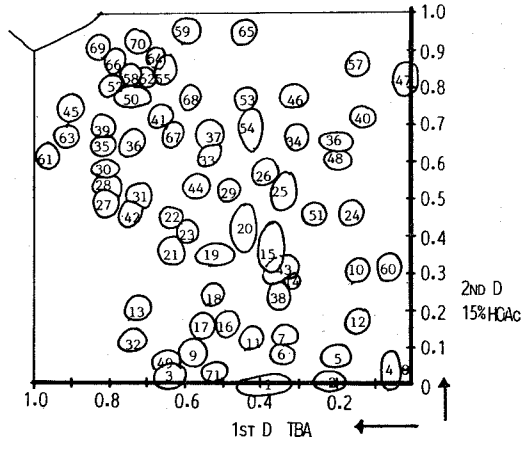

Fig. 1. Paper chromatogram of phenolic compounds in 10 species of bamboo leaves studied.

Distribution of Peroxidase and Esterase Isozymes in the Bamboo Leaves

The peroxidase zymogram patterns of eight species are shown in Fig. 2, of which 9 bands were found in the genus *Arundinaria,* 7 bands in *Pseudosasa,* 7 bands in *Semiarundinaria,* 5 bands in *Shibataea,* 5 bands in *Sinobambusa,* and 21 bands in *Yushania* (Table 2). The peroxidase patterns in the bamboo leaves differed with species studied, particularly *Yushania* exhibited a unique pattern with 21 bands and many bands not found in the remaining genera were located in high Rf value.

Table 1. *Distribution of phenolic compounds in genera* ***Arundinaria, Pseudosasa, Semiarundinaria, Sinobambusa, Shibataea,*** *and* ***Yushania*** *obtained by paper chromatography*

Spot no.	Rf of PC[1]		Color reaction[2]		Speices[3]									
	TBA	15% HOAc	UV	UV/NH_3	Ag	Ah	As	Psj	Psu	Sef	Shk	Sik	Sit	Yn
1	0.39	0.00	YG	B	+			+		+			+	
2	0.22	0.02	YG							+			+	
3	0.65	0.03	P	FY	+	+	+	+	+	+	+	+	+	+
4	0.07	0.09	YG	FB	+	+	+	+	+	+	+	+	+	
5	0.26	0.08	FY	Y	+	+	+	+	+	+	+	+	+	+
6	0.35	0.09	P	Y	+	+	+	+			+		+	
7	0.36	0.15	Y	Y	+	+	+			+	+	+		
8	0.03	0.04	YG	YG							+			
9	0.58	0.08	Y					+			+			
10	0.14	0.32	Y	Y					+		+	+		+
11	0.44	0.12		Y				+	+		+	+		
12	0.16	0.17		Y				+	+		+			
13	0.74	0.22	Y		+	+	+			+				
14	0.33	0.26	P	FY	+	+	+	+		+		+		
15	0.38	0.36	P	Y			+	+	+	+	+		+	+
16	0.49	0.16	Y						+				+	
17	0.56	0.18	Y	YG							+	+		
18	0.53	0.26	P						+		+			
19	0.54	0.35	P	Y	+	+	+	+	+	+	+		+	
20	0.45	0.43	P	Y	+	+	+	+	+	+	+	+	+	
21	0.63	0.36	P	P	+					+		+	+	
22	0.65	0.44	P	P				+			+	+	+	
23	0.60	0.43	P	Y					+					
24	0.17	0.46		Y				+					+	
25	0.35	0.52	P	Br-Y	+	+	+	+		+	+	+	+	
26	0.39	0.58	P	Br-Y	+	+	+	+	+	+	+	+	+	+
27	0.82	0.49		FP				+		+		+		
28	0.85	0.51		FP	+	+	+	+	+		+	+	+	
29	0.48	0.52	P									+		
30	0.82	0.56	B	FB				+		+		+	+	
31	0.73	0.53	FP	FP	+	+	+		+		+			
32	0.73	0.13	P					+	+					
33	0.64	0.63	P	Br-Y		+		+			+	+	+	
34	0.31	0.63	Y	Y	+	+	+	+	+		+	+	+	+
35	0.83	0.63	Y	Y	+	+	+	+				+		
36	0.20	0.66		FB	+	+	+		+	+				
37	0.54	0.65	Y	Y		+					+		+	+

Spot no.	Rf of PC[1]		Color reaction[2]		Speices[3]									
	TBA	15% HOAc	UV	UV/NH_3	Ag	Ah	As	Psj	Psu	Sef	Shk	Sik	Sit	Yn
38	0.35	0.26		Pk		+								
39	0.83	0.69	FB	FP	+	+	+					+		
40	0.14	0.72	Y				+	+	+				+	
41	0.66	0.73	B		+		+	+	+	+	+	+		+
42	0.74	0.47	B		+			+	+					
43	0.36	0.32	Y		+	+	+	+						
44	0.59	0.54	YG				+							
45	0.87	0.75	YG		+	+	+				+	+	+	
46	0.33	0.79	Y	FP		+		+			+		+	+
47	0.01	0.83	Y		+	+	+	+	+		+	+	+	
48	0.20	0.01		FP			+	+						
49	0.67	0.06	Y					+	+					
50	0.74	0.77	P			+		+		+			+	
51	0.27	0.46	P	P					+					
52	0.80	0.80		FP		+	+							
53	0.44	0.63	B						+	+	+		+	
54	0.44	0.71	P	Br-Y					+	+				
55	0.67	0.85		FP		+					+	+		+
56	0.75	0.65	F	FB					+					
57	0.15	0.86	YG										+	
58	0.76	0.82	B		+									
59	0.60	0.96	P			+		+	+			+	+	+
60	0.07	0.31	Y							+				
61	0.97	0.62	Y							+				
62	0.73	0.83		YG			+							
63	0.92	0.67	B							+				
64	0.68	0.87		FP	+		+							
65	0.45	0.95	Y			+								
66	0.79	0.88	YG			+								
67	0.64	0.68	YG					+						
68	0.59	0.78		Y				+						
69	0.82	0.92	YG				+							
70	0.73	0.93	YG				+							
71	0.53	0.03		YG							+			
Total number of spot					26	30	30	35	28	23	31	26	28	11

(1) TBA = *t*-butanol: glacial acetic acid: water=3 : 1 : 1 (v/v/v); 15% HOAc = 15 ml glacial acetic acid in 85 ml distilled water.

(2) Color reaction: B=blue, Br=brown, F=fluorescence, G = green, Pk = pink, P=purple, Y=yellow, YG=yellowish green.

(3) The abbreviations of species: Ag=*Arundinaria graminea*, Ah=*A. hindsii*, As=*A. simionii*, Psj=*Pseudosasa japonica*, Psu = *P. usawai*, Sef = *Semiarundinaria fastuosa*, Ssk = *Shibataea kumasasa*, Sik=*Sinobambusa kunishii*, Sit = *S. tooksik*, Yn = *Yushania niitakayamensis*. The abbreviations hereafter in the following tables are the same expcept otherwise mentioned.

Table 2. *The distribution of peroxidase isozymes in leaves of eight bamboo speices*

Band	Rf	Ah	As	Psj	Psu	Sef	Shk	Sit	Yu
1	0.12	+	+			+			+
2	0.16				+		+	+	+
3	0.21								+
4	0.33								+
5	0.36					+			
6	0.38	+	+			+			
7	0.41	+	+						
8	0.42			+	+	+			
9	0.43	+	+	+	+	+	+		
10	0.45							+	
11	0.46	+			+				+
12	0.47		+	+		+		+	+
13	0.48						+		+
14	0.49	+	+		+		+	+	+
15	0.50								+
16	0.53	+	+						
17	0.55								+
18	0.59								+
19	0.63								+
20	0.67								+
21	0.69	+	+						
22	0.70								+
23	0.71			+	+	+	+	+	
24	0.75								+
25	0.79								+
26	0.81				+				+
27	0.84								+
28	0.86								+
29	0.88								+
Total number		8	8	4	7	7	5	5	21

On the other hand, the zymogram patterns of esterase are shown in Fig. 3, in which 25 bands were found. Ten bands were found in the genus *Arundinaria*, 11 bands in *Pseudosasa*, 10 bands in *Semiarundinaria*, 8 bands in *Shibataea*, and 13 bands in *Sinobambusa*. Of them, 4 bands, no. 14, 21, 22, and 23 are commom to all species studied (Table 3). Because the smaple of *Yushania* accidently lost, the result of esterase analysis was not given at the present time, but we would supplement the data later.

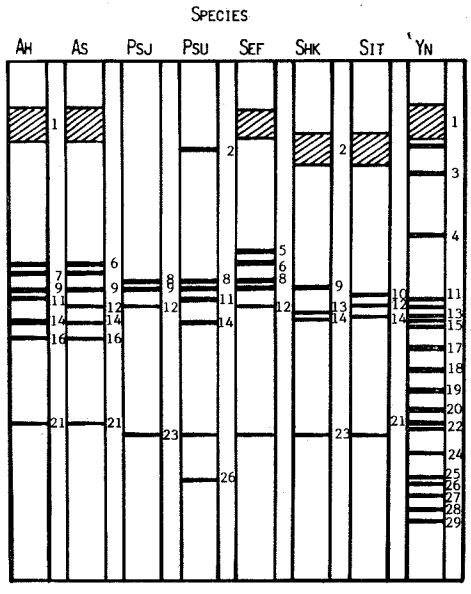

Fig. 2. The zymogram patterns of peroxidase present in fresh leaves of 8 species of bamboos, namely Ah=*Arundinaria hinsii*, As = *A. simionii*, Psj = *Pseudosasa japonica*, Psu=*P. usawai*, Sef= *Semiarundinaria fastuosa*, Shk = *Shittataea kumasasa*, Sit=*S. tootsik*, Yn= *Yushania niitakayamensis*.

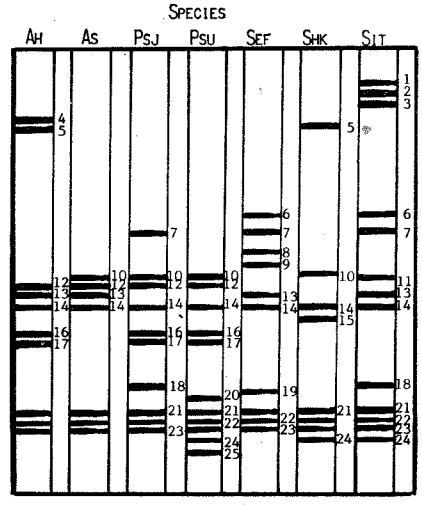

Fig. 3. The zymogram patterns of esterase present in seven species studied. The abbreviations of species see Fig. 2.

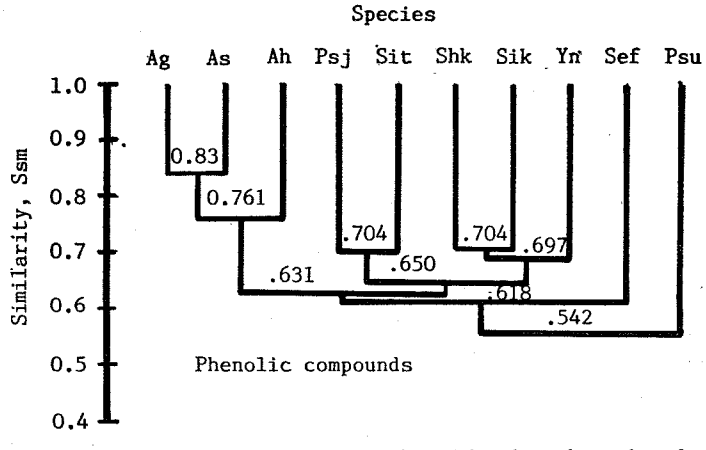

Fig. 4. The phenograms of 10 species of bamboo plants based on chromatographic data of phenolic compounds. The abbreviations of species see Table 1.

Phylogenetic Relationship of the Bamboo Plants

Based on chromatographic data shown in Table 1, simple matching coefficients of 10 species studied are shown in Table 4, which derives a phenogram of Fig. 4. From the phenogram it was found that the phenetic relationship between three species of the genus *Arundinaria* is very close together with high Ssm=0.791. *Pseudosasa japonica* and *Sinobambusa tootsik* belong to one cluster with Ssm=0.704. *Shibataea kumasasa* and *Sinobambusa kunishii* also showed a high similarity of Ssm=0.704. The later two clusters seem to be very close together with Ssm=0.650. However, *Psudosasa usawai* and *Semiarundinaria fastuosa* belong to a third cluster, which seems to be genetically far from the other two clusters mentioned.

Table 3. *The distribution of esterase isozymes in leaves of 7 bamboo plants*

Band	Rf	Ah	As	Psj	Psu	Sef	Shk	Sit
1	0.08							+
2	0.11							+
3	0.13							+
4	0.16	+						
5	0.18	+					+	
6	0.38					+		+
7	0.42			+		+		+
8	0.46					+		
9	0.49					+		
10	0.51		+	+	+		+	
11	0.52							+
12	0.53	+	+	+	+			
13	0.56	+	+			+		+
14	0.58	+	+	+	+	+	+	+
15	0.61						+	
16	0.64	+		+	+			
17	0.66	+		+	+			
18	0.76			+				+
19	0.78					+		
20	0.79				+			
21	0.82	+	+	+	+	+	+	+
22	0.84	+	+	+	+	+	+	+
23	0.86	+	+	+	+	+	+	+
24	0.88				+		+	+
25	0.90				+			
Total number		10	7	10	11	10	8	13

Table 4. *The matrix of simple matching coefficients of the phenolic compounds in 10 species of bamboo leaves based on paper chromatography*

OTU	OTU (Operational taxonomic unit)									
	Ag	Ah	As	Psj	Psu	Sef	Shk	Sik	Sit	Yn
Ag	—									
Ah	0.775	—								
As	0.831	0.746	—							
Psj	0.620	0.592	0.592	—						
Psu	0.606	0.521	0.577	0.620	—					
Sef	0.704	0.563	0.620	0.549	0.592	—				
Shk	0.620	0.676	0.592	0.634	0.648	0.549	—			
Sik	0.718	0.690	0.634	0.648	0.577	0.648	0.704	—		
Sit	0.634	0.662	0.577	0.704	0.606	0.648	0.704	0.662	—	
Yn	0.620	0.648	0.592	0.577	0.676	0.662	0.690	0.704	0.676	—

(1) The abbreviations of OTU see Table 1.

Table 5. *The matrix of simple matching coefficients of the zymogram patterns of peroxidase in 8 species of bamboo leaves*

OTU	OTU (Operational taxonomic unit)							
	Ah	As	Psj	Psu	Sef	Shk	Sit	Yn
Ah	—							
As	0.931	—						
Psj	0.655	0.724	—					
Psu	0.690	0.621	0.828	—				
Sef	0.690	0.759	0.897	0.724	—			
Shk	0.690	0.690	0.828	0.862	0.724	—		
Sit	0.621	0.690	0.828	0.793	0.724	0.862	—	
Yn	0.276	0.276	0.207	0.310	0.172	0.310	0.310	—

(1) The abbreviations of OTU see Table 1.

On the other hand, based on the zymogram pattern of proxidase the similarity coefficients between species are shown in Table 5, which derives a phenogram of Fig. 5. It was found that species of the genus *Arundinaria* have high similarity of Ssm=0.931; *Pseudosasa japonica* and *Semiarundinaria fastuosa* also have high similarity; *Hibataea kumasasa* and *Sinobambusa tooktsik* also showed high similarity of Ssm=0.862. However, *Yushania niitakayamensis* has low similarity, Ssm = 0.262, from the other genera. Based on esterase data, a matrix of similarity coefficient is given in Table 6, which derives a phenogram of Fig. 6. It was obvious that species of *Arundinaria* followed into a cluster, *Pseudosasa* the second cluster.

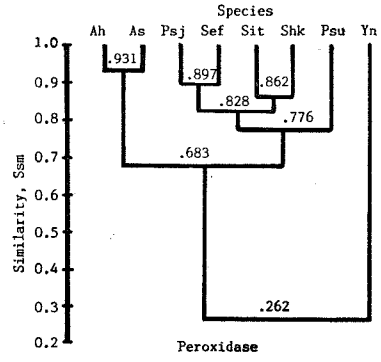

Fig. 5. The phenograms of 8 species of bamboo plants based on electrophoresis of peroxidase isozozyme. The abbreviations of species see Table 1.

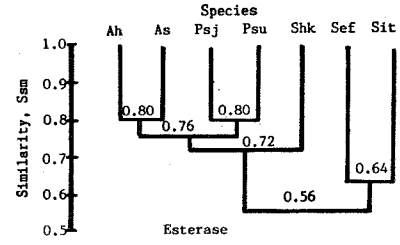

Fig. 6. The phenograms of 7 species of bamboo plants based on the data of esterase isozyme. The abbreviations of species see Table 1.

Table 6. *The matrix of simple matching coefficients of the zymogram patterns of esterase in 7 species of bamboo leaves*

OTU	OTU (Operational taxonomic unit)						
	Ah	As	Psj	Psu	Sef	Shk	Sit
Ah	—						
As	0.80	—					
Psj	0.76	0.80	—				
Psu	0.72	0.76	0.80	—			
Sef	0.60	0.72	0.60	0.48	—		
Shk	0.68	0.80	0.68	0.72	0.60	—	
Sit	0.48	0.60	0.56	0.44	0.64	0.56	—

(1) The abbreviations of OTU see Table 1.

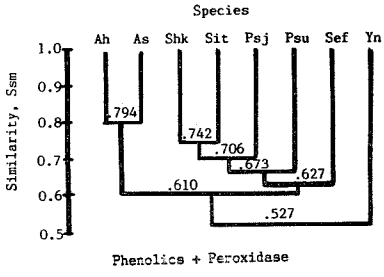

Fig. 7. The phenograms of 8 species of bamboo plants based on the data of phenolics and peroxidase isozymes. The abbreviations see Table 1.

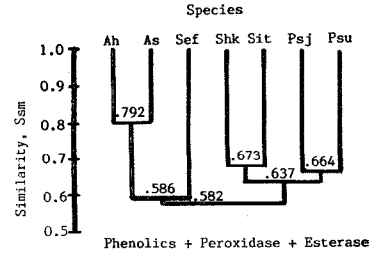

Fig. 8. The phenograms of 7 species of bamboo plants based on the data of phenolics and isozymes of peroxidase and esterase.

These two clusters can even group into one cluster, and *Shibataea* is also close to them. These three genera revealed a high similarity of Ssm=0.72. Genera *Semiarundinaria* and *Sinobambusa* are close enough and belong to the third cluster with a similarity coefficient of Ssm=0.64. The former two clusters can even belong to a cluster. Computing the data of phenolic compounds and peroxidase, a matrix simple matching cofficients obtained is given in Table 7, which derives a phenogram of Fig. 7. In addition, the data of phenolics, peroxidase and esterase were all combined, a matrix of similarity coefficients is shown in Table 8, which leads to a phenogram of Fig. 8. The combination of the phenolics and isozyme characters provided a better understanding of phylogenetical relationship between plants studied. This concludes that species of *Arundinaria* and *Semiarundinaria*

Table 7. *The matrix of simple matching coefficients of the characters based on the phenolics and peroxidase isozymes in 8 species of bamboo leaves*

OTU	OTU (Operational taxonomic unit)							
	Ah	As	Psj	Psu	Sef	Shk	Sit	Yn
Ah	—							
As	0.794	—						
Psj	0.598	0.619	—					
Psu	0.557	0.577	0.670	—				
Sef	0.588	0.649	0.639	0.619	—			
Shk	0.670	0.600	0.680	0.701	0.588	—		
Sit	0.639	0.598	0.732	0.649	0.660	0.742	—	
Yn	0.546	0.505	0.454	0.557	0.505	0.567	0.557	—

(1) The abbreviations of OTU see Table 1.

Table 8. *The matrix of simple matching coefficient of characters based on the phenolics and isozymes of peroxidase and esterase in 7 species of bamboo leaves*

OTU	OTU (Operational taxonomic unit)						
	Ah	As	Psj	Psu	Sef	Shk	Sit
Ah	—						
As	0.773	—					
Psj	0.591	0.618	—				
Psu	0.545	0.573	0.664	—			
Sef	0.545	0.627	0.591	0.545	—		
Shk	0.636	0.609	0.645	0.673	0.545	—	
Sit	0.564	0.555	0.664	0.564	0.618	0.673	—

(1) The abbreviations of OTU see Table 1.

belong to a cluster, *Pseudosasa, Shibataea,* and *Sinobambusa* belong to the second cluster, whereas *Yushania niitakayamensis* apparently belongs to the third cluster.

Discussion

In a series of phylogenetic study of bamboo plants in Taiwan, we have previously reported that there was a substantial number of phenolic compounds and numerous bands of isozyme peroxidase present in leaves of bamboo plants. These characters were used for clustering analysis, leading to an understanding of phylogenetic relationship between taxa of Bambusaceae. We have found that there are three major clusters in genera *Phyllostachys* and *Bambusa* (Chou *et al.*, 1984a, 1984b). Chou *et al.* (1984b) and Hsiao (1980, 1981) pointed out that the isozyme patterns revealed less confidence as compared with the phenolic compounds as far as phylogenetic viewpoint was concerned. This is primarily due to the number of isozyme bands of peroxidase is less than that of phenolic compounds, and sometimes both major characters could not well agree with each other. The increased number of isozyme analysis such as esterase will increase the number of characters, which are of great advantage for the clustering analysis and provides a more confident phenogram. It is interesting to note that the phylogenetic position of *Semiarundinaria* is not clear. When the phenolic characters were used as parameter to run similarity analysis, the genus was found to be close to genus *Pseudosasa,* while the peroxidase or esterase was used as a parameter it followed respectively into the cluster of *Pseudosasa* or *Sinobambusa.* However, as a result of combination of all characters, such as esterase, peroxidase, and phenolic compounds, the genus *Semiarundinaria* became phylogenetically close to the genus *Arundinaria.* This finding agrees fairly well with the conventional taxonomic treatment. Based on this study we prefer to treat the genus *Semiarundinaria* in the genus *Arundinaria.* On the other hand, the genera *Pseudosasa, Shibataea,* and *Sinobambusa* belong to an another cluster, whereas *Y. niitakayamensis* is apparently phylogenetically far from them.

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臺灣竹科植物血緣關係之生化學研究

四、苦竹屬、矢竹屬、崗姬竹屬、唐竹屬、葉平竹及玉山箭竹

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在臺灣大學溪頭實驗林及臺北植物園的竹類植物標本園及合歡山上的竹林區內，選竹類植物計苦竹屬（*Arundinaria*）三種，矢竹屬（*Pseudosasa*）二種，崗姬竹屬（*Shibataea*）一種，唐竹屬（*Sinobambusa*）二種，葉平竹（*Semiarundinaria*）及玉山箭竹（*Yushania niitakayamensis*）。取其新鮮的竹葉以萃取葉中之酚類化合物並以紙色層，薄色層及紫外光譜儀鑑定葉中酚類化合物的分布，結果得苦竹屬中含30個酚類化合物，矢竹屬35個，崗姬竹屬31個，唐竹屬28個，葉平竹23個，然玉山箭竹中僅含11個。另以聚丙烯醯胺電泳法分析過氧化酶（peroxidase）及酯酶（esterase）而發現，上述竹葉中含過氧化酶之同功異構酶有29個帶，酯酶者25個，其圖譜不同地分布於上述種屬間。以上述酚酸化合物及酶譜分布之數據做統計分析得種間之相似度關係。其結果可得知上述十種植物之親緣關係是苦竹屬之三種及葉平竹間之相似度值高（Ssm＝0.586），應屬一分類羣，矢竹屬，崗姬竹屬及唐竹屬之相似度值亦高（Ssm＝0.637）屬一分類羣，另外玉山箭竹與其他種屬之相似度值低應屬另一分類羣。依上述生化的方法來檢定竹類植物之血緣關係是相當有意義的。其數據可進一步提供分類學者對竹類植物演化之參考。