Characterizing taro using isozymes and morpho-agronomic descriptors

V. Lebot, S. Hartati, N.T. Hue, N.V. Viet, N.H. Nghia, T. Okpul, J. Pardales, M.S. Prana, T.K. Prana, M. Thongjiem, C. M. Krieke, H. VanEck, T.C. Yap and A. Ivancic

Introduction

According to the FAO, in 2008 more than 1.6 million hectares of taro [*Colocasia esculenta* (L.) Schott] were being cultivated, producing 11.7 million tonnes of corms (FAOSTAT 2010). Several problems limit taro development: often irregular corm, threat of leaf blight disease caused by the fungus *Phytophthora colocasiae*, and viruses that affect yields. The success of taro improvement depends strongly on its genetic resources, and the breeding process is much easier when adequate and appropriate genetic resources are available. Although taro is a vegetatively propagated species, it is highly polymorphic. Growing areas are characterized by a wide range of environments and a great diversity of cultivars. Each cultivar is adapted to specific environmental conditions and generally it is cultivated to satisfy distinct and particular uses.

Taro morphological variability is one of the main reasons for different botanical classifications, but little is known of the genetic diversity of the species. Purseglove's (1979) system of systematization includes one species with two botanical varieties: *C. esculenta* var. *esculenta* (named dasheen) and *C. esculenta* var. *antiquorum* (named eddoe), with the main difference between the two being the length of the sterile appendix of the spadix. The sterile tip of the spadix of *antiquorum* is usually much longer than that of *esculenta*. However, the differences in this character are far from obvious because of rare flowering of most plants. The relevance of this taxonomic system has not been demonstrated yet.

Present breeding programmes are in most cases national. International cooperation among breeders and the procedure of germplasm exchange are yet to be fully established. There is no international breeding centre for taro, nor is there a large international germplasm collection. The Taro Network for Southeast Asia and Oceania (TANSAO), a 4-year project (1998–2001), was established to enhance the competitive position of taro in traditional cropping systems of the region. Cultivars were selected for desired agronomic characteristics, exchanged between participating countries (Indonesia, Malaysia, Papua New Guinea, the Philippines, Thailand, Vanuatu and Vietnam) and evaluated in diverse agroecological environments.

In 1998–99 TANSAO, supported by the International Cooperation with Developing Countries programme (INCO-DC) of the European Union, conducted an ecogeographic survey of the genetic variation existing in the region and systematically characterized national collections. This paper presents the extent of morpho-agronomic variation measured in cultivars, within and between seven countries of Southeast Asia and Oceania. It also analyzes the isozyme variation in taro and its relevance for the management of genetic resources. An intraspecific classification of *C. esculenta* is proposed to assist breeders in the selection of core subsets that could be used directly for genetic improvement.

Materials and methods

Germplasm collections

Collections of local cultivars and wild forms were assembled in Vietnam (VASI, Hanoi), Thailand (HRI, Phichit), Malaysia (UPM, Serdang), Indonesia (LIPI, Bogor), the Philippines (PRCTRC, Baybay), Papua New Guinea (NARI, Lae) and Vanuatu (VARTC, Santo). Accessions thought to be representative of the genetic diversity existing within each country were collected.

Morphological descriptions

Descriptions were conducted during 1998–99 on TANSAO collections using 23 standardized morphological descriptors. Each trait was scored with qualitative data. National databases were developed in Excel format.

Isozyme analysis

Accessions electrophoresed on starch gels and six enzyme systems—malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), isocitrate dehydrogenase (ICD), 6-phosphogluconate dehydrogenase (PGD), mallic enzyme (ME) and shikimic dehydrogenase (SKDH)—were revealed successfully. Distinct zymogram variants were revealed for each enzyme system and each was identified with a distinct letter. Zymotypes were determined by the respective variants for each system. Isozyme data from Papua New Guinea and Vanuatu were derived from previous work conducted by Lebot and Aradhya (1991).

Data analysis

Statistical analyses of the qualitative data obtained from morpho-agronomic characterization were performed on the data matrix obtained for each country, and for the region, using hierarchical agglomerative classifying algorithm (UPGMA) with SM and DICE coefficients. Statistical analyses of the binary isozyme data (presence = 1, absence = 0) were performed on the data matrix obtained for each country and for the region. Multivariate analysis of zymotypes (PCA) was confirmed by cluster analysis based on the DICE coefficient of association among cultivars using UPGMA.

Results

Morpho-agronomic variation

Detailed results obtained from the morpho-agronomic descriptions conducted in each country are presented for the most important morpho-agronomic traits (Table 1), leaf traits (Table 2), colour variation of aerial parts (Table 3) and corm traits (Table 4).

Germplasm type

Only two groups are distinguished: cultivars and wild forms. Morphologically, there is no strict separation between these two groups. Wild forms can have the attributes of cultivated dasheen or eddoe types. The morphological variability between wild forms is quite limited in comparison with cultivars. Wild plants are adapted to natural environmental conditions with strong selection pressure. Their main characteristics are long stolons, small elongated corms, continuous growth and a predominantly high concentration of calcium oxalate that makes them inedible. However, a few cultivars are also inedible and represent ornamental morphotypes or varieties cultivated for medicinal purposes.

Botanical variety

The main difference between dasheen and eddoe is in shape and size of the main corm and cormels. Dasheen genotypes are characterized by a larger central or main corm and smaller side cormels. Eddoe genotypes usually have a relatively smaller central and fibrous corm and well-developed side cormels. Most accessions can be clearly differentiated as dasheen (80.50%) or eddoe types (15.40%) and only 3.96% are intermediate types. These intermediates could be hybrids between the two botanical varieties or accessions that are difficult to classify because of the unusual shape of their corms. However, the classification based on the underground architecture of the plant appears to be practical and sufficiently discriminating. Most accessions collected in this vast geographical area are clearly dasheen types. Eddoe types do not exist in Melanesia (Papua New Guinea and Vanuatu). In Malaysia they represent 93% of the accessions but only in Vietnam are eddoes cultivated for sale in markets.

Growing conditions

Two major adaptation traits are distinguished: flooded (32.46%) and rain-fed (64.75%) growing conditions. Flooded types tend to perform poorly in rain-fed cropping systems. Most flooded genotypes require at least minimum water circulation. In dry conditions, growth is reduced and as a result yield is low. Corms of typical wetland cultivars are elongated, a trait that seems to remain stable even in dry conditions. Size, however, is reduced significantly by stress. The plants adapted to drier environments do not perform well under submerged conditions. Cultivars adapted to flooded conditions are always dasheen types, whereas eddoes are always found to be cultivated in rain-fed conditions.

Altitude

In most countries, taro is cultivated at low or mid elevations (<1000 m) and in only one country (Papua New Guinea) were more than 14% of the accessions collected above 1000 m of elevation and, surprisingly, all were dasheen types. In Indonesia, the very few plants found cultivated above 1000 m were all eddoe types.

Flowering

More than 38% of accessions do not flower. Consequently it is not possible to systematically evaluate the taxonomic classification system based on the size of the sterile appendix. However, more than 31% of the plants do flower and show that the length of the sterile tip of their spadix is not determined by the botanical variety, i.e. numerous eddoe types exhibit sterile tips of their spadix shorter than dasheen types. The intravarietal variability of this floral trait is remarkable for both eddoes and dasheens and does not appear to be in agreement with the classification of var. *esculenta* versus var. *antiquorum*. Intermediate types also exhibit variation of this trait.

Formation of stolons

In most accessions (62.18%), stolons are present and in only 37.12% of the accessions are stolons totally absent. The presence of stolons often was found to be associated with undesirable traits such as poor corm shape and taste quality. All wild forms have stolons, an attribute apparently necessary for the asexual survival of wild populations in the absence of seeds or when seeds fail to germinate.

TABLE 1. Geographical distribution of the most important morpho-agronomic traits presented as percentages of accessions

Country: †	PH	VN	TH	MY	ID	PG	VU	Total
No. of accessions	172	350	300	135	685	278	378	2298
Germplasm type								
Traditional cultivars	77.91	94.00	78.33	45.93	61.46	100	100	79.94
Wild genotypes	7.56	0.86	21.67	54.07	23.65	0	0	13.75
Feral material	13.37	5.14	0	0	6.57	0	0	3.74
Breeding lines	1.16	0	0	0	0	0	0	0.09
Not determined	—	—	—	—	8.32	—	—	2.48
Botanical variety								
Dasheen	80.81	40.00	78.33	6.67	98.69	98.56	100	80.50
Eddoe	16.86	35.71	21.67	93.33	1.17	0.36	0	15.40
Intermediate	2.32	24.29	0	0	0	0.72	0	3.96
Not determined	—	—	—	—	0.14	0.36	—	0.09
Growing conditions								
Flooded	0	59.14	21.67	20.74	54.74	0	18.78	32.46
Rain-fed	100	40.86	78.33	79.26	37.22	96.76	81.22	64.75
Not determined	—	—	—	—	8.03	3.24	—	2.78
Altitude								
Low (<500 m)	68.02	59.14	87.00	100	70.07	52.52	100	75.02
Mid (500–1000 m)	18.02	32.86	13.00	0	21.75	28.42	0	17.97
High (>1000 m)	9.88	7.43	0	0	0.14	14.03	0	3.61
Not determined	4.07	0.57	—	—	8.03	5.03	—	3.39
Flowering								
Never flowering	53.49	46.86	93.33	86.67	0	0	58.99	38.12
Flowering	46.5	14.00	6.76	13.4	19.22	100	41.01	31.81
Not determined	—	39.14	—	—	80.88	—	—	30.07
Formation of stolons								
Absent	40.70	90.86	0.33	5.92	21.90	17.62	67.99	37.12
Present	59.3	9.14	99.67	94.07	77.66	77.7	32.01	62.18
Not determined	—	—	—	—	0.44	4.68	—	0.70

Country: †	PH	VN	TH	MY	ID	PG	VU	Total
Maturity period								
Early (4–8 months)	93.6	63.5	79.0	4.4	83.5	99.7	0	67.24
Late (8–11 months)	6.4	16.2	21.0	95.6	9.8	0	91.1	29.2
Undetermined (wild)	0	0	0	0	3.21	0	0	0.96
Not determined	—	0.29	—	—	3.50	0.36	8.99	2.61

† Philippines (PH), Vietnam (VN), Thailand (TH), Malaysia (MY), Indonesia (ID), Papua New Guinea (PG) and Vanuatu (VU).

TABLE 2. Geographical distribution of the variation of leaf traits, presented as percentages of accessions

Country: †	PH	VN	TH	MY	ID	PG	VU	Total
No. of accessions	172	350	300	135	685	278	378	2298
Growth habit								
Erect	79.65	74.28	3.00	60.74	18.39	1.08	83.07	40.51
Semi-erect	20.35	25.71	89.33	37.78	81.46	78.06	6.88	54.17
Semi-prostrate	0	0	7.67	1.48	0	18.70	1.06	3.52
Prostrate	0	0	0	0	0	1.44	0	0.17
Not determined	–	–	–	–	0.14	0.72	8.99	1.61
Plant height								
Dwarf (<50 cm)	9.30	12.86	0.33	25.18	4.82	1.08	0.53	5.83
Medium (50–100 cm)	76.74	75.14	70.00	66.67	70.07	92.80	31.48	67.53
Tall (100–150 cm)	13.95	10.28	27.67	3.70	24.67	5.75	52.38	23.11
Very tall (>150 cm)	0	1.71	2.00	4.44	0.29	0	6.61	1.95
Not determined	—	—	—	—	0.14	0.36	8.99	1.67
Position of leaf lamina								
Erect, apex up	0	0	0.33	0.74	0	0	0	0.09
Semi-erect, apex up	0	0	0.33	4.44	1.46	0	0.26	0.78
Erect, apex down	5.23	2.57	1.33	11.11	17.81	0	55.82	16.10
Semi-erect, apex down	93.02	95.43	97.34	62.96	76.93	98.92	29.63	77.68
Semi-horizontal	0.58	1.71	0.67	17.78	2.77	0.36	2.12	2.65

Country: †	PH	VN	TH	MY	ID	PG	VU	Total
Horizontal	1.16	0.29	0	2.96	0.58	0.36	3.17	1.04
With drooping edge	0	0	0	0	0.29	0	0	0.09
Not determined	—	—	—	—	0.15	0.36	8.99	1.57
Shape of leaf lamina								
Plain (flat)	98.26	20.57	0	10.37	0.58	13.31	87.30	27.24
Drooping lobes	0	0.57	100	2.96	1.17	0	0	13.66
Drooping edge	0	0.57	0	5.18	1.02	0.72	0	0.78
Cup shaped	1.74	78.00	0	71.11	97.08	85.61	3.44	56.05
Umbrella shaped	0	0.29	0	10.37	0	0	0.26	0.70
Not determined	—	—	—	—	0.15	0.36	8.99	1.57
Leaf lamina margin								
Entire	1.16	3.14	0	2.22	0.73	0.36	15.87	3.57
Sinuate	16.86	0.29	5.00	38.52	17.23	0.36	41.27	16.19
Undulate	81.40	96.00	95.00	58.52	81.31	98.92	33.86	78.33
Not determined	0.58	0.57	—	0.74	0.73	0.36	8.99	1.91

† Philippines (PH), Vietnam (VN), Thailand (TH), Malaysia (MY), Indonesia (ID), Papua New Guinea (PG) and Vanuatu (VU).

TABLE 3. Geographical distribution of the colour variation of aerial parts, presented as percentages of accessions

Country: †	PH	VN	TH	MY	ID	PG	VU	Total
No. of accessions	172	350	300	135	685	278	378	2298
Lamina colour								
Whitish	0.58	0	0	2.96	0.29	0	0.26	0.35
Yellow	0	0	0	0	0.44	0.36	0	0.17
Normal green	67.44	61.14	97.33	44.44	57.08	37.41	48.15	59.14
Dark green	31.40	38.86	2.00	50.37	33.43	61.87	41.80	35.81
Light purple	0.58	0	0.67	1.48	0.58	0	0.53	0.48
Purple	0	0	0	0.74	0.15	0	0.26	0.13
Not determined	—	—	—	—	8.03	0.36	8.99	3.92

Country: †	PH	VN	TH	MY	ID	PG	VU	Total
Lamina variegation								
Absent	99.42	99.71	100	98.52	98.54	80.93	85.71	94.73
Present	0	0	0	0.74	0.88	18.70	5.29	3.44
Not determined	0.58	0.29	—	0.74	0.58	0.36	8.99	1.83
Vein junction colour								
Whitish	0	0.29	0.33	2.22	1.46	0.36	1.85	1.00
Yellow	5.81	14.29	11.67	5.93	30.80	0	4.23	14.36
Light green	16.86	18.00	10.67	27.41	3.21	28.78	0.53	11.53
Dark green	0.58	3.14	0.33	7.41	3.21	28.42	0.26	5.44
Light purple	23.84	22.86	50.33	41.48	1.46	28.42	29.90	23.06
Dark purple	52.91	14.57	19.67	14.81	33.28	11.15	33.33	26.37
Red	0	0.29	7.00	0	18.69	2.52	17.46	9.70
Not uniform	0	26.29	0	0	1.17	0	3.44	4.92
Not determined	—	0.29	—	0.74	6.72	0.36	8.99	3.61
Basic colour of petiole								
Light green	25.00	41.71	86.67	56.30	5.84	8.27	36.77	31.64
Dark green	23.26	34.86	3.33	22.22	31.97	12.23	23.81	23.72
Red	0.58	0.29	0.33	0.74	19.12	23.38	3.17	9.22
Light purple	20.93	5.43	5.67	7.41	29.78	55.76	8.20	20.54
Dark purple	20.93	16.57	4.00	5.93	0.29	0	5.56	5.96
Brown or brown-purple	9.30	1.14	0	7.41	3.65	0	13.49	4.61
Not determined	—	—	—	—	9.34	0.36	8.99	4.31

† Philippines (PH), Vietnam (VN), Thailand (TH), Malaysia (MY), Indonesia (ID), Papua New Guinea (PG) and Vanuatu (VU).

Maturity period

Most accessions (67.2%) reach maturity early (in <8 months) compared with 29.2% of late-maturing cultivars (>8 months). Maturity period of wild forms is rather difficult to assess accurately because leaf regeneration is very fast and the growth is almost perennial.

Growth habit and plant height

More than 94% of the accessions are erect or semi-erect types and only 3.69% are prostrate or semi-prostrate types. The majority of the plants described (67.53%) have a medium height between 50 and 100 cm.

TABLE 4. Geographical distribution of corm traits, presented as percentages of accessions

Country: †	PH	VN	TH	MY	ID	PG	VU	Total
No. of accessions	172	350	300	135	685	278	378	2298
Taste quality of corms								
Not edible	7.56	2.29	19.00	0	8.91	0	16.40	8.75
Poor quality	13.37	4.29	2.33	64.44	7.01	0.72	3.44	8.49
Acceptable	66.86	30.57	49.00	0	25.99	84.53	29.89	38.95
Good	5.81	48.86	29.33	20.74	53.14	1.44	32.54	34.29
Very good	5.23	11.43	0.33	5.93	1.46	2.88	2.91	3.79
Excellent	1.16	2.29	0	0	0	1.80	3.97	1.30
Not determined	—	0.29	—	8.98	3.50	8.63	10.85	4.44
Corm weight								
Very small (<0.25 kg)	27.33	54.00	0	5.93	0	0	9.79	12.23
Small (0.25–0.5 kg)	61.05	42.29	21.67	0	16.50	0.36	17.99	21.76
Medium (0.5–2 kg)	11.63	3.71	78.33	42.22	76.50	98.92	59.79	58.75
Large (2–4 kg)	0	0	0	0.74	3.07	0	1.59	1.22
Very large (>4 kg)	0	0	0	42.22	0.29	0.36	0	2.61
Not determined	—	—	—	8.89	3.65	0.36	10.85	3.44
Corm shape								
Unbranched	99.42	50.58	18.66	91.11	87.01	86.69	70.37	70.94
Branched	0	49.71	61.33	0	9.20	2.52	18.78	20.8
Extremely elongated	0	1.14	0	0	0	2.16	0	0.43
Flat	0.58	4.57	0.33	0	0	0	0	0.78
Clustered	0	0	19.67	0	0	0	0	2.57
Not determined	—	—	—	8.89	3.80	8.63	10.85	4.48
Corm flesh colour								
White	47.67	96.00	3.67	62.96	39.71	16.91	42.06	43.17
Yellow	12.21	2.29	25.67	14.07	54.01	5.04	8.99	23.63
Orange	9.88	0.29	0	14.07	0	38.85	1.32	6.53
Pink	9.30	0	1.33	0	1.75	15.11	16.14	5.87
Red	0	0	4.67	0	0	10.79	0	1.91

Country: †	PH	VN	TH	MY	ID	PG	VU	Total
Red-purple	0	0	4.00	0	0.15	1.08	5.82	1.65
Purple	0.58	0.57	60.67	0	0.88	1.44	10.85	10.27
Colour not uniform	20.35	0.57	0	0	0	2.16	3.97	2.52
Not determined	—	0.29	—	8.98	3.50	8.63	10.85	4.44

† Philippines (PH), Vietnam (VN), Thailand (TH), Malaysia (MY), Indonesia (ID), Papua New Guinea (PG) and Vanuatu (VU).

Position and shape of lamina

Leaf laminae can be from 30 to more than 80 cm long and from 20 to more than 50 cm wide. Leaf petioles are stout, clasping at the base. Petiole length varies depending on genotype; from less than 30 cm to more than 1.5 m. Leaf size is strongly influenced by the environment. Maximal dimensions of taro leaves are usually associated with the beginning of flowering. Most accessions (77.68%) had a semi-erect position of the leaf lamina and more than 78.33% had an undulate leaf lamina margin.

Petiole and leaf colours

The colour of leaves is genetically controlled and represents one of the most useful traits for describing genotypes. It varies from a whitish yellow to a very dark purple, depending on the genotype. It can be uniform or show variations (lines, spots or blotches of different pigmentations). Leaf petioles and leaf laminae do not always have the same colour. The basic colour of the petiole is extremely variable (Table 3) and tremendous variation of the patterns (lines, stripes, blotches, dots, etc.) and secondary colour of the petioles is also observed (data not shown).

Corm quality, weight, shape and flesh colour

Corm quality of the majority of the accessions (73.24%) described was acceptable to good; only 8.75% are considered inedible and 5.09% were very good or excellent. Surprisingly, the majority of wild accessions are also edible. It is therefore possible that they are in fact escapees from past cultivation. Most accessions (58.75%) produce corms and cormels that yield between 0.5 and 2 kg of marketable weight. Unbranched corms present the most variable shapes (round, dumb-bell, conical, elliptical, and cylindrical) and 70.94% of the accessions produce unbranched corms. More than 20% of the accessions have a branched corm, an undesirable trait. The pigmentation observed in corm cross-section is white (43.17%), yellow (23.63%), orange to pink, red and purple. In addition, there can be combinations of white with purple or red blotches or white parenchyma with darker pigmented fibres (2.52% of accessions). The colour of the root system is usually white or it may contain anthocyanins. Some genotypes can have both pigmented and not pigmented roots.

Tolerance to *Phytophthora colocasiae*

This pathogen species, the causal agent of Taro Leaf Blight, thought to have originated in Southern China, was introduced to Papua New Guinea during the Second World War, but has not reached Vanuatu yet, although it is present in the Solomons, Samoa and Hawaii. Table 5 presents the results

obtained from the characterization of accessions in each country. Overall, 45.82% of the accessions are susceptible to leaf blight and 20.76% are tolerant. Resistant (9.96%) and immune accessions (6.44%) are, in most cases, wild forms.

Data analysis

The qualitative data obtained from each country's database were analyzed using, first, UPGMA and the simple matching coefficient (SM). The resulting dendrograms were found to be misleading because many of the characters, although useful for differentiating morphotypes (i.e. colours, shapes), have limited informative value on the genetic structure of the germplasm. A second analysis was conducted on the same data matrices using, after transformation of the data, the DICE coefficient (presence or absence of a particular variable for each character scored). The resulting dendrograms were found to be more informative. However, quite a few clusters were also found to aggregate cultivars that appeared genetically distant (i.e. dasheen cultivars clustered with eddoe cultivars, flooded cultivars with rain-fed cultivars, with and without stolons, etc.).

TABLE 5. Geographical distribution of the tolerance to leaf blight caused by *Phytophthora colocasiae*, presented as percentages of accessions

Country: †	PH	VN	TH	MY	ID	PG	VU‡	Total
No. of accessions	172	350	300	135	685	278	378	2298
Very susceptible	4.07	0	0	0	0.15	0	—	0.35
Susceptible	21.51	1.71	94.67	0	65.40	100	—	45.82
Tolerant	73.84	34.86	0.33	4.44	32.26	0	—	20.76
Resistant	0.58	41.14	5.00	43.70	1.46	0	—	9.96
Immune	0	22.29	0	51.85	0	0	—	6.44
Not determined	—	—	—	—	0.73	—	100	16.67

† Philippines (PH), Vietnam (VN), Thailand (TH), Malaysia (MY), Indonesia (ID), Papua New Guinea (PG) and Vanuatu (VU).
‡The taro germplasm collection of Vanuatu could not be evaluated because of the absence of leaf blight.

Isozyme variation

Isozymes were analyzed to study the extent of allelic diversity existing within and between countries. Six enzyme systems (MDH, PGI, ICD, PGD, ME, SKDH) were successfully revealed and 2081 accessions were fully characterized (Table 6). Numerous electromorphs and distinct zymograms were identified for each enzyme system.

Overall, 319 distinct zymotypes exist in the region. With 194 distinct zymotypes, Indonesia appears to host significant genetic diversity. Although the number of zymotypes is lower in Malaysia (57 morphotypes for 30 zymotypes), Thailand (322 morphotypes for 64 zymotypes) and Vietnam (210 morphotypes for 74 zymotypes), these three countries also host significant allelic diversity. In

comparison, the countries located in the Pacific part of this geographical region (the Philippines, Papua New Guinea and Vanuatu) appear to contain limited allelic diversity.

Different zymotypes can be considered as different genotypes assessed at the isozyme level. Therefore, it is possible to appreciate the extent of genetic variation existing between and within countries using a simple variability index, that is the number of distinct zymotypes divided by the number of distinct morphotypes present in each country. This index ranges from 0.52 in Malaysia to 0.05 in Vanuatu (Table 6).

Table 7 presents the geographical distribution of accessions exhibiting the 21 most frequent zymotypes over a total of 319 distinct zymotypes. In Vanuatu, for example, only three zymotypes assemble 100% of the accessions electrophoresed; in Papua New Guinea, 8 zymotypes of 82% of the accessions and in the Philippines, 5 zymotypes of 87% of the accessions. Surprisingly, throughout this vast region, only 6 zymotypes represent more than 51% of the total number of accessions electrophoresed and only 21 zymotypes represent more than two- thirds (70%) of the total number of accessions. Furthermore, the genetic variation existing between the 6 major zymotypes is rather limited. This indicates that the genetic base of most cultivars existing in these seven countries is very narrow.

TABLE 6. Isozyme variation in Southeast Asia and Oceania

Country:	ID	MY	TH	VN	PH	PG	VU	Total
No. morphotypes†	688	57	322	210	198	452	154	2081
No. zymotypes‡	194	30	64	74	10	51	8	319
Variability index§	0.28	0.52	0.20	0.35	0.05	0.11	0.05	0.15
Unique zymotypes¶	138	7	32	39	4	39	0	
% unique††	72	23	50	53	40	75	0	
MDH variants‡‡	16	3	5	5	3	1	1	16
PGI	8	3	4	3	1	3	1	8
ICD	5	3	4	4	2	4	1	6
PGD	17	4	9	10	2	9	1	19
ME	8	6	6	7	2	3	2	10
SKDH	6	5	3	4	3	5	3	7
Total variants	61	24	31	33	13	25	9	66

†Number of different morphotypes electrophoresed (no. of accessions morphologically distinct).
‡Number of different zymotypes identified in the collection.
§Number of distinct zymotypes divided by no. of distinct morphotypes.
¶Number of zymotypes unique to a particular country: Philippines (PH), Vietnam (VN), Thailand (TH), Malaysia (MY), Indonesia (ID), Papua New Guinea (PG) and Vanuatu (VU).
†† Percentage of unique zymotypes to total no. of zymotypes in a particular country.
‡‡Number of different zymograms observed for a particular enzyme system.

Numerous zymotypes are unique to particular countries, but in many cases these are attributed to local wild forms. The 21 most frequent zymotypes are common to the most popular cultivars found in various countries, indicating that cultivars were probably extensively distributed as clones throughout the region in the past. However, there is also a significant geographical bias. For example, zymotypes AAAAAA, AAAAAB, AAAABB and AAAABA are predominantly from Melanesia. In fact, zymotype AAAABB exists only in Papua New Guinea and nowhere else. More than 75% of the zymotypes existing in Papua New Guinea are unique to this country, where some cultivars exhibit zymotypes identical to local wild forms (AAAAAA). In Indonesia, 72% of the zymotypes are also unique to this country but again, these zymotypes are associated with local wild forms or rare and unpopular cultivars.

Unlike Southeast Asia, there are no variants for malate dehydrogenase (MDH) in Melanesia. For the five other enzyme systems, many zymograms are also unique to Papua New Guinea. Multivariate analysis of 319 zymotypes conducted on the binary data matrix of the coded electromorphs (Figure 1), reveals two distinct groups. This grouping was confirmed using DICE coefficient and UPGMA hierarchical classification but the dendrogram is not presented here. Each of these two groups corresponds to the zymotypes unique to the two distinct geographical regions, Southeast Asia and Melanesia.

Discussion

Although taro cultivars are always vegetatively propagated, the present study has demonstrated that morphological variability is extremely high in Southeast Asia and Oceania. Several thousands of cultivars probably exist in this region and the diversity presently maintained in national germplasm collections indicates that it might be difficult to characterize all possible morphological variations. However, the use of 23 standardized morphological descriptors has shown that these are sufficiently discriminating to describe most morphotypes. There is a striking difference between the great polymorphism of cultivars and the limited morphological variation observed within wild forms. Wild taros are well adapted to their environments and they do not appear to be endangered in any of the seven countries surveyed. In most cases, they are thriving components of the existing flora.

Isozymes have been quite useful to assess the extent of allelic diversity existing in *C. esculenta*. In 1991, Lebot and Aradhya studied the isozyme variation in 1417 cultivars and wild forms of taro collected mostly in the Pacific (Micronesia, Polynesia and Melanesia) and compared them with a few Asian accessions. Their results showed greater variation in Asia than in Oceania, with

TABLE 7. Geographical distribution of accessions exhibiting the 21 most frequent zymotypes

Enzyme system†							Country‡							
MDH	PGI	ICD	PGD	ME	SKDH		ID	MY	TH	VN	PH	PG	VU	Total
A	A	A	A	A	A		15	–	7	2	153	157	136	470
A	A	C	A	A	C		43	3	109	18	14	1	–	188
A	A	C	A	A	A		65	4	32	23	5	2	–	131
A	A	A	A	A	B		9	–	2	–	–	101	6	118
A	A	A	A	A	C		33	–	8	11	19	33	12	116
A	A	D	A	A	C		15	2	26	7	–	–	–	50
E	A	C	A	A	A		44	3	–	–	–	–	–	47
A	A	D	A	A	A		31	–	6	9	–	–	–	46
A	A	A	A	B	B		–	–	–	–	–	42	–	42
A	A	C	J	A	C		19	1	14	2	–	–	–	36
A	A	A	A	B	A		1	–	–	–	–	30	–	31
F	A	D	A	A	A		25	–	–	4	–	–	–	29
E	A	C	A	A	C		19	5	3	–	–	–	–	27
A	A	C	A	A	B		8	5	5	1	2	4	–	25
A	A	C	J	A	A		18	–	2	–	–	–	–	20
A	A	C	A	F	C		10	–	7	1	–	–	–	18
A	A	C	P	A	A		2	6	2	8	–	–	–	18
E	A	D	A	A	C		11	1	6	–	–	–	–	18
A	A	C	P	A	C		2	5	3	6	–	–	–	16
A	A	D	A	F	C		5	–	8	1	–	–	–	14
E	A	C	A	A	B		12	2	–	–	–	–	–	14
No. of accessions						387	37	240	93	193	–	370	154	1474
Percentage						56	65	75	44	97	–	82	100	70
Total number of zymotypes						20	11	16	13	5	–	8	3	21

†Malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), isocitrate dehydrogenase (ICD), 6-phosphogluconate dehydrogenase (PGD), mallic enzyme (ME) and shikimic dehydrogenase (SKDH)
‡Indonesia (ID), Malaysia (MY), Thailand (TH), Vietnam (VN), Philippines (PH), Papua New Guinea (PG), and Vanuatu (VU).

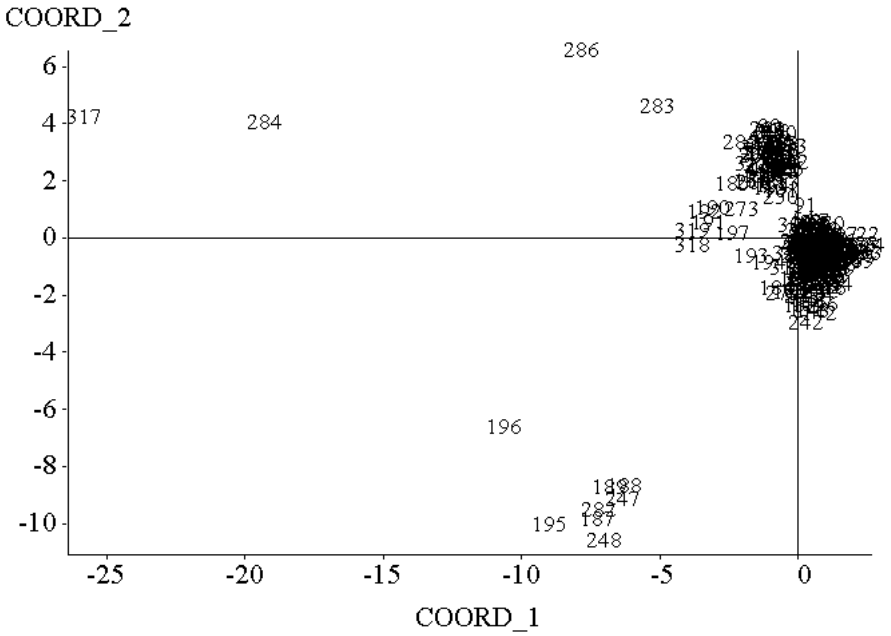

Figure 1. Distribution of 319 taro zymotypes in plane of a principal components analysis of isozyme variation in 2081 cultivars originating from seven countries of Southeast Asia and Oceania. Two major zymotypic groups are identified by cluster analysis using DICE coefficient and UPGMA on the matrix OTUs x electromorphs. The two groups correspond to Melanesian and Southeast Asian genepools. The distant accessions are wild forms.

Indonesia being the area of greatest diversity. Multivariate analyses of their isozyme data indicated that the majority of the Indonesian cultivars were different from the cultivars existing in the Philippines and the Pacific. They also found that zymotype 1 (AAAAAA) was the most widespread and cultivated genotype in this large geographic region, from Hawaii to Papua New Guinea. The present isozyme survey not only confirms their findings, but also provides more comprehensive information on the diversity existing within and between seven Southeast Asian countries and two Melanesian countries.

Although cultivars are morphologically very variable, the results obtained indicate that they share a narrow genetic base with limited allelic diversity. It is probable that sexual recombinations among cultivars are very rare. Human selection pressure has obviously generated numerous morphotypes and the majority of cultivars are most likely clones of a common source. Because somatic mutations occur constantly and are retained, cultivars that appear to be morphologically different may be genetically similar. Very few genes are probably involved in anthocyanin pigmentation that results in various petiole and corm colours.

Although morphologically similar, wild taros contain most of the allelic diversity revealed with six enzyme systems. Rare and poorly improved cultivars also show significant isozyme variation. The most widespread cultivars, on the other hand, exhibit limited isozyme variation, and many cultivars representing distinct morphotypes appear to have identical zymotypes. It is likely that TANSAO germplasm collections might contain accessions that, although morphologically similar, have different genetic origins and vice versa, share the same genetic background. Identical morphotypes have different names in different collections and countries owing to the numerous vernacular languages found in the region.

The great genetic diversity of Southeast Asian cultivars may reflect the lack of improvement made to this crop. Taro cultivation is still practised in the traditional way, and the cultivars indeed differ from one another. In this region, several morphotypes often exhibit several wild characters including frequent flowering, stolon production or poor eating quality. Because taro is not a staple, it is most likely that human selection pressure was lower than in Melanesia where the crop is of utmost importance for the local populations. Southeast Asia is most likely the area of origin of many cultivars that have co-evolved with one of their most serious pathogens, *Phytophthora colocasiae*. In Melanesia, cultivars were, until recently (circa 1945) selected in an environment free of *P. colocasiae* allowing human selection to operate on various and diverse morphological traits. Now that the pathogen has been introduced into the Pacific, it will be necessary to broaden the genetic base of germplasm collections.

Structuring the great diversity existing in taro is necessary to optimize the use of germplasm by breeders. Our survey could not confirm that eddoe types correspond to var. *antiquorum* and that dasheen types correspond to var. *esculenta*. It appears that floral attributes are unreliable to classify accessions, because variation within each variety is so important that variation between the two becomes insignificant and cannot be used for systematic classification. Rationalization of the collections is, however, essential as soon as there are more than 100 accessions. It is consequently necessary to propose a common classification system that could be adopted by breeders. Accessions need to be systematically grouped so that duplicates can be removed and so that core subsets can be established based on accurate data. These core subsets allow breeders to organize the diversity existing in their germplasm collection and to have direct use of it.

Cluster analyses conducted directly on the data matrices composed of the 23 standardized descriptors used for our study did not produce meaningful clusters and useful dendrograms. An alternative hierarchical approach was adopted and a branching method based on the use of five major characters produces meaningful groups. These characters are, in order of decreasing importance: (1) germplasm type (wild or cultivated); (2) botanical variety (dasheen, eddoe or intermediate); (3) adaptation (flooded or rain-fed); (4) stolons (presence or absence); (5) maturity (early or late). This approach is based on assumptions about the structure of taro genepools. It does not take into consideration the geographic origin of the accessions, because it is assumed that many genotypes were dispersed as clones in the past. It is, however, assumed that these five major characters allow predictions of the genetic diversity existing in national germplasm collections.

In the present study, 2298 accessions were described morphologically; however, only 2232 accessions had no missing data and were therefore used to develop a classification. Our stratification

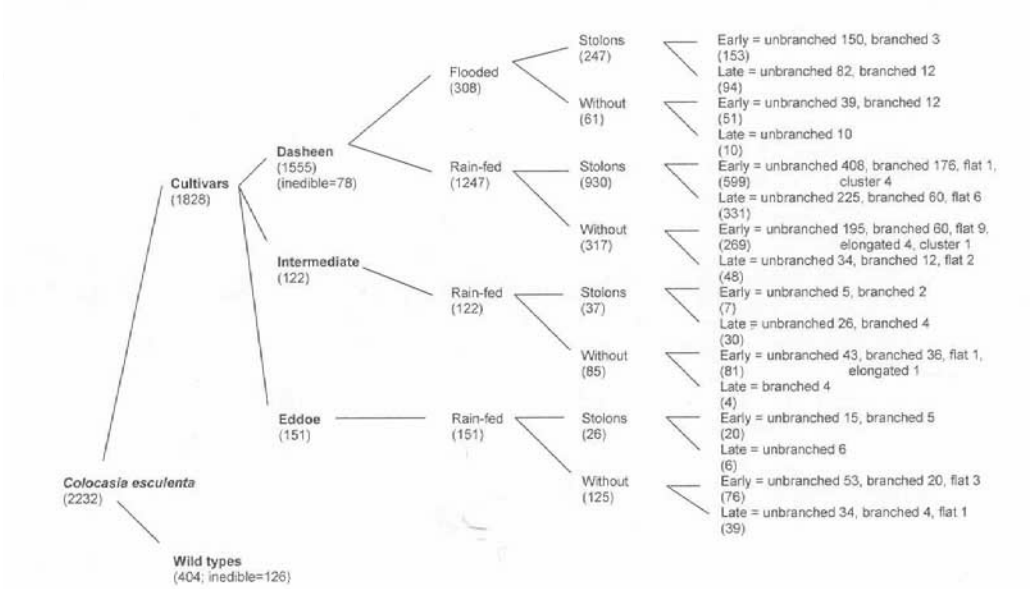

Figure 2. Branching method used to classify accessions in taro germplasm collections.

allows 16 groups to be differentiated and permits the classification of 1828 cultivars. All wild forms (404 accessions) are classified in only one group (Figure 2). The use of a sixth character—corm shape—allows further groupings. This classification, used at the national and regional levels, can contribute to the rationalization of germplasm collections. Once these groups are identified, it is relevant to use UPGMA methods within groups to identify clusters of related morphotypes.

Isozyme data cannot be used to stratify accessions because the data do not correspond to the morphological variation that is useful to breeders. Data can be used, however, to assess the extent of allelic diversity existing within morphological groups and to assist in the sampling strategy that will result in setting up core subsets. To choose potential parents, it is recommended to avoid the selection of individuals that exhibit identical zymotypes. The combination of the two approaches, using first the morphological data and second the isozyme data, should contribute to a reliable stratification.

Conclusions

This study has demonstrated that, although remarkable morphological variation is distributed throughout the region, the extent of allelic diversity varies greatly from one country to another. This might be the result of the occurrence of sexual recombinations in some areas and not in others. It could be the consequence of more active pollinators, a better distribution of the wild forms and/or the fact that cultivars, being less improved, have accumulated fewer mutations inhibiting their sexuality. Zymotypes can be considered as good indicators of genetic distances existing within

and between country collections. Chances of getting significant variation by crossing identical zymotypes are probably quite limited.

The variability of cultivars is probably the result of some degree of genetic differentiation among wild taros due to geographical and insular isolation of populations within Southeast Asia and Melanesia. Given the isozyme data, it appears that two distinct genepools are differentiated and may represent the result of two distinct domestication processes. In Melanesia, taro is a staple and cultivars have been improved to satisfy a diversity of uses and needs. It can be said that improved types (i.e. compact corms, no stolons, few suckers, high yields) are represented by many accessions, but that, until recently, most were selected in an environment free of alien pathogens. In Asia cultivars are probably less improved, but geneflows have been quite frequent, as demonstrated by the allelic diversity observed. In this area, most cultivars have also co-evolved with their most serious pathogen *P. colocasiae*.

The hierarchical choice applied to major morphological characters is made according to the ideotypes that breeders want to develop. For example, dasheen types are always improved as dasheen cultivars, because it is what farmers and markets request and the same can be said for eddoe types. The numerous intermediate types found in the collections are probably the result of natural crosses and all of them were found to be uninteresting morphotypes. Dasheen and eddoe correspond to two different products and, considering the variation available, there is no reason why breeders should want to intercross the two types. The same is true for rain-fed and flooded types. There is no reason why breeders would cross these two distinct types considering the variation available within each group. The presence or absence of stolons is a clear indication of the genetic load present in the accessions. Taro being highly heterozygous, undesirable characters might segregate profusely in crosses, implicating accessions with stolons. The maturity period being a polygenic trait that is difficult to improve, it seems reasonable to consider it as well for classifying accessions.

The proposed stratification presents a useful classification for breeders. This is a first attempt to develop a system relevant to taro genetic diversity and enhance its direct use. It allows systematic groupings but is not definitive. It will be improved when more characterization data are available. It will also be evaluated at the DNA level.

Acknowledgements

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2 [Editor's note: World production data for taro and other root and tuber crops can be compared by selecting: (1) 'Production' then 'Crops' in main menu, then (2) 'World+' in the country list, 'Roots and Tubers, Total>' in the item list, 'Production Quantity' in the element list, and '2000' in the year list, then (3) clicking on the button 'show data'.]