Collecting and evaluating taro *(Colocasia esculenta)* for isozyme variation

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Introduction

Taro (*Colocasia esculenta* (L.) Schott.) is the major staple food for both the Melanesian and Polynesian peoples. Breeding programmes have been initiated in the Solomons, Fiji, Western Samoa and Hawaii to provide farmers with improved cultivars.

Several authors have attempted to study the genetic variation in this crop (Kuruvilla and Singh, 1981; Tanimoto and Matsumoto, 1986; Coates *et al.*, 1988). Their results suggested that x = 14 is the base number for *C. esculenta*, and that diploids and triploids exist. Apart from cytological investigations, little is known of the genetic variation existing among taro cultivars.

Very few isozyme studies have been conducted with *C. esculenta*. In a recent study (Lebot and Aradhya, 1991), we attempted to characterize taro germplasm and to assess the extent of its genetic variation using isozymes. In the present paper, isozyme variation and its relevance to future taro breeding programmes are discussed.

Methods

The accessions used in our study came from the following germplasm collections:

- Hawaii: University of Hawaii Kauai Branch Station, Harold Lyon Arboreteum, Waimea Botanical Garden, and Hawaiian Studies Institute Ethnobotanical Garden.
- French Polynesia: Station de Papara, Tahiti.
- The South Pacific: South Pacific Commission (SPC) tissue culture collection in Suva, Fiji, and Koronivia Research Station, Department of Agriculture in Nausori, Fiji.
- New Caledonia: Institut de Recherches en Agronomie Tropicale, Station de Poindimié.
- Vanuatu: Agriculture Department, Santo.
- The Solomons: Dodo Creek Research Station, Guadalcanal.
- Papua New Guinea: Department of Primary Industries, Bubia Research Station, Morobe Province.
- Indonesia: Indonesian Institute of Sciences, Bogor, Java.
- Japan: National Research Institute of Vegetables, Ornamental Plants and Tea, Anô, Mie.
- The Philippines: Rootcrop Research and Training Center, Baybay, Leyte.

Accessions from India, Thailand and Malaysia were collected in market places.

Young suckers or tubers collected from New Caledonia, Tahiti, Pohnpei, the Philippines, Indonesia, Malaysia, Thailand, Japan, India and Vanuatu and tissue cultured accessions obtained from the SPC laboratory based in Suva, Fiji were introduced to the Horticulture Department of the University of Hawaii, Honolulu.

For taro accessions originating in Papua New Guinea and the Solomons, leaves were collected in the field and preserved in liquid nitrogen in order to avoid transportation of propagules thought to be infected with the Alomae and Bobone virus complex. Portions of young leaves were rolled and sealed in disposable microcentrifuge tubes, immersed in a liquid nitrogen shipping container in the field, and transported to the University of Hawaii for isozyme electrophoresis.

Twenty enzyme systems were assayed using a variety of buffer systems, but only histidine citrate, pH. 6.5, was found to be useful. Leaf extracts were obtained using modified Bousquet's buffer (Bousquet *et al.*, 1987; Lebot *et al.*, 1991) and loaded on to starch gels (12.5 %).

Samples were electrophoresed at 4°C. Running conditions were 15V/cm and 40-50 mA for six hours. After electrophoresis, the gels were sliced horizontally and stained for malate dehydrogenase (MDH), phosphoglucose isomerase (PGI), isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6-PGD), malic enzyme (ME), shikimic dehydrogenase (SkDH) and alcohol dehydrogenase (ADH).

Because genetic interpretation of zymograms was complex, the electrophoretic bands (electromorphs) were used as isozymic descriptors. Each electromorph was considered as a character, with presence and absence being the only two possible modalities. A total of 56 distinct electromorphs was used as isozymic descriptors. Isozyme variation existing within country collections was estimated by calculating the percentage of electromorphs not common to all cultivars (percentage of dissimilarity = 100 minus percentage of identical electromorphs).

Results

More than 2000 accessions were electrophoresed during our study (see Lebot and Aradhya, 1991). The number of cultivars studied per country of origin is indicated in Table 1. A total of 620 cultivars have been collected throughout the Pacific and in several Southeast Asian countries. They have been introduced to the germplasm collection of the University of Hawaii, in Kauai for field evaluation. The University germplasm collection consists of 702 cultivars at present.

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Table 1. Geographical origins of cultivars electrophoresed

Origin	Cultivars	Zymotypes	Dissimilarity[1]
Polynesia			
Hawaii	82	1	0
French Polynesia	35	3	5.7
Easter Island	9	1	0
The Cooks	3	1	0
Niue	5	1	0
Western and American Samoas	43	1	0
New Zealand	6	1	0
Micronesia			
Pohnpei	11	3	5.7
Melanesia			
Papua New Guinea	452	70	53
The Solomons	262	43	51
New Caledonia	82	18	51
Vanuatu	154	8	15.1
Fiji	47	3	5.7
Indonesia	52	38	80
Malaysia	3	3	64
Thailand	1	1	0
India	5	4	66
The Philippines	146	3	5.4
Japan	18	7	73
China	1	1	0

[1] Percent of dissimilarity = 100 minus percent of identical electromorphs.

Polynesia

Only three zymotypes were identified for this vast geographic area (Hawaii, French Polynesia, the Samoas, the Cooks, Niue, Easter Island and New Zealand). All three were variants of SkDH and 91% of the cultivars exhibited the same zymotype. Isozymic variation was very low, with only 5.7% of electromorph dissimilarity.

In Hawaii, 343 accessions, representing 82 cultivars, and 145 F1 hybrids were identical for all the enzyme systems studied. Similar observations were made for 145 accessions originating from the rest of Polynesia (the Marquesas, the Gambiers, the Australs and Easter Island, and from the Samoas, the Cooks, Niue and New Zealand).

The isozymic variation is nil in Hawaii and in most islands of Polynesia, suggesting that the genetic base of this crop is extremely narrow. Several factors could be responsible for this. In Polynesia cultivars rarely flower naturally, and active insect pollinators are not observed. The variegated forms frequently found in farmers' fields could be a result of somatic mutations. The majority of the Polynesian cultivars are probably clones from a common source. The fact that all the Polynesian cultivars are identical with respect to the enzyme systems studied indicates that the variation observed in morphological characters is probably controlled by a few genes that are not linked with loci-controlling isozyme markers. The genetic base of taro in Polynesia is so narrow that this germplasm is very vulnerable to future pests and disease epidemics.

Micronesia

Three zymotypes were identified in Pohnpei, all variants of SkDH. Isozyme variation was very low, with cultivars exhibiting only 5.7% electromorph dissimilarity (Table 1). Variation may be a result of recent introductions from other geographic areas.

Melanesia

Papua New Guinea has the most important germplasm collection, with 452 cultivars (Table 1). A total of 70 zymotypes was identified, and several are unique to that country. Although the collections from the Solomons and New Caledonia are smaller, their isozymic variation is comparable to Papua New Guinea, with 51, 51 and 53% dissimilarity respectively (Table 1). In Papua New Guinea and the Solomons several accessions were collected from wild populations established along streams, and their zymotypes were identical to those exhibited by some cultivars. The great variation observed in New Caledonia could be the result of recent introductions from Southeast Asia. In Melanesia, Vanuatu and Fiji show low isozymic variation, with cultivars exhibiting 15.1% and 5.7% electromorph dissimilarity respectively (Table 1).

Melanesian germplasm is much more diverse than Polynesian. Many cultivars flower naturally, insect pollinators are very active and hybridization occurs. As a result, in Papua New Guinea and the Solomons naturally set seed is not uncommon in farmers' fields, and it is almost certain that cross-pollination has been and still is occurring.

In the Solomons, field screening for Alomae and Bobone virus complex diseases is very difficult. Unfortunately, no significant differences could be identified between zymotypes of tolerant and susceptible cultivars, making isozymes unsuitable for screening populations for reactions to these diseases.

Indonesia

In Java 48 cultivars were collected and in Irian Jaya (Jayapura), four. Isozymic variation was very high with 38 distinct zymotypes and 80% dissimilarity. Although malate dehydrogenase (MDH) showed no variation in Polynesia, Micronesia and Melanesia, in Java alone seven different zymograms were observed for this enzyme system. Cultivars collected around Jayapura in Irian Jaya exhibited zymotypes similar to those from Java and were thought to be recent introductions resulting from Javanese migrations.

The great diversity of the Indonesian cultivars may reflect the lack of selection and improvement made to this crop. Morphotypes observed in the germplasm collection at Bogor, Java, exhibited several wild characters including frequent flowering and significant stolon production.

The high proportion of unique zymotypes in Melanesia and Indonesia may be caused by a combination of factors. Isozymic variation could be the result of intra- or interlocus heterodimer formations. These could be genetic recombinations of existing alleles or the product of the introduction of new alleles due to sexual reproduction. New

alleles may also have resulted from mutations of existing ones.

The Philippines

The Rootcrop Research Centre at Baybay, Leyte holds 146 cultivars, collected throughout the country, in its germplasm collection. All cultivars were analyzed. They exhibited very little isozyme variation. Only three zymotypes were identified and all were variants at the IDH loci. Dissimilarity between electromorphs was 5.35%.

Japan

Among the 18 cultivars received from Japan, isozymic variation was high, with seven distinct zymotypes and 73% dissimilarity. All the cultivars originating from the Ogasawara Islands exhibited zymotype 1, distributed throughout the Pacific.

Isozyme data revealed seven zymotypes. Because sexual propagation of taro does not occur under natural conditions in Japan, variations among cultivars may be due to somatic mutations and introductions. Surprisingly, the most widespread zymotype in the Pacific is also found in Japan and the Ogasawara Islands. Some Japanese cultivars might have been introduced from Indonesia and the Pacific as a result of the Japanese occupation during the second world war.

The data constituted by the matrix zymotypes x electromorphs were subjected to multivariate analysis (Lebot and Aradhya, 1991). The information gained was used to identify four groups in the distribution of zymotypes on planes (axes 1 and 2) of the principal components analysis. Group 1 contained 95 zymotypes representing the Oceanian cultivars distributed from the Philippines and Papua New Guinea in the west to Hawaii in the east. Oceanian zymotypes appeared to be very closely related. Cluster analysis indicated that the majority of the Indonesian cultivars were clustering together and were significantly different from Oceanian cultivars. Group 2 comprised 30 Indonesian zymotypes and exhibited greater variation. Group 3 included three Indonesian zymotypes and group 4, five zymotypes from Japan and Indonesia. However, these last two zymotypic groups contained such a small number of cultivars (nine) that it is unlikely that they would still exist after a broader geographical sampling.

Conclusions

Identical cultivars may have different names in different collections and countries. This is especially true in Melanesia where numerous vernacular languages increase the chances of synonymy and duplication. Isozymes are often used as genetic markers to fingerprint accessions and identify duplicates. Our results show, however, that isozymes cannot be used to identify taro cultivars. The fingerprinting performance of this technique depends upon the number of polymorphic loci that can be identified.

The fact that, despite the number of systems used, taro cultivars exhibit poor isozyme variation in the Pacific indicates a common, narrow genetic base. Since taro is propagated vegetatively, each clone is isolated from the others and as there is no exchange of genes the drift and mutation are independent and result in an increased genetic divergence (Kuruvilla and Singh, 1981).

Taro is monoecious, with the female flowers more receptive one day before pollen is shed on the same inflorescence. The protogynous behaviour of taro is such that it promotes cross-pollination, but pollination between different inflorescences of the same clone is probably very frequent. Genetically this is self-fertilization even though different plants are involved. However, there is also some receptivity two days before pollen-shed and sometimes on the same day. Therefore, self-fertilization is possible within the same inflorescence, when rain washes pollen down from the upper part of the inflorescence.

Our results confirm the observation made by Tanimoto and Matsumoto (1986) that diploids and triploids cannot be separated by their zymotypes. This is not surprising and would indicate that taro triploids are auto-polyploids. The same set of chromosomes is replicated three times, and identical genes synthesize proteins with identical charges which produce identical zymograms.

No correlations could be found between morphological characters and isozyme banding patterns. The data obtained suggest that in *C. esculenta*, when morphological variation is the product of somatic mutation, it is not possible to correlate it with isozyme variation. Morphological character and isozyme production are controlled by independent genes. On the other hand, we have shown that isozyme variation is observed in areas where taro reproduces sexually and when new cultivars are selected from seedlings presenting genetic recombinations.

This work has contributed to our understanding of taro genetics. Isozymes appear to be useful indicators of genetic distances existing among cultivars in germplasm collections. There is greater variation in Asia than in Oceania, and Indonesia appears as the area of greatest genetic diversity for taro. It may also be an area of origin, although a screening of Indian germplasm would allow a comparison of the existing zymotypes as well as their variation. It is important to collect and evaluate more comprehensive germplasm collections from these two geographic areas.

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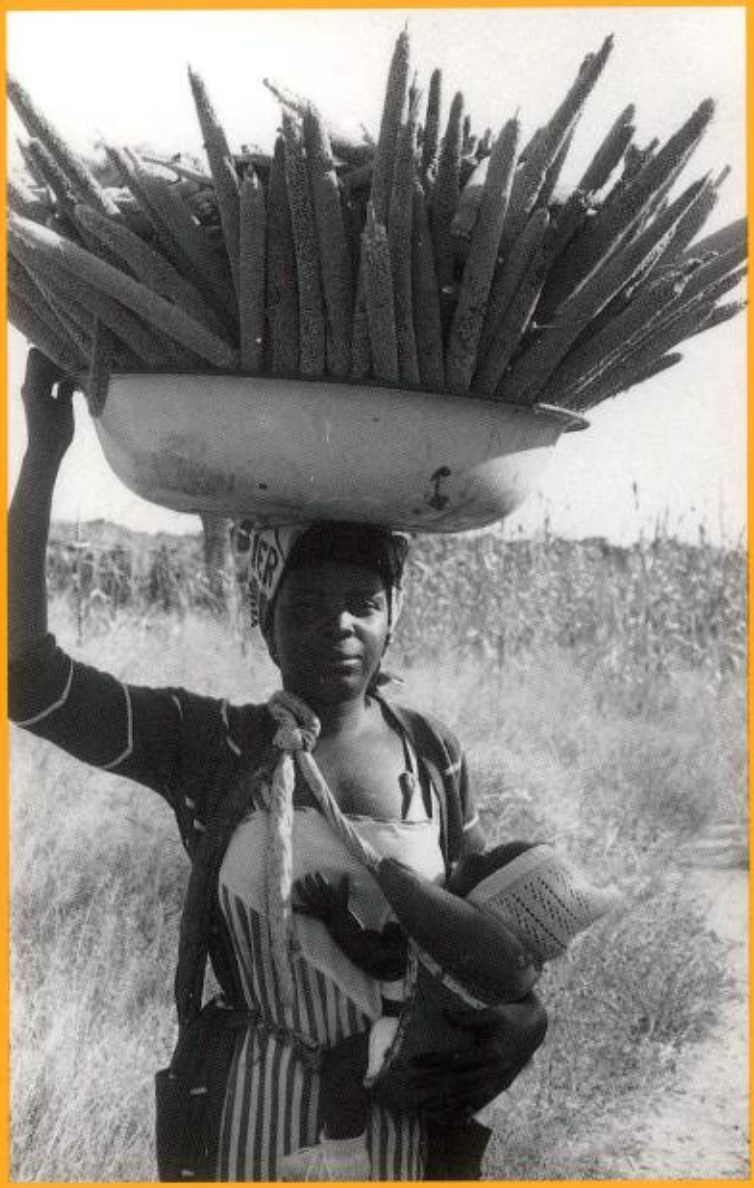

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