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Phylogenetic Analyses of Taro (*Colocasia esculenta* (L.) Schott) and Related Species based on Esterase Isozymes

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Phylogenetic relationships among the 84 accessions of taro (*Colocasia esculenta* (L) Schott), *C. gigantea* Hook, *Alocasia macrorrhiza*, *A. odora*, *Xanthosoma sagittifolium* (L.) Schott and *X. violaceum* Schott were investigated using isozyme polymorphism of esterase. The phylogenetic tree estimated by the UPGMA analyses revealed that taro accessions formed a single cluster and *C. gigantea* was more closely related to *Alocasia* species than to taro. Taro accessions from Yunnan tended to share band patterns with those from various areas, which indicates that the Yunnan area might have been important for taro evolution.

Key words : *Colocasia esculenta*, phylogeny, isozyme, esterase

Introduction

Taro, *Colocasia esculenta* (L) Schott, is one of the major starchy food plants in certain tropical parts of the world. In Japan, this crop was ranked sixth in production area among the major vegetables harvested in 1993. Despite such agricultural importance, no extensive studies of phylogeny on taro have ever been conducted. Even in a conventional taxonomy of the genus *Colocasia*, there seem to be some discrepancies among the classifications proposed by different botanists, Hotta[2], Kumazawa[6] and Kitamura[4].

Isozyme analysis has provided useful data for plant systematics and evolution studies. Isozyme polymorphism observed as banding patterns has been used to characterize crop cultivars and estimate phylogenetic relationships among germplasm[1]. There have been a few isozyme studies on taro[3,7,11], and related species, *Arisaema* (Araceae)[8,9]. Lebot and Aradhya studied isozyme variation among 1,417 taro accessions from Asia and Oceania and found seven polymorphic enzyme systems[7]. Based on the presence or absence of isozyme bands of these seven enzymes, they classified the accessions and estimated phylogenetic closeness among the accessions. Tanimoto and Matsumoto analyzed peroxidase and esterase isozymes of Japanese taro cultivars and observed variation in banding patterns[11]. They concluded that cluster analysis based on the banding patterns was a suitable method for classifying cultivars into groups. Isshiki et.al. studied inheritance of isozyme variations for asparate aminotransferase, shikimate dehydrogenase and glucose-6-phosphate isomerase of wild taro introduced from Bangladesh[3].

The present study was conducted in the beginning of our taro phylogeny investigation using isozymes. The objective of the present study was to estimate the phylogenetic relationships among the accessions of taro and related species from southeastern Asia based on isozyme band patterns of esterase (EC 3.1.1.).

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Materials and Methods

Plant materials

The plant materials used in this study were 69 accessions of taro (*C. esculenta* (L.) Schott), four accessions of *C. gigantea* Hook, two accessions of *Alocasia macrorrhiza*, four accessions of *A. odora*, four accessions of *Xanthosoma sagittifolium* (L.) Schott and one accession of *X. violaceum* Schott (Table 1). These materials were collected in several expeditions to Nepal, Thailand, Yunnan, Ryukyu and other places in southeastern Asia during 1973 to 1996 and have been vegetatively maintained at Okayama University[15]. These accessions were classified according to Hotta[2]. Chromosome numbers in root tip cells were counted for *C. esculenta* and *C. gigantea* accessions[5].

Enzyme extraction and electrophoresis

The isozyme analysis was performed on extracts from young leaves which were sampled in the growing seasons during 1995 to 1997. Approximately 100 mg of young leaf tissue was extracted in a 1.5 ml polypropylene tube with 0.3 ml of 0.1 MTris-HCl buffer pH 7.5, containing 20 % (v/v) glycerol, 1 % (v/v) mercaptoethanol and 0.005 M EDTA. The extracts were centrifuged for 10 minutes at 4000 rpm and 25 μl of the supernatant was used for the electrophoresis. The enzymes were resolved by vertical slab gel electrophoresis in which a separation gel contained 7.5 % and a stacking gel contained 3.75 % (w/v) of acrylamide.

Eletrophoreses were carried out at 4-7 °C with constant current of 15 mA per gel in 0.025 M Tris-glycine electrode buffer (pH 8.3) until bromophenol blue tracking dye migrated to the bottom of the gel.

Staining for isozyme activity

The staining solutions and buffers used for the esterase system were 50 mg of α naphthyl acetate, 50 mg of β naphthyl acetate and 150 mg of Fast Blue RR Salt in 100 ml of 0.1 M phosphate buffer pH 6.3. The gels were incubated in staining solution at 34 °C in the dark until the isozyme bands developed to a sufficient intensity. The experiments were repeated three times using independent samples of the same plant materials and only repeatable results were reported here.

Data analyses

Esterase activities were detected as an isozyme band at 22 positions of the electrophoresis gel for the materials used in this study. A similarity measure for each pair of accessions was estimated by a simple matching method: the number of band positions where the bands of two accessions were either both present or absent divided by the total number of band positions. The distant matrices for the 84 accessions were appraised by subtracting the similarity measure from unity. The matrices were subjected to the UPGMA (Unweighted Pair-Group Method with Arithmetic mean) analyses to assess the phylogenetic relationships among the accessions based on esterase variations. To estimate esterase variations of *C. esculenta* within a geographical area of collection, the accessions were grouped according to geographical origins and the mean similarity measure for each area was calculated by averaging the similarity measures over the accession pair combinations.

Results and discussion

Chromosome observations showed that the 40 accessions of *C. esculenta* were diploid (2x=28) and 29 were triploid (3x=42). All four accessions of *C. gigantea* were found diploid (2x=28) (Table 1).

Among the 22 band positions of esterase detected in this study, clear bands appeared at seventeen, five, fifteen and nine positions for the accessions of *C. esculenta*, *C. gigantea*, two *Alocasia* species and two *Xanthosoma* species, respectively (Fig. 1). Tanimoto and Matsumoto reported eight positions of esterase bands on 38 strains of taro and 3 strains of *C. gigantea*[11]. Both α- and β-

Table 1 Plant materials used for analyses of esterase variation in *Colocasia* and related genera

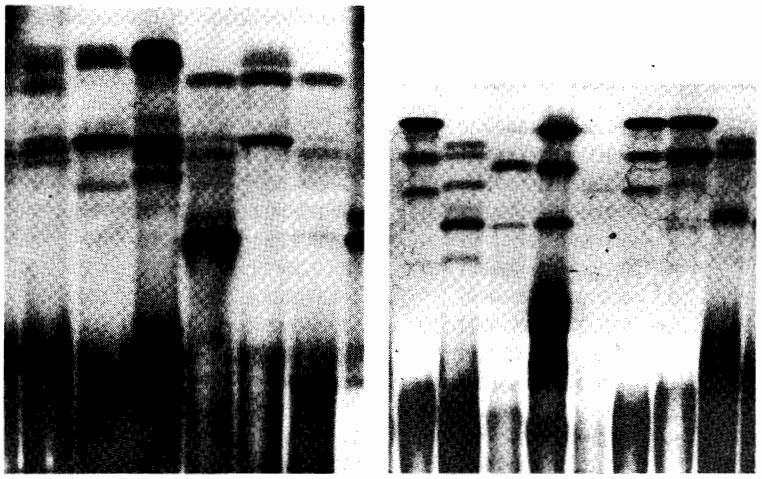
No.	Accession	Species	Origin	Type	Chromosome	Presence/absence of esterase bands	Zymotype No.
1	C81125	Cee	N	CV	28	00011000011000010010	9
2	C81081	Cea	N	W	28	00011110011101000011	31
3	C81079	Cea	N	W	28	00011110011101010011	34
4	C81135	Cea	N	W	28	00011110011101000011	31
5	C81080	Cea	N	W	28	00011110011101010011	34
6	C81019	Cea	N	W	28	00011110011101000011	31
7	TC83001	Cea	TC	W	28	00011000011000000001	7
8	C81027	Cea	N	W	28	00011000101010010011	20
9	J9507	Cee	JI	CVT	28	00011000011010010001	11
10	CN95009	Cea	CN	W	28	00011000011000000001	7
11	CN95002	Cea	CN	W	28	00011000011010010001	11
12	NG9502	Cee	PGN	CV	28	00011000011000010011	10
13	NG9504	Cee	PGN	CVT	28	00011000011000000001	7
14	NG9506	Cee	PGN	CV	28	00011000011000000001	7
15	C81023	Cea	N	W	28	00011110011101000011	31
16	C81012	Cea	N	W	28	00011110011101000011	31
17	C81045	Cea	N	W	28	00011110011101010011	34
18	C8030	Cea	N	W	28	00011000011101000011	17
19	C81114	Ce	N	W	28	00011000101101010010	24
20	C9520	Cee	N	CV	42	00011110011101011111	36
21	101	Cee	N	CV	42	00011110011101011011	35
22	113	Cee	N	CV	42	00011110011101010001	32
23	KUYE373	Ce	N	W	42	00000110011101011011	2
24	111	Cee	N	CV	42	00011110011101011011	35
25	KUYE442	Cee	N	CV	42	00000110010001011011	1
26	005	Cee	N	CV	42	00011000011010010111	14
27	118	Cee	N	CV	42	00011110011101011111	36
28	C81126	Ce	N	W	28	00011000010001110001	6
29	KUYE474	Ce	N	W	28	00011000011101000001	16
30	C81073	Ce	N	W	28	00011000010001001001	5
31	C9501	Cee	N	CV	42	00011110011000011011	30
32	CN95004	Cee	CN	CV	42	00011000011010011111	15
33	CN95005	Cee	CN	CV	28	00011000011010010011	12
34	CN95006	Cee	CN	CVT	42	00000110011111111011	3
35	CN95010	Cea	CN	W	28	00011000011000010011	10
36	CN95011	Cea	CN	W	28	00011000011000000011	8
37	CN95015	Cee	CN	CV	42	00011000011111110011	19
38	CN95022	Cea	CN	W	28	00011000011000000001	7
39	CN95029	Cee	CN	CV	42	00011110011101010001	33
40	CN95031	Cee	CN	CV	28	00011000011101010001	18
41	CN95044	Cee	CN	CVT	42	00011000111010010011	27
42	C96005	Cee	VN	CV	42	00011000010000000011	4
43	C96006.4	Cee	VN	CV	42	00011000111000000011	26
44	C96007.4	Cee	VN	CV	42	00011000101010011001	22
45	C96002	Cea	VN	CV	42	00011000001000000001	4
46	TC83013	Cee	TC	W	28	00011000101101010001	23
47	TC83014	Cea	TC	W	28	00011000011000000001	7
48	TC8601.2	Cee	TC	CV	28	00011000011010010001	11
49	TC8611.2	Cee	TC	CV	42	00011000101010011010	21
50	C81142	Cee	TC	CV	42	00011110111101011111	39
51	C84001-3	Cee	BI	CV	28	00011110011000000001	28
52	C93100.4	Cee	E	CV	42	00011110101010111111	38
53	C9521a	Cee	E	CV	42	00011000011010010111	13
54	C87008	Ce	S	W	42	00011000011010010001	11
55	C87001	Cea	S	W	28	00011000011101000001	16
56	CL83006	Cee	RJ	CV	42	00011110011111111011	37
57	CL8203.1	Cea	RJ	W	28	00011000011000000001	7
58	CL87015	Cea	RJ	W	28	00011000111000000001	25
59	CL83022	Cea	RJ	W	28	00011000011000010011	10
60	CL83019	Cea	RJ	W	28	00011000011000000011	8
61	CL83027	Cea	RJ	W	28	00011000011010010001	11
62	CL87019	Cea	RJ	CV	28	00011000011000000001	7
63	C7409-3	Cee	RJ	CV	42	00000110011111111011	3
64	Egu-imo	Cee	J	CV	42	00000110011111111011	3
65	Takenoko	Cee	J	CV	28	00011110011000000010	29
66	C95501	Cee	J	CV	42	00011000011000000001	7
67	C95502	Cee	J	CV	42	00011000011010010011	12
68	C95503	Cee	J	CV	42	00011000011000000001	7
69	C86001	Cei	J	CV	28	01011110101000010001	40
70	C87018	Cg	RJ	CV	28		
71	CN95021	Cg	CN	CV	28		
72	C95101	Cg	J	CV	28		
73	C96004	Cg	VN	CV	28		
74	J9508	Xs	JI	CV	26		
75	NG9505	Xs	PNG	CV	—		
76	C96003	Xs	VN	CV			
77	VN96201	Xs	VN	CV			
78	VN96204	Xv	VN	CV	—		
79	Am9512.3	Am	J	W	28		
80	Ao9516	Ao	J	W	28		
81	NG9503	Am	PNG	W	28		
82	Ao9519	Ao	J	W	28		
83	CN95001	Ao	CN	W	—		
84	VN96206	Ao	VN	W	—		

Abbreviations:

Species:
- Ce: *C. esculenta* (L.) Schott,
- Cee: *C. esculenta* (L.) Shott var. *esculenta*,
- Cea: *C. esculenta* (L.) Schott var. *aquatilis*,
- Cei: *C. esculenta* (L.) Schott var. *illustris*,
- Cg: *C. gigantea* Hook,
- Am: *A. macrorrhiza*,
- Ao: *A. odora*,
- Xs: *Xanthosoma sagittifolium* (L.) Schott,
- Xv: *Xanthosoma violaceum* Schott.

Source:
- E: Ethiopia,
- BI: the Bali Island,
- CN: Yunnan (China),
- J: Japan,
- JI: the Java Island,
- N: Nepal,
- PGN: Papua New Guinea,
- VN: Vietnam,
- RJ: the Ryukyu Islands (Japan),
- S: Seychelles,
- TC: Thailand.

Type:
- CV: Cultivar,
- CVT: Cultivated type,
- W: Wild.



(a) (b)

Fig. 1 Esterase zymograms for *C. esculenta* (a) and *A. macrorrhiza*, *A odora* and *X. sagitiifolium* (b).

naphthyl acetates were used as an esterase substrate for isozyme staining in our study. This may have increased the number of detected bands.

Phylogenetic relationship estimated by the UPGMA method is shown in Fig. 2. The 69 accessions of *C. esculenta* formed a single cluster which contained no accessions of other species analyzed in this study. Four accessions of *C. gigantea* had an identical band pattern even though these accessions were collections from far distant areas. The *C. gigantea* entries formed a cluster with *A. odora* and *A. macrorrhiza*, and this cluster merged with one consisting of *Xanthosoma* and *Alocasia* species (Fig. 3). This indicates that *C. gigantea* is related to some of the *Alocasia* species more closely than to *C. esculenta*. The accessions of *Alocasia* species in this study had large variability in the esterase isozyme and appeared to consist of three distinctive groups: those distantly related to *C. gigantea*, one associated with *Xanthosoma*, and *A. odora* accessions completely separate from the rest of the accessions in this study.

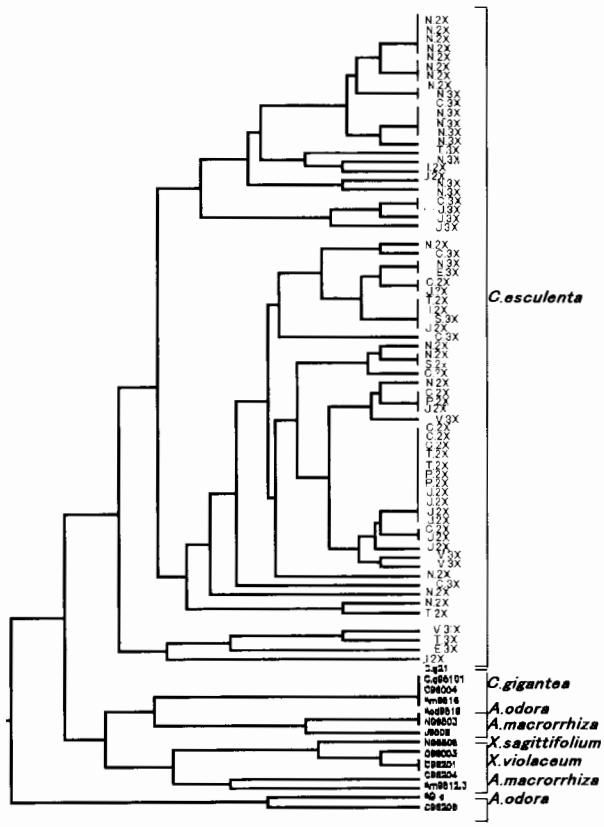


Fig. 2 Dendrogram for accessions of taro related species obtained by the UPGMA analyses based on the esterase banding patterns.

Within a cluster of the 69 accessions of *C. esculenta*, Nepalese collections tended to cluster together and the cluster was separate from the accessions of other origins. Except for this, accessions grouped by the esterase zymogram similarity appeared not to have any common characteristics related to origins or ploidy levels.

The geographic distribution of the *C. esculenta*

accessions and their zymogram types were summarized in Table 2. The number of zymogram types were almost equal to the number of the accessions in each geographical area indicating that most of the accessions had unique band patterns and large variability existed in the esterase isozyme among the accessions within each area. One third of the Nepalese collections had the same zymogram types. However, the mean similarity measure for the Nepal area was similar to those of other areas, suggesting the genetic variability among the *C. esculenta* plants in this area might not be different from those of other areas.

In order to investigate geographical dispersal of taro, band patterns common to two geographic areas were counted for the areas with four or more zymogram types (Table 2). The Yunnan area of China shared common band patterns with all areas except for Vietnam, in which no common pattern was found to any of the five areas (Fig. 4). Nepal showed only one connection to the Yunnan out of five areas and the common zymogram on that connection was only one type, even though sixteen different zymogram types were

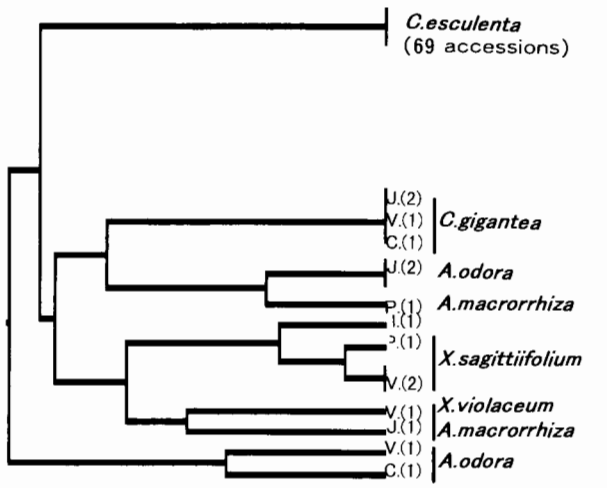


Fig. 3 Dendrogram for accessions of taro related species obtained by the UPGMA analyses based on the esterase banding patterns.

Table 2 Geographical origins of accessions, zymogram types and similarity measure

Origin	Accessions	Zymogram types	Mean similarity measure
Nepal	24	16	0.7882
China	12	11	0.7702
Japan	7	5	0.6623
Ryukyu	7	6	0.7939
Thailand	6	5	0.6667
Vietnam	4	4	0.8258
Papua New Guinea	3	2	—
Seychelles	2	2	—
Java Island	1	1	—
Bali Island	1	1	

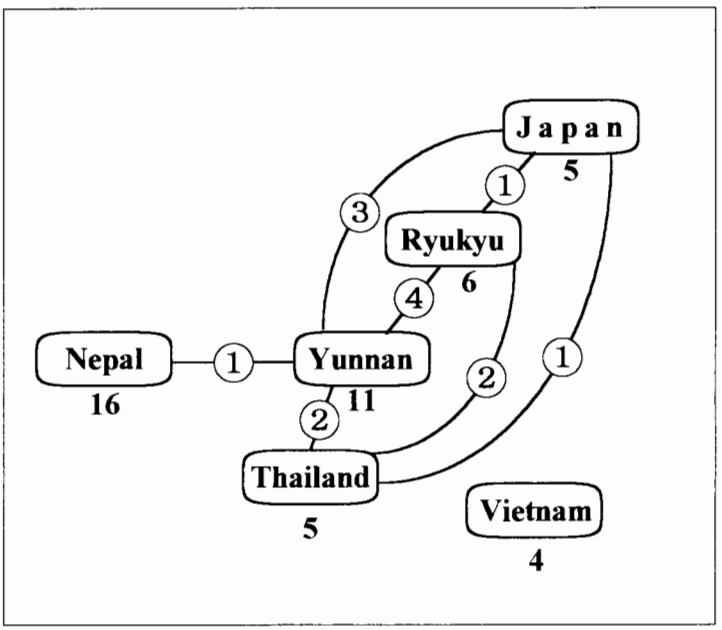


Fig. 4 Geographical distribution of esterase zymogram types.
The number under each area is the number of zymogram types from accessions originating in that area.
The circled numbers indicate the number of zymogram types common to two areas connected by the line.

found in that area. Genetic variability of taro exists in Nepal; however, taro plants in that area may be genetically isolated from those in other parts of southeastern Asia. These results agree with those by the UPGMA analyses. Although taro is considered to have originated in Assam or Burma or India[10], the results of this study imply that the Yunnan area might have played an important role in evolution and dispersal of taro.

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エステラーゼ・アイソザイムによるサトイモの系統分類

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東アジアを中心に採集したサトイモ(*Colocasia esculenta* (L) Schott)とその近縁野生種 *C. gigantea* Hook, *Alocasia macrorrhiza*, *A. odora*, *Xanthosoma sagittifolium* (L.) Schott and *X. violaceum* Schott の84系統について，エステラーゼのアイソザイムの多型を基に類縁関係の推定を行った．UPGMA 法によって系統樹を求めたところ，サトイモ69系統は一つの独立したクラスターを形成した．また，ハスイモ(*C. gigantea* Hook)は，サトイモよりも *Alocasia* 属の種とより近縁であることを示唆する結果を得た．サトイモについて，東アジアの各系統のアイソザイムのバンドパターンを比較したところ，中国雲南省で収集したものは，東アジア各地のサトイモと共通のパターンを示すものが多かったことから，この地域がサトイモの進化に重要な役割を果たしていることが推察された．