Bioversity International

The Global Diversity of Taro

Ethnobotany and Conservation

V. Ramanatha Rao,
P. J. Matthews,
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Bioversity International is an independent international scientific organization that seeks to improve the well-being of present and future generations of people by enhancing conservation and the deployment of agricultural biodiversity on farms and in forests. It is one of 15 centres supported by the Consultative Group on International Agricultural Research (CGIAR), an association of public and private members who support efforts to mobilize cutting-edge science to reduce hunger and poverty, improve human nutrition and health, and protect the environment. Bioversity has its headquarters in Maccarese, near Rome, Italy, with offices in more than 20 other countries worldwide. The organization operates through four programmes: Diversity for Livelihoods, Understanding and Managing Biodiversity, Global Partnerships, and Commodities for Livelihoods.

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Cover illustration: Transporting wild taro leaves used for pig fodder, northern Myanmar, October 2006 (Photo: P. J. Matthews).

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Via dei Tre Denari, 472/a
00057 Maccarese
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Bioversity International is the operating name of the International Plant Genetic Resources Institute (IPGRI).

This book is dedicated to the memory
of Eric Quarcoo and Nurzuhairawaty,
who sadly passed away in 2000 and 2004 respectively.
We lament the loss of our colleagues, but are also inspired
and challenged by the great contributions they made
to taro research in Africa and the Pacific.

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Foreword

Taro, a flexible partner in evolving farming systems and food culture

Taro (*Colocasia esculenta*) is deeply rooted in the agriculture and food culture of Japan. Its name in Japanese - *satoimo* - is composed of the words “sato” meaning village or home and “imo” meaning root or tuber, reflecting the central role it plays as a staple food crop in small-scale farming systems. It is also thought that the Japanese owe their predilection for slimy foods, such as glutinous or sticky rice, to the high appreciation they hold for taro.

Taro was introduced to Japan around 1000 B.C., long before the advent of rice. Owing to its high and stable productivity and due to the absence of other major food crops at the time, it was quickly adopted in the Japanese agricultural production system. It was later supplanted by rice, which shares similar cultivation methods (i.e. use of wetland and terrace systems). However, it still plays a major role in small-scale farming and is used as a food security crop when rice production drops. Consequently, rice and taro have co-evolved in farming and food systems.

Due to the ease with which it adapts to diverse farming systems and food cultures, taro has played a central role in the evolution of agro-ecosystems in many countries, and has helped maintain food security in continuously-evolving rice production systems. As in other crops, genetic diversity in taro has facilitated evolution of the crop. Scientific understanding of taro’s genetic diversity and management will further facilitate its use in providing global food security. We welcome this volume as an important step in this direction, particularly for the way it highlights the global benefits that taro can provide in times of change.

Japan is a leader in the application of science to taro and hopes that other countries will be supported to make the most of this undervalued cornerstone of many tropical and subtropical agricultural systems.

Masa Iwanaga

Director General,
National Institute of Crop Science,
Tsukuba, Japan

Contributors

Akita, Motomu, Department of Biotechnological Science, Kinki University, Uchita, Naga, Wakayama, Japan
Alfonso, Leonor Castiñeiras, Fundamental Research Institute on Tropical Agricultural (INIFAT), Santiago de las Vegas, C. Habana, Cuba
Ayad, W.G., Genetic Resources and Technology Group, IPGRI, Rome, Italy
Bennett-Lartey, S.O., Plant Genetic Resources Centre, Bunso, Ghana
Chi, Vu Linh, Vietnam Agricultural Science Institute, Thanhtri, Hanoi, Vietnam
DeLacy, I.H., School of Land and Food Sciences, The University of Queensland, Brisbane, Australia
Delp, C., Alafua Campus, The University of the South Pacific, Samoa
Dewei, Zhu, The Institute of Vegetables and Flowers (IVF), Chinese Academy of Agricultural Sciences (CAAS), Beijing, China
Di, Shen, The Institute of Vegetables and Flowers (IVF), Chinese Academy of Agricultural Sciences (CAAS), Beijing, China
Eyzaguirre, Pablo B. Diversity for Livelihoods Programme, Bioversity International, Rome, Italy.
Fonoti, P., Ministry of Agriculture (MAFFM), Apia, Samoa
García, Magaly García, Research Institute on Tropical Root and Tubers (INIVIT), Santo Domingo, Villa Clara, Cuba
Godwin, I.D., School of Land and Food Sciences, The University of Queensland, Brisbane, Australia
Guarino, Luigi, Global Crop Diversity Trust, Rome, Italy.
Gutiérrez, María I. Román, Research Institute on Tropical Root and Tubers (INIVIT), Santo Domingo, Villa Clara, Cuba
Hartati, S., R & D Center for Biotechnology, Indonesian Institute of Sciences (LIPI), Cibinong, Bogor, Indonesia
Hue, Nguyen Thi Ngoc, Vietnam Agricultural Science Institute (VASI), Thanhtri, Hanoi, Vietnam
Hunter, Danny G., Bioversity International, Rome, Italy.
Iosefa, T., Alafua Campus, The University of the South Pacific, Samoa
Ivancic, A., University of Maribor, Slovenia
Jackson, G.V.H., Secretariat of the Pacific Community (SPC), Suva, Fiji
Jianchu, Xu, The Kunming Institute of Botany, Chinese Academy of Science (CAS), Kunming, Yunnan, China
Krieke, C. M., WAU, Dept. of Plant Breeding, Wageningen, The Netherlands
Lebot, V., CIRAD, Port Vila, Vanuatu, South Pacific
Llerena, Julia Albert, Research Institute on Tropical Root and Tubers (INIVIT), Santo Domingo, Villa Clara, Cuba
Mace, E.S., Primary Industries and Fisheries (DPI), Queensland, Australia
Manzano, Arlene Rodríguez, Fundamental Research Institute on Tropical Agricultural (INIFAT), Santiago de las Vegas, C. Habana, Cuba
Maolin, Li, Biotechnology Research Center, CAAS, Beijing, China
Markwei, Carol, Botany Department, University of Ghana, Legon, Ghana
Martínez, Luis Ruiz, Research Institute on Tropical Root and Tubers (INIVIT), Santo Domingo, Villa Clara, Cuba
Mathur, P.N., Bioversity International, South Asia, New Delhi, India
Matthews, Peter J., National Museum of Ethnology, Osaka, Japan

Mayor, Zoila Fundora, Fundamental Research Institute on Tropical Agricultural (INIFAT), Santiago de las Vegas, C. Habana, Cuba
Montiel, Maryluz Folgueras, Research Institute on Tropical Root and Tubers (INIVIT), Santo Domingo, Villa Clara, Cuba
Morales, Sergio Rodríguez, Research Institute on Tropical Root and Tubers (INIVIT), Santo Domingo, Villa Clara, Cuba
Nghia, N.H., VASI, Thanh Tri, Hanoi, Vietnam
Nodals, Adolfo A. Rodríguez, Fundamental Research Institute on Tropical Agricultural (INIFAT), Santiago de las Vegas, C. Habana, Cuba
Ohta, Yoshimoto, Department of Biotechnological Science, Kinki University, Nishimitani, Uchita, Naga, Wakayama, Japan
Okpul, T., NARI, Bubia, Lae, Papua New Guinea
Pardales, J., PRCTRC, Baybay, Leyte, the Philippines
Pérez Hernández, Ramón E., Research Institute on Tropical Root and Tubers (INIVIT), Santo Domingo, Villa Clara, Cuba
Prana, M.S., R&D Center for Biotechnology, Indonesian Institute of Sciences (LIPI), Bogor, Indonesia
Prana, T.K., R&D Center for Biotechnology, Indonesian Institute of Sciences (LIPI), Bogor, Indonesia
Quarcoo, Eric, Bioversity International, Benin
Rao, V. Ramanatha, Bioversity International, South Asia, New Delhi, India
Rodríguez, Miguel Portieles, Research Institute on Tropical Root and Tubers (INIVIT), Santo Domingo, Villa Clara, Cuba
Rugang, Li, Biotechnology Research Center, CAAS Beijing
Singh, D., International Maize and Wheat Improvement Centre (CIMMYT), Nairobi.
Sivan, Param, Secretariat of the Pacific Community (SPC), Suva, Fiji
Taylor, Mary B., Secretariat of the Pacific Community (SPC), Suva, Fiji
Thongjiem, M., HRI, Dept. of Agriculture, Bangkok, Thailand
VanEck, H., WAU, Dept. of Plant Breeding, Wageningen, The Netherlands
Viet, Nguyen Van, Vietnam Agricultural Science Institute, Thanhtri, Hanoi, Vietnam
Xixiang, Li, The Institute of Vegetables and Flowers (IVF), Chinese Academy of Agricultural Sciences (CAAS), Beijing, China
Yap, T.C., UPM, Plant Genetic Resources Center, Serdang, Selangor, Malaysia
Yongping, Yang, The Kunming Institute of Botany, Chinese Academy of Science (CAS), Kunming, Yunnan, China
Zhou, Mingde, Chinese Academy of Agricultural Sciences, Beijing, China

Ethnobotany and global diversity of taro

V. Ramanatha Rao, Danny Hunter, Pablo B. Eyzaguirre and Peter J. Matthews

Taro is one of the world's oldest food crops, dating back over 9,000 years. This ancient crop, first domesticated in Southeast Asia, has continued to spread throughout the world and is now an important crop in Asia, Pacific, Africa and the Caribbean. It is a crop that has been maintained by farmers for millennia and taro genetic resources have remained largely under the control of local communities. The history of its adaptation, use and diversification can teach us many things about using and conserving genetic resources. Much has been written, from different disciplinary perspectives, on the uses and distribution of taro in specific countries and regions. This book on taro is among the first to offer a global approach, covering all regions, disciplinary perspectives and uses of the plant. Our contributors from different disciplines and geographic regions offer the reader a multidisciplinary and evolutionary perspective on taro that shows how one of the world's oldest domesticated plant species continues to evolve and acquire new uses. The book also demonstrates how the story of taro can serve as a model for the *in situ* conservation and use of a staple crop whose global importance is evidenced at the local level in traditional food systems, and not in global commodity markets or trade.

Worldwide, taro ranks fourteenth among staple vegetable crops with about 12 million tonnes produced globally from about 2 million hectares with an average yield of 6.5 t/ha. (FAOSTAT 2010 estimates). Its corms are baked, roasted, or boiled and the leaves are frequently eaten as a vegetable and represent an important source of vitamins, especially folic acid. The blades and petioles of leaves can be preserved or dried, and are an important food in times of scarcity. Petioles and stolons are also eaten fried or pickled. The inflorescence (a flowering stalk) is a delicacy in some food cultures of Asia and the Pacific. The corms and leaves are also used for medicinal purposes. Taro in many cultures is a sacred plant with high prestige and strong cultural and symbolic importance – it may be presented on formal occasions, in domestic or agricultural rituals, in religious and other feasts, and as bride price or compensation.

Global scope and importance

Until recently taro genetic resources have been largely maintained by farmers who have selected thousands of varieties adapted to a wide range of agro-ecologies, covering wetland and dryland, marginal, complex and often harsh environments. Taro is embedded in many cultures as a result of its selection for a wide variety of uses. It is often viewed as intrinsic to cultural identity, as in Hawaii where taro is believed to be an ancestor by the indigenous people. Similar origin stories are common in other societies that have used taro for thousands of years, in Melanesia and Micronesia, and the plant commands an important role in local legends, stories, chants and proverbs. Taro is a crop for which farmers have remained the main custodians of the genetic and cultural diversity and recent events in Hawaii, regarding ownership and research on taro genetic resources, indicate how strong this connection remains.

Taro has received relatively little attention from the international agricultural research community and is frequently referred to as an 'orphan' crop. There is no international centre or collection

dedicated to taro. Despite this neglect, some countries and organisations have a tradition of taro research and continue to maintain national taro collections and explore ways to strengthen the *in situ* conservation of the crop. More recently, the 1990s saw the emergence of new research paradigms for taro that address issues of international research and recognize the benefits of sharing taro genetic diversity between distant geographic areas.

Development of taro research

Historically, small island states have been in the forefront of taro research. The Universities of the South Pacific (USP), West Indies (UWI), Florida (UF), Hawaii (UH) and UNITECH-PNG, and organizations such as the National Agricultural Research Institute (NARI-PNG), the Caribbean Agricultural Research and Development Institute (CARDI) and the Secretariat of the South Pacific (SPC-Fiji) have played a leading role in taro research. India and China also carry out significant research on taro at the Central Tuber Crops Research Institute (CTCRI) India, as does the Chinese Academy of Agricultural Sciences-Society of Horticultural Science. Botanists, crop scientists and anthropologists at diverse institutions in Australia, New Zealand and Japan have also done important work on the taxonomy, conservation, agronomy and ethnobotany of taro. The number of actors and stakeholders involved in taro research continues to grow. More recently, the universities of Guam (UG), Ghana, Tsubuka, Queensland (UQ), Sydney (US), Queensland University of Technology (QUT), Wageningen Agricultural University (WAU), Central Queensland University (CQU) and a growing number of Asian institutes such as the Indonesian Institute of Sciences, Vietnam Agricultural Science Institute and Kunming Institute of Botany have all been involved in taro related activities covering breeding, agronomy, ethnobotany, pathology, molecular characterization and conservation.

Other organizations and agencies have played an important role in supporting activities relating to taro conservation and use, they include: the Australian Agency for International Development (AusAID), Australian Centre for International Agricultural Research (ACIAR), British ODA/DFID, CIRAD in France, the European Union (EU), New Zealand AID, HortResearch, Japan International Research Center for Agricultural Science (JIRCAS), United Nations Development Programme (UNDP) and the Food and Agriculture Organisation (FAO), Bioversity International, and Agricultural Development in the American Pacific (ADAP).

The growing demands for limited resources and funds in agricultural development, which is likely to get worse in the short-term, and the continuing low priority given to taro by donors, has contributed to a paradigm shift in how international research on taro is undertaken. Recent developments have seen the emergence of international collaborations through the establishment of taro crop networks. The Taro Genetic Resources: Conservation and Utilisation (TaroGen) network was set up in early-1998 following the outbreak of taro leaf blight (TLB) in Samoa a few years earlier. TaroGen's aims included developing and implementing a regional strategy for taro genetic resource conservation and taro improvement to redress the extensive taro genetic erosion occurring in Oceania. Substantial taro collection, characterization and conservation were undertaken. Regional breeding programmes were established to address the threat of TLB. A taro core collection and improved resistant taro germplasm were made available for the benefit of participating, and other, countries. The Taro Network for Southeast Asia and Oceania (TANSAO), another international network with similar aims to TaroGen, was established in 1998. TANSAO's aims were to improve the competitive position of taro in cropping systems and markets. Some of the research outcomes from both networks are published in this book.

Both networks actively promoted and facilitated the sharing of information, resources and capacity and provide a useful model to replicate at a wider or global scale. Such international network approaches offer a research and development model that allows pooling of scarce resources, more effective use of limited funding, standardisation of cultivar descriptions, the development of core collections, secure storage, central databases and mechanisms for dealing with germplasm exchange, and better international sharing and collaboration between countries and organisations (Jackson, 2003; Lebot *et al.*, 2001).

Taro vulnerability and improvement

Despite the considerable effort and resources that have gone into safeguarding taro genetic resources through breeding and *ex situ* conservation over the years, future efforts surely need to focus on developing core collections representative of the widest possible genetic diversity for taro improvement and using this to strengthen *in situ* or on farm conservation. After all, it has been the farmers and communities that have depended on taro cultivation to meet their daily needs and obligations for millennia that have nurtured and adapted the crop. However, farmers continue to face many risks because of taro's vulnerability to biotic and abiotic problems, and global climate change.

We now know that the genetic base of much taro, outside of Southeast Asia, is narrow and vulnerable to numerous problems. This is largely the result of farmer selection of a limited number of clones, which they carried with them when moving to new lands, and subsequent selection in environments where stresses, such as pests and diseases, have been absent. In the Pacific, and in relatively new taro cultivation regions, this narrow genetic base has made the taro crop vulnerable to a range of very damaging biotic stresses such as taro beetles (*Papuana* spp.), taro viruses such as the Alomae-Bobone virus complex (ABVC), and most importantly taro leaf blight (TLB), caused by *Phytophthora colocasiae* Racib. It is the latter which has had the greatest impact on taro production. The earliest records for the appearance of the disease in the Pacific Islands date from the 1920s, and the disease has had dramatic effects on taro productivity and diversity. Severe declines in cultivation, changes in cropping and dietary patterns, genetic erosion and catastrophic impacts on livelihoods have all accompanied the arrival of TLB in taro growing areas.

The outbreak of *Phytophthora* in Samoa in 1993 highlights starkly the impact of the disease in places reliant on one or two varieties for local and export markets. The country is still recovering from the devastating cultural and financial impact of the disease almost twenty years on. More recently, the introduction of TLB to the Caribbean in 2004 led to the almost total collapse of the taro crop in the Dominican Republic, Cuba and Puerto Rico. A number of countries and communities may face the same fate unless they take action to monitor crop introductions and ensure that diverse and resistant taro germplasm is made available to farmers.

The good news is that we now have models of participatory and decentralised taro breeding that can potentially put much greater taro genetic diversity in the hands of farmers anywhere in the world, from which they can select for different agro-ecological conditions and their own preferences. Further, decentralised breeding can do this faster and more efficiently than conventional breeding approaches where one or two clones might be evaluated for limited criteria and wide adaptability on research stations for up to a decade before release. The new approaches offer farmers great scope for adapting to changing environments and changing climate. Breeding taro is relatively easy

and farmers have shown themselves capable of handling and selecting from large segregating populations of taro. With greater international cooperation and exchange of taro germplasm, such as that facilitated by the TANSAO and TaroGen networks, there is considerable potential to broaden the genetic base of taro, including for durable resistance to important pests and diseases. Core collections representing wide genetic diversity are for the first time available for farmer evaluation, as are a number of taro clones and varieties specifically bred for resistance to TLB. The possibility of introducing this diverse, geographically distant taro germplasm for farmer evaluation and breeding is exciting and must be seen as a global priority for the crop, a win-win situation.

Further, introducing such resilience into taro cropping systems is now a safe possibility with recent advances in taro virus characterisation and detection, along with *in vitro* propagation, which can greatly facilitate the safe international movement of taro germplasm. At the same time, there is the prospect of supplying countries and farmers with taro genetic diversity through true seed, which also reduces the possibility of introducing unwanted taro viruses. Science and understanding of taro has come a long way in the last 10 years and we can now employ many tools and methods to put greater taro genetic diversity in the hands of farmers, and to help farmers create their own diversity, which can ensure that they continue to nurture and adapt this ancient and important crop well into the future. In addition, we have a greater understanding of how and why farmers and communities maintain taro diversity on farm through studies carried out in Nepal, Vanuatu and the Solomon Islands.

Organisation of the book

The book begins with a consideration of taro's history as a food and then travels from Ghana, through Indonesia, Vietnam, China and Cuba, finally finishing up in the Pacific Region. On this journey, we assess the uses and role of taro in food cultures around the world and the status and potential of taro genetic resources in taro improvement. We present research on the distribution of genetic diversity and adaptation of taro in each of the areas visited, which represent the main producing regions, then provide a brief review of new technologies and complementary approaches to develop and conserve taro genetic resources for diversified uses. Our aim is to share information on recent taro research in many parts of the world in the hope that it will strengthen future international collaboration and cooperation to improve the sustainable conservation and utilisation of taro genetic resources. This book highlights the synergies between science and thousands of years of farmer knowledge of taro genetic resource management, and the enormous potential of taro. Our continuing co-evolution with taro can provide a positive lesson for future food security and the adaptive management of tropical root crop systems.

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1 [Editor's note: World production data for taro and other root and tuber crops in 2000 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'.]

An introduction to the history of taro as a food

Peter J. Matthews

Earliest uses and cultivation of taro

Taro [*Colocasia esculenta* (L.) Schott] is believed to be one of the world's most ancient food crops, with a history of more than 2000 years in cultivation. This dating is based on the earliest written records for taro (Matthews 2006), and is open-ended because the plant is difficult to detect archaeologically. Even very recent archaeological evidence for the use of taro is lacking, except in the Hawaiian and other Pacific islands where archaeological features such as terraces, irrigation ditches and drains can be securely linked to taro cultivation and use. Taro is an important crop in the Pacific region today and has featured in many debates on agricultural history in Southeast Asia and the Pacific.

At Kilu Cave in the Solomon Islands, near Papua New Guinea, starch granules on stone tools have been tentatively identified as coming from *C. esculenta*. The tools were found in a stratigraphic unit dated to between ca. 28 700 years BP and 20 100 years BP (Loy *et al.* 1992). The tools were apparently used to scrape and/or cut fresh (i.e. raw) taro, and it was assumed that taro formed part of the prehistoric diet. This evidence may relate to wild or cultivated plants, and the possibility of medicinal or other non-food uses cannot be excluded. At the Kuk archaeological site, in a swamp in the Western Highlands Province of Papua New Guinea, earthworks, geomorphological evidence, and plant remains indicate a sequence of agricultural practices from around 9000 years ago. Taro is one of the main candidates for early use and cultivation at this site (Bayliss-Smith 1996; Denham *et al.* 2003).

Before its modern introduction to Central and South America, taro was cultivated at temperate and tropical latitudes in both hemispheres, and at longitudes from West Africa to Eastern Polynesia. Taro was thus the world's most widely distributed starchy food plant before the modern era of rapid international transport. Early cultivation and domestication of taro could have started independently in many places over a large geographical range, from India and southern China to Indonesia and Papua New Guinea. In these regions, apparently natural populations of wild taro can be found today. Little is known about the relationships between wild and cultivated forms of taro, but the species has clearly had a long and complex history of domestication and dispersal. Present-day cultivars of taro vary greatly in their genetic constitution, agronomy, morphology, phytochemistry and food qualities.

Presumably, the earliest cultivation of taro was preceded by the gathering and use of wild-type (natural) forms from wild habitats. These could have been used in multiple ways—as food (for the starch and green leaves), as medicine and as a cooked fodder (perhaps for pigs). Wild-type taros in tropical Asia and the Pacific are notable for having relatively little starch, long stolons and high acridity (Matthews 1995, 1997). Barrau and Peeters (1972) argued that in Australasia, the development of food preparation methods was an essential starting point for the use and storage of root crops, and that ways to remove the acridity of taro and other food plants had to be found before the plants were worth cultivating. This argument seems reasonable, but we cannot be sure how

much acridity had to be removed. Human tolerances for toxicity in food could have been greater in the past, innately and/or through habituation. Much remains to be learned about acridity and other phytochemical (plant chemical) qualities in taro. These qualities and how taro is eaten may have been changing in closely linked ways since ancient times. While the history appears to be complex, present-day methods and tolerances permit the safe consumption of all cultivated forms of taro, with the exception of wild-types (natural forms) that are cultivated as ornamental plants.

In this review, my main concerns are the nutritional qualities, phytochemistry and food culture of taro. Phytochemical variation in the crop today is believed to partly reflect a balance of selection pressures in two opposing directions, toward (1) increased palatability and digestibility for humans, and (2) decreased palatability and digestibility for herbivores that may attack the crop during growth and storage. Phytochemicals that protect against microorganisms are presumably also involved. In conclusion, I argue that to preserve taro as a globally distributed food resource, fundamental historical research is needed in both the natural and human sciences. More specifically, I suggest that preserving culinary knowledge is essential for preserving genetic diversity in the crop.

Nutritional qualities

Information on the nutritional qualities of taro is scattered in local reports published in many countries. These usually describe cultivars from local or regional sources (production areas, markets or research collections). In the absence of standard reference cultivars, it is difficult to compare the results of different authors working in different countries. Useful reviews can be found in Wang (1983), O'Hair and Asokan (1986), Bradbury and Holloway (1988), O'Hair (1990), Maga (1992), Pollock (1992) and Nip (1997).

Taro has good nutritional qualities, but is not a nutritionally complete food. Losses of minerals and vitamins are large when water used for cooking taro is discarded (Bradbury and Holloway 1988:95). Although all cooking methods reduce the nutritional value of taro, cooking of some sort is almost always required to detoxify the corms and leaf parts, and to make them softer and physically palatable. Here I summarize the nutritional qualities of corms and other edible parts. Quantitative details for each nutritional component can be found in the reviews cited above.

Corms

Taro corms cooked in the absence of other ingredients provide a bland, slightly savoury starch. Taro is therefore well suited to daily consumption and can be combined with an almost unlimited range of other ingredients. The mouth feel of fully cooked, mature pieces of corm can be (a) soft and melting, (b) firm and dry, (c) soft and sticky, or (d) firm and sticky. Perceptions of stickiness are likely to reflect variation in the chemical composition of taro starch (see Nip 1997), but may be influenced by the presence of mucilage. Mucilage from the corm often produces a slimy feeling in the mouth. Although this may be tolerated or enjoyed by some people, most ways of preparing taro reduce the sliminess.

The corms are primarily a source of energy in the form of easily digested starch. They are high in carbohydrates and low in fat and protein. Fresh corms are composed of about 69% moisture, 25% starch, 1.5% dietary fibre, 1.1% protein and 1% sugar (rounded averages from Bradbury and Holloway 1988:58). Percentages for the last three components vary greatly between cultivars, relative to the average values, but are generally low (see reviews cited above). Dietary fibre consists of plant cell walls and wall components, and the fibre from taro may be beneficial for health (Ferguson *et al.*

1992; Harris *et al.* 1992). In one experiment, raw taro starch served in a sweet pudding was found to be 99% digestible by human subjects (Langworthy and Deuel 1922). Since cooking increases the digestibility of starch, in general, cooked taro starch must be very easily digested under normal circumstances of consumption.

The corms also provide a good range of vitamins, amino acids and minerals. Among the essential amino acids (i.e. those that cannot be synthesized in the human body), phenylalanine and leucine are relatively abundant. Bradbury and Holloway (1988:61) found lysine and threonine to be the first and second limiting amino acids, on average (results vary among cultivars and authors). Among minerals, potassium is abundant and iron is lacking. Although low, the sugar content of corms is sufficient to affect flavour and varies according to maturity and storage conditions: sugars are converted to starch as corms mature, and starch is converted back to sugars when stored corms begin to sprout. The main sugar present is sucrose (Agbor-Egbe and Rickard 1990; Maga 1992).

Lipids, proteins and amino acids, and sugars all give rise to volatile compounds when taro is cooked, and these compounds contribute to the flavour. MacLeod (1990) identified nearly all volatile compounds in extracts from cooked taro, and described the flavour as buttery, earthy and musty/mouldy, with odour notes reminiscent of boiled/baked potatoes, cooked rice and roasted cereal. In Cyprus and elsewhere, flavour is regarded as better in taro that has been fertilized with natural manure (animal dung) rather than simple mixtures of nitrate, phosphate and potassium (N–P–K). This suggests that micronutrients (lacking in simple N–P–K mixtures) are important for the development of flavour.

Although the overall protein content is low, large servings of taro corms can be a significant source of protein. A large quantity of the starch from corms can be consumed and digested by one person in one day. Standal (1983) found that taro corms can serve "as a dietary source of carbohydrates and potassium for all ages, and as a major protein source for adults who depend on taro as their staple food". In some parts of Papua New Guinea, and on many Pacific islands, taro was traditionally consumed in large quantities. Pollock (1992:214) estimated that on most days, taro in the traditional (i.e. pre-modern) diet of a Pacific islander could have provided some 1500 calories (kcal). This statement implies a quantity of about 1.25 kg fresh taro corm per day (c.f. Table 17 in Pollock 1992). Taro was also consumed in the form of occasional snacks and during special feasts.

Blades

Taro leaf blades are a good source of protein, minerals and vitamins. The protein content (average 4.2% of fresh weight) is much higher than in the corms. In the South Pacific Islands, taro leaves (blades) were previously one of the few green vegetables available (Bradbury and Holloway 1988). For each cultivar, whether or not leaves are eaten depends on the degree of acridity, local knowledge and food preferences. Bradbury and Holloway (1988) found no major differences in the nutrient content of edible and inedible leaves (blades) among cultivars of taro in Fiji.

Petioles

Taro leaf stems (petioles) are eaten in many areas, but there is relatively little information on their nutritional qualities and use. Standal (1983) reported that the stem (petiole) contains fewer nutrients than the corms and leaves (blades), but also has a generous amount of potassium. From the data summarized by Sunell and Arditti (1983), it appears that petioles always have less protein than the blades, but sometimes have more protein than the corms.

A second species of taro, *Colocasia gigantea* (*hasu-imo* in Japan), produces insignificant corms and is used as a petiole source in Southeast Asia and Japan. No information has been found on the nutritional qualities of any part of *C. gigantea*.

Other parts

The stolons and flower heads (inflorescences) of taro are eaten in Southern China, Southeast Asia and the Pacific Islands, but these are generally very minor uses. In Hawaii, Greenwell (1947) found that spadices (from inside the flower heads) were previously baked as a delicacy with fish or pork. In Papua New Guinea, Sillitoe (1983) observed that leaves and inflorescences are cooked in bamboo tubes over embers (a steaming method). In Malaysia, Furtado (1940) stated that parboiled leaves, leaf stalks (petioles) and runners (stolons) may be fried with egg or other ingredients, in a Chinese-style. Jianchu *et al.* (2001) described cultivars in Yunnan, China, that provide edible inflorescences and stolons. The nutritional qualities of stolons, inflorescences and fruit have not been investigated to any significant extent.

Phytochemicals involved in plant defence

As well as having many positive nutritional qualities, taro contains a range of phytochemicals that can be variously described as poisonous, toxic, antinutritional or simply unpleasant. Those that are discussed here are involved in plant defence, or are suspected of such involvement. Acridity is the most obvious and unpleasant phytochemical quality of taro. The sensation of acridity is experienced at its worst when raw or undercooked pieces of corm or leaf are placed in the mouth. Acridity is the main factor determining the palatability of taro, but other factors are also involved. Around the world, generations of cooks have developed numerous ways to cope with acridity and other negative qualities in taro. With suitable caution and an informed approach, there is no need to fear eating this plant.

The phytochemicals discussed below all have negative implications for taro as a food, yet they also have positive implications for taro as a crop that can be grown with minimal use of fungicides and pesticides. The main phytochemicals of interest are: (1) protease, (2) oxalic acid, (3) cyanide, (4) alpha-amylase inhibitors, (5) trypsin and chymotrypsin inhibitors, (6) lectins (major storage proteins), (7) mucilage, and (8) tannins and other phenolic compounds.

Protease (the cause of acridity)

Acridity in taro is thought to be important as a natural defence against grazing animals (Bradbury and Holloway 1998). Acridity is found in the corms and leaves, and is experienced as a severe itching, stinging or burning sensation in the mouth and throat, followed by swelling—or as a less severe irritation or itching of external skin, on hands and arms for example. In one study, human subjects (volunteers!) chewed on raw samples from the corm of a highly acrid cultivar (Osisiogu *et al.* 1974). Within a few seconds, ‘stinging’ effects were felt on the lips and then tongue; salivation increased, and after swallowing some saliva, ‘stinging’ extended to the throat; the tympanic membrane (of the ear) also seemed affected; hearing was somewhat blocked, and there was a slight headache; the ‘stinging’ effects lasted about 25 minutes, the hearing impairment about 45 minutes, and then the headache ceased.

Furtado (1940:12) noted claims that adding acidic ingredients or sodium bicarbonate can help to reduce acridity during cooking, and that milk, thick syrup of sour lime or tamarind, or solid tamarind

with or without salt, can help alleviate the ill effects of acridity. May (1984) recommended lime juice as an antidote, while Hollyer *et al.* (1997) suggested several possible antidotes, including a wash with baking soda. Systematic investigation of the various suggested antidotes might be very useful for learning about the acrid principle or principles, and human perceptions of acridity.

According to Bradbury and Nixon (1998), acridity is caused by a protease (protein-degrading enzyme) that is attached to raphides, thus forming a functional complex described as 'Nature's poisoned spear'. The raphides are sharp, arrow-like crystals of calcium oxalate, and are abundant in taro. Raphides in taro and other aroids are not always acrid, and the degree of acridity in these plants is not always correlated with the abundance of raphides, or the amount of calcium oxalate present (calcium oxalate is also found in other crystal forms). The uncertain or variable correlations might reflect differential production, attachment or activation of the acridity-causing protease, or other factors. Previous explanations for acridity in taro have not been entirely proven or disproven (see reviews in Bradbury and Holloway 1988; Maga 1992; Tang and Sakai 1983). Osisiogu *et al.* (1974) found a positive correlation between the abundance of raphides and irritant effects, but also found evidence for the existence of a volatile irritant in the highly acrid cultivar referred to above.

Oxalic acid

In large quantities, oxalic acid is poisonous to humans and can also reduce the nutritional value of a food by binding with calcium to form calcium oxalate. Calcium oxalate crystals are abundant in taro and many other plants but are only poorly digested by humans. Because oxalic acid and oxalates are present, Standal (1983) concluded that the bioavailability of calcium in taro leaves was uncertain, although the leaves had been evaluated as a generous source of calcium. Bradbury and Holloway (1988:114) subsequently determined the amounts of total oxalate, soluble oxalate, calcium oxalate, and free calcium in taro corms and leaves, and found that (a) the amount of soluble oxalate is quite small and of the same magnitude as that found widely in vegetables and fruits, and (b) the amount of free calcium is adequate for human nutrition.

Since calcium oxalate is involved in acridity (see above), there could be a connection between oxalic acid balance and acridity in taro. Soil conditions are sometimes said to affect acridity in taro, and a mechanism proposed for the tolerance of excess aluminium in acid soils suggests how oxalic acid balance might be involved (Ma and Miyasaka 1998). In theory, the production or use of oxalic acid for this role might either promote or retard the formation of insoluble calcium oxalate raphides, and the influence—in either direction—might be greatest when raphides are being formed from soluble components in the new tissues of growing plants.

Cyanide

Bradbury *et al.* (1995) found that taro can produce HCN gas when leaf or corm tissues are macerated, but the amounts produced were not considered sufficient to be of concern for human nutrition. Only a small number of wild and cultivated forms of taro were tested, after cultivation in pots. Further investigation is needed to learn whether or not cyanide production is ever sufficient to deter humans, or natural herbivores, under field conditions.

Alpha-amylase inhibitors

Alpha-amylases are enzymes that help animals and humans to digest starch. The enzymes are found in saliva and the small intestine, and can be inhibited by enzyme-specific inhibitors from many

plants. The activity of alpha-amylase inhibitors in extracts from taro corms varies greatly among cultivars (Rekha *et al.* 1997). Seltzer and Strumleyer (1990) purified an alpha-amylase inhibitor from taro and named it *esculentamin*. Others have found that alpha-amylase inhibitors from taro (not necessarily identical with *esculentamin*) can inactivate human salivary and pancreatic amylases (Maga 1992). Seltzer and Strumleyer (1990) found that *esculentamin* is very resistant to heat, losing no activity *in vitro* after boiling for 30 minutes, and very little after heating at 100°C for 3 hours. The latter treatment exceeds most cooking times for taro. If usual cooking practices do not deactivate these inhibitors, then they are presumably tolerated by humans, and may be of little concern. This argument needs to be tested experimentally.

It has been suggested that a number of alpha-amylase and proteinase inhibitors have common origins as storage proteins in plant storage organs (Seltzer and Strumleyer 1990). Lectins, another class of taro storage protein discussed later, are apparently also related to the proteinase inhibitors, as well as to taste-modifying proteins (see Castro *et al.* 1992; Bezerra *et al.* 1995). Proteinase inhibitors are discussed next.

Trypsin and chymotrypsin inhibitors

These inhibitors are proteins that inhibit the activities of trypsin and chymotrypsin, enzymes that degrade proteins. Such enzymes are known as proteinases (or proteases) and are digestive enzymes found in the stomachs of humans and animal herbivores. By inhibiting these digestive enzymes, trypsin and chymotrypsin inhibitors can discourage herbivores. The likely protective role of proteinase inhibitors in taro and other aroids has been discussed by Bradbury and Hammer (1990).

In *Alocasia*, a genus closely related to taro, the gene for a trypsin and chymotrypsin inhibitor was cloned in order to learn more about its potential for engineering biochemical defence in other crops (Mathews 1996). In taro, the two kinds of inhibition may or may not be caused by different protein molecules, and the amount of inhibition displayed varies among cultivars (see Sasikiran *et al.* 1997a,b *in* Bradbury and Hammer 1990). The inhibitors constitute some 1- 4% of the total crude protein in corms (much less than the lectins discussed below), and are absent or inactive in leaves.

When corms are cooked, trypsin inhibitor activity increases at first, but is eventually lost. Twenty minutes of boiling is sufficient to remove trypsin activity (and is also sufficient to remove the acridity in many taro cultivars). In raw taro corms, the amount of trypsin activity may be enough to slow the growth of pigs or other animals (Bradbury and Holloway 1988; Bradbury and Hammer 1990; Bradbury *et al*. 1992).

Lectins

Plant lectins are an extremely heterogeneous group of proteins that have only one property in common, namely their ability to bind carbohydrates (Peumans and Van Damme 1993). In experimental assays, they are commonly distinguished according to their ability to cause the agglutination of cells from various animal tissues and fluids. Agglutination assays reflect the natural role of lectins as compounds that attach to carbohydrates on cell membrane surfaces in a more-or-less specific fashion. When very abundant in plant storage organs, lectins may act as storage proteins that support new growth during the plant lifecycle, and also as biochemical defence molecules that anticipate attack by microorganisms or plant-eating organisms such as nematodes, insects, other

invertebrates and higher animals (Peumans and Van Damme 1993). The lectins in many raw foods, including grains, legumes and some root crops, have deleterious effects on human gastric mucosa (the tissue lining of the stomach). Some lectins may be beneficial to humans, but many are strongly poisonous or inflammatory or both (Bhalla 1990).

Van Damme *et al.* (1995) noted that lectins are the most prominent proteins in the storage tissues of taro and other Araceae, and suggested that aroid lectins are storage proteins with the additional function of biochemical defence. Bezerra *et al.* (1995) gave the name *tarin* to a family of proteins in taro, suggested that these are lectins, and estimated that they represent about 40% of total corm protein. In an earlier functional study, Seo *et al.* (1990) gave mice purified taro lectin and control samples, by force-feeding, and found that the lectin retarded growth and reduced physical activity. Heating the lectin to 100ºC for 20 minutes (a common length of time for cooking taro) eliminated its ability to reduce mouse growth, but not its ability to agglutinate blood cells *in vitro*. *In vitro* activities of lectins in pure or crude extracts were little affected by heating to 80ºC for 60 minutes (Kamitani *et al.* 1987; Seo *et al.* 1989). Lectins in other aroids appear similarly resistant to heat (see Sandhu *et al.* 1990).

Although the toxicity of taro lectin was less than that of lectins from a bean (*Phaseolus vulgaris*), the taro lectin was more heat resistant. Seo *et al.* (1990) concluded that lectin in taro is a major toxicity factor that needs further investigation. So far, no definitive tests have been carried out to determine whether or not lectins in taro are antinutritional for humans (Peumans, pers. comm. 2000). The reactions of some people to taro, discussed later in relation to culinary knowledge and food culture, suggest that lectins do affect human perceptions of the crop.

Mucilage

When raw taro corms are cut, the exposed surfaces often exude droplets of a slimy substance, the mucilage. When cut pieces of corm are washed or placed in water, a great quantity of mucilage is quickly released, making the water viscous. The crude mucilage is a complex mixture composed mainly of neutral polysaccharides (sugar polymers), with small quantities of fibre and protein (El-Mahdy and El-Sebaiy 1984; Nip 1997). Using histochemical staining techniques, Harris *et al.* (1992) found arabinogalactan-proteins (AGPs) in the mucilage ducts, where secretory cells are located. They noted that in human nutrition, AGPs are regarded as soluble dietary fibres, and may have positive or negative effects for human health.

The natural function of taro mucilage is unknown, but its slimy effects often deter people from eating taro, especially if slimy or sticky foods are not a familiar part of the diet. Because of these unfavourable sensory effects, mucilage can be regarded as antinutritional. However, the sliminess is greatly reduced by many methods of preparation. Mucilage can be removed by discarding the water used for cooking, or acid ingredients such as lemon juice can be used to neutralize the slimy effect, or the taro can be cooked without water (e.g. fried or baked) in a manner that leads to partial dehydration and a dry texture.

Tannins and other phenolic compounds

Tannin cells are scattered through the corm tissues, and browning associated with tannin cells has been observed after subjecting corms to chilling injury at 4°C (Rhee and Iwata 1982). Some darkening after peeling has also been reported, and polyphenolic compounds (the precursors for enzymatic browning) have been found concentrated near the corm surface (Maga 1992).

A remarkable feature of taro leaves is the very rapid browning reaction that takes place when petioles or leaf veins are cut and exposed to air. This is presumably also an enzyme-catalyzed oxidation of tannins or other phenolic compounds. The most obvious harm caused by this leaf reaction is the permanent staining of cloth and clothes exposed to sap from the leaves [a practical warning to this effect was made by Furtado (1940)].

With histochemical staining, Harris *et al.* (1992) found unidentified phenolic components in all organs of taro, but did not comment on their possible functions or effects. However, certain polyphenolic compounds in corms are oxidized enzymatically when corm tissues are invaded by fungi (Ohazurike and Arinze 1996). This suggests a role of some sort in protection against fungi.

In general, tannins are toxic substances whose main function may be to make plant tissues less palatable for herbivores. For humans, they are the cause of astringency, an unpleasant effect in the mouth that is believed to result from the precipitation of saliva components by tannins. If tannins in raw taro corms and leaves have a plant defence role, then cooking must render them harmless more effectively than it does acridity: astringency due to tannins has never been reported for taro, while acridity is well known.

Variation in cultivars and their uses

As indicated above, a complex phytochemical cocktail influences the use of taro in many ways. Other factors that have not been discussed here include sterols and aflatoxin potential (see Sunell and Arditti 1983; Maga 1992). Although certain phytochemicals in taro are associated with negative eating qualities, taro is well regarded in many societies. However, it is not universally well regarded, and some of the reasons for this are discussed below.

Culinary knowledge and food culture

In areas where taro is an ancient and popular crop, safe and reliable methods for preparing taro are known by most cooks. Because taro is propagated vegetatively, any cultivar can be maintained indefinitely with little or no genetic change. This has permitted practical culinary knowledge to develop around specific, well-known cultivars over long periods of time. When the people responsible for preparing food adhere strictly to established methods, and apply them to well-known cultivars, the people eating taro are not always aware that other taro cultivars can be poisonous. This has been noted by the author in Cyprus, Egypt and Japan.

Taro is not popular in all areas where it has a long history. Ideological aspects of local food culture are sometimes advanced as an explanation for this, but low popularity could also reflect a local predominance of poor cultivars (with relatively high acridity, for example). In some areas, recent introduction or failure to flower and produce seed may have limited the possibilities for developing better cultivars. Even where the plant is able to breed—in fallow fields in the tropics, for example—a relaxed selection regime or excessive gene flow from wild-type populations might block the development of better cultivars.

In Nigeria, the lack of popularity apparent in one survey might have reflected both ideology and cultivar quality. Oke and Ojofeitimi (1980) questioned a group of 80 nursing students and found that 61 were familiar with taro. Among the latter, 60% said they do not eat it because it is forbidden in their family, or because it is a poor man's food, and a further 26% claimed that taro causes "piles

or stomach upset", although they had never experienced this themselves. The authors expressed concern that taro had such a bad reputation, despite its high nutritional value. Although I share this concern, our knowledge of phytochemical variation in taro is still very limited. In some areas, and depending on the cultivars available, there may be good reasons for taro having a bad reputation.

Could "piles and stomach upset" be symptoms of lectin toxicity? This possibility is suggested by Bhalla's (1990) indication of a link between human intolerance for lectins in raw food and an enhanced susceptibility to duodenal ulceration (i.e. in the small intestine, just after the stomach). It is also suggested by occasional reports (to the author) of discomfort in the stomach after eating taro. When taro is cooked, it is likely that temperatures and times that reduce acridity are also able to reduce lectin activity. However, lectins are abundant in corms, and have some resistance to heat, so some lectin activity might survive treatments that remove most acridity.

In Japan and eastern Asia generally, taro is an ancient and widespread crop. The Japanese archipelago spans a long distance from north to south, and apparently tropical cultivars of taro reach their northern limit in Kyushu at about 33°N, while temperate-adapted cultivars extend to about 41°N, in northern Honshu. The tropical cultivars produce large central corms with relatively few side corms, and the temperate cultivars produce many small side corms from a small central corm. Although temperate cultivars are grown in Kyushu and further south, they are predominant in northern areas. The different cultivars vary in size, shape, acridity, texture after cooking, and other qualities. This variation partly explains the great diversity in taro cooking methods in Japan. These methods are described in great detail in the encyclopaedia *Japanese Food Eating Styles* (Rural Culture Association 1997). For this huge work, elderly people in all prefectures of Japan were interviewed in the 1980s. Records were made of food rituals and recipes known during the first half of the 20th century, and there are almost 2000 entries on taro. Many or most of the recipes recorded are still used today.

In recent decades, Japanese research and writing on taro have been extensive. New and old recipes for taro are frequently published in popular books, magazines and newspapers, and their publication often coincides with the autumn harvesting period for taro. Food is an immensely popular topic in modern Japan, and it is quite common to see taro on TV in cooking programmes, in documentaries about local events, and in period dramas featuring traditional food scenes. Small taro corms or side corms are commonly cooked with a liquid stock from which the starch absorbs flavour, in dishes such as *nimono* (vegetables simmered in soy sauce) and *miso-shiru* (soup with miso paste) (Figure 1).

The continuing popularity of taro in Japan can be attributed to a generally high regard for traditional foods, relatively low prices compared with many other starchy foods, and the continuous dissemination of new and old cooking methods in popular media. This dissemination may be both a cause and a response for declining oral transmission, from older to younger people.

Internationally, taro is mainly produced, sold and bought by people for whom the plant is already familiar. This is true in countries where the crop is ancient, and in countries where recent immigrants from growing regions buy imported taro or grow it for their own consumption. Specific information on how to use the sold product is rarely available for new buyers. Since most taro cultivars are poisonous unless cooked properly, it is difficult for new buyers to prepare the sold product safely

and well without personal guidance from an experienced cook. In general, English-language cooking books cannot be relied upon for preparing the particular corm or leaf that a buyer finds. Such books reveal wild variation in recommended cooking times, and rarely refer to specific cultivars.

These difficulties are compounded when sellers use vernacular names for taro and other plants loosely, or when buyers are not familiar with the vernacular names, or when visual clues are not enough to distinguish cultivars and species in the market. For example, the corms of taro and *Xanthosoma sagittifolium* (a tropical American relative of taro) are often difficult to distinguish after their leaves have been removed. The taste, texture and acridity of taro vary according to growing conditions, maturity and storage conditions. They also vary among different cultivars of taro, and between taro and other edible aroids. From Ghana, Doku (1966:23) reported a dramatic example of how acridity can vary among the edible aroids:

"Because [the *kokoo* or *twi* variety of *C. esculenta*] is very soft, it is preferred by old men and women, and on account of its poisonous qualities, it has to be boiled for about 12 hours before it becomes edible as compared with the 20 minutes or so required for cooking the Amankani varieties [of *Xanthosoma sagittifolium*]".

Buyers often ignore unfamiliar foods in markets, and this is a sensible response when taro is unfamiliar and no reliable guidance is available. Because taro has good nutritional qualities, and can be grown in diverse environments, there is much potential to develop new markets and production areas. For this to happen, however, new buyers must have access to cultivar-specific information, and must be offered reliable methods that incorporate both traditional culinary knowledge and a modern understanding of phytochemical variation in the crop.

Recognizing the edibility of different plant parts

Only the skin of the taro corm, and the true anatomical roots, have not been reported as food. All other parts of the plant are potentially edible (corms, blades, petioles and inflorescences). The nutritional values of these parts have been noted above. The full potential of taro as a food is not known by all people who use the plant, even in areas where taro has been long cultivated. Local practices in relation to a set of local taro cultivars are not necessarily a full guide to the potential of those cultivars.

In Cyprus, for example, only the corms are used (Figure 1), yet boiling for just a few minutes is sufficient to remove acridity from the petioles (author's observation, 1996). In Bangladesh, Chowdhury and Hussain (1979) observed that the leaves (blades) and petioles of upland varieties of taro are edible, but are not eaten. In Japan, it is usually only the petioles (*zuiki*) of *C. esculenta* (*sato-imo*) and *C. gigantea* (*hasu-imo*) that are eaten. Petioles are commonly peeled and cooked in soups, where they absorb flavour from other ingredients, provide an easily chewed substance, and add visual interest (Figure 1). They are valued despite their relatively low nutritional value. According to a widespread folk tradition, it is good for women to eat *zuiki* after childbirth (Rural Culture Association 1997).

According to Heyne (1950:425), the fruits of wild *C. gigantea* in Java were used as a flavouring agent, and Furtado (1940) noted the existence of a cultivated form of *C. gigantea* that was known in Malaysia as *lambok* or *keladi ulam*. This cultivar had petioles that could be eaten raw or cooked

after peeling. The Japanese cultivar (*hasu-imo*) also can be used raw or cooked: thin slices of raw, peeled petiole are served with raw fish and rice, and sections of petiole are peeled and cooked as described above. The very minor food status of *C. gigantea*, and the lack of cultivar differentiation in this species, suggest that Malaysian and Japanese cultivars might belong to one low-acridity clone. If they do, then the switch to low acridity might not have required many genetic changes and a long process of breeding and selection. The switch could have been as simple as a single-gene mutation in one cultivar. In the case of *C. esculenta*, various degrees of acridity are found in cultivars that are clearly not all one clone, so the genetic mechanisms involved may be diverse and more complex.

Methods for coping with acridity

When preparing taro corms or petioles for eating, the two most common steps for removing acridity are peeling and heating. If not too acrid, the petioles, young rolled blades and mature expanded blades of taro leaves can be eaten. In the Pacific Islands it is common to remove the main ribs and tips from mature blades before cooking them; these actions are said to reduce acridity. Younger leaves are generally less acrid than older leaves. Bradbury and Holloway (1988:61) found that there is no difference in the nutrient content of edible and non-edible taro leaves. Fermentation of corm tissues and additions of certain ingredients (see Table 1) are also believed to reduce acridity.

In the Pacific islands, (raw) taro is grated and then left in pits, for months or years (Yen 1973; Kirch and Yen 1982). Comparison with the fodder trials suggests that such long fermentation may remove acridity, although the final product is eventually cooked. In Hawaii, taro corms are peeled, cooked (or cooked and then peeled), mashed, and then fermented for various periods to produce *poi*, a popular food for humans. Acridity is generally greatest in the cortex of taro corms, and the acrid principle is sensitive to heat. When *poi* is made, it is likely that most acridity in the corms is removed by peeling and boiling before fermentation. The short period of fermentation (usually overnight to three days) might also remove some acridity. As substrates for fermentative microorganisms, raw and cooked starch are very different. This contrast does not appear to have been investigated in relation to aerobic and anaerobic fermentations of taro starch.

Interest in the use of taro as fodder for cattle and other domestic herbivores has led some researchers to compare the growth of animals fed with raw, cooked or fermented (ensiled) taro, and other kinds of fodder. An important result of placing taro corms (raw and unpeeled) and leaves (raw) in anaerobic silage pits was the neutralization or destruction of the acrid factor or factors in taro (Carpenter and Steinke 1983). Exactly how the acridity was removed was not explored. Anaerobic fermentation by lactobacilli produced a large quantity of lactic acid, little butyric acid, and low pH values (pH 3.5–4.0). The final fermented product was optimally palatable for pigs and cattle after 30 days of fermentation

Next, for contrast, I describe food preparation methods that have been applied to the most acrid and least acrid forms of taro.

The highly acrid wild-type taro

In my own experience, the acridity of corms and leaves from wild-type taro is extremely resistant to long periods of cooking. In Papua New Guinea, Japan and other countries, acrid wild taros are usually not eaten, but they may serve as famine food. In northern Australia, Aborigines used (and may still use) a series of cooking, pounding and leaching steps to render wild-type taro edible. A rare

Figure 1. Taro cooking in Cyprus and Japan. *Peter J. Matthews*

a. An expert restaurant cook chipping taro (kolokasi) to make a taro stew, kolokasi yiakhni (Cyprus, September 1996).

b. Kolokasi yiakhni, a popular taro dish using chipped pieces from the main corm (mappa), here served in a restaurant specializing in Cypriot dishes. Yoghurt and lemon juice are added as condiments, and bread is eaten with the sauce (Cyprus, November 1996).

c. Chikuzenni, a common dish using taro side corms (ko-imo) and other vegetables (konnyaku, lotus root, burdock, carrot, mushroom, and soy beans). These are all lightly fried, then simmered in a savoury stock of soy sauce and mirin (Japan, August 1995).

d. Miso-shiru, a soup made with fresh taro petioles (zuiki), miso (fermented soy bean paste), and fish stock (Japan, August 1995).

and detailed description of these steps was recorded by R. Tucker (pers. comm., 1987, cited in full by Matthews 1990:396–402), and is summarized here:

- Bake corms (whole) in *amai* (earth and stone oven) wrapped or unwrapped.
- Peel cooked corms.
- Pound cooled corms into paste, something like Polynesian *poi*.
- Place paste in very fine *punya*, a bag made of *Lomandra* leaf fibres, and soak in running water for at least 1 day.
- Drain excess water from the soaked paste (*mai-i*), usually by hanging in a tree.Shape the drained paste into (a) cakes or balls and "dry-fry" them on a hot rock in the centre of a fire, (b) cylinders rolled in leaves or bark and baked in hot sand, ashes or in an *amai*, or (c) balls for boiling in coconut milk or turtle stew to make a rich sauce-like soup, in a pot (or traditionally an *alup*—bailer shell).

This information was obtained from people with whom Tucker lived in Cape York. They no longer harvested wild taro, but did treat other wild food plants in the same way: the underground parts of *Amorphophallus* and *Dioscorea*, and the stems of a climbing aroid, *wunki* (*Rhaphidophora pinnata*). Very acrid and apparently wild-type taros also can be found wild in the Hawaiian islands, and are known there by the name *aweu*. Whitney *et al.* (1939) noted that *aweu* was used for *poi* in the past, but only when other food was scarce. Moriarty (1976) reported that *aweu* was an emergency food that Hawaiians always replanted whenever it was harvested. It is not known if *aweu* can be made into good *poi* using an ordinary sequence of cooking, pounding and fermentation.

Cultivars with very low acridity

In contrast to the very acrid wild taros, the cultivar Bun Long in Hawaii has exceptionally low acridity. According to Whitney *et al.* (1939) Bun Long was introduced into Hawaii from China. The introduction is likely to have been made by Chinese immigrants during the late 19th or early 20th centuries, before the 1939 publication just cited. Bun Long is now a major commercial cultivar in Hawaii and is remarkable because the peeled corm can be eaten raw without ill effect. Nevertheless, I have found no report of Bun Long actually being eaten raw. In practice, it is always cooked, and the cooking makes the corm soft and palatable. Acridity in Bun Long is not completely absent, and might vary according to age and environmental conditions. Other antinutritional factors could be present. In Hawaii it is known as a 'table variety', which means that the corms can be eaten after very simple preparation such as boiling or steaming. Whitney *et al.* (1939) reported that the young leaves are considered desirable for *lu'au* (taro leaf) because of their large size, tenderness and relatively low acridity. In Hawaii today, Bun Long is one of the major varieties grown for *lu'au*. The leaf blades are used for *lau lau*, a native Hawaiian dish in which various combinations of pork, fish and chicken are wrapped in taro leaf, forming an edible bundle that is further wrapped in *ti* leaves (*Cordyline fruiticosa*) and then steamed. The corm of Bun Long is used for making taro chips by slicing and frying, and as a garnish in upscale gourmet restaurants. To make a garnish, corms are grated into long thin strips like spaghettini, deep fried and placed on top of an entrée (J. Cho, pers. comm., 2000).

Cultivars with very low acridity appear to be widespread in mainland Asia. During field work in 1999, Cho found cultivars similar to Bun Long to be the major varieties grown for consumption in Thailand and Vietnam. Standal (1983:145) stated that a vegetable (i.e. leaf) variety similar to Bun

Long is found in India, and that taro petioles (from the same variety?) are eaten raw as a snack in the Khasi and Jainta Hills. For the snack, young stems are peeled, cut into 1 cm pieces, and then mixed with pieces of lemon or lime, salt and chilli pepper.

Preparation and storage as a food

Basic elements of preparation and storage that have been applied to corms and leaves, the main edible parts of taro, are listed in Table 1. This table is based on a wide survey of ethnographic and culinary literature, and the author's own observations. Storage methods, for the period from harvest to consumption, are also noted because they affect culinary qualities, and are relevant to the later discussion of taro as a traded food commodity.

In practice, the permutations and combinations of basic elements are innumerable. In different versions of one recipe, and in different recipes, the same elements may be arranged in different sequences, or they be may be expressed in different ways. Similar elements have been recognized by other authors concerned with the processing of food plants. Stahl (1996) recognized the following: (1) grinding, pounding, grating; (2) soaking/leaching; (3) drying; (4) heat, and (5) chemical, including fermentation. In Table 1, water treatments have been placed within the chemical category, and fermentation has been made a distinct general category. The latter is a complex biological process, involving the action of living microorganisms, rather than a relatively simple physical or chemical process.

Letting taro ferment also has a storage function, and the practice may have originated in attempts to store taro. In the Pacific Islands, fermentation of taro in pits has been described as both a method of storage and as a method of food preparation. Pollock (1992) noted that storage techniques did not just have to succeed in storage, but also had to result in a taste that people liked. She argued that fermented taro and other fermented foods were held not just for storage, but also to add variety to traditional diets.

Johns and Kubo (1988) and Johns (1990) recognized seven basic techniques for detoxifying plants: (i) heating, (ii) solution, (iii) fermentation, (iv) adsorption, (v) drying, (vi) physical processing, and (vii) pH change. In Table 1, I regard solution (the use of water) and pH change as chemical treatments. Water is a chemical, and pH change usually depends on the presence of acids or bases in water solution. The removal of toxins by adsorption (Johns' category 4) is a physical and chemical process that depends on the large surface areas presented by porous or particulate materials such as charcoal and clay. The use of adsorption has not been reported for taro, but this does not exclude the possibility of its use in the past.

Overall, the diversity of methods used for corms and side corms is greater than for leaves. This can be explained in two ways: (1) starchy tissues may have more potential for being stored and transformed into different edible forms, and (2) the high energy value of starch provides a strong incentive to elaborate the methods for using it. Whatever the case historically, there is definitely still room for innovation in how all parts of the plant are prepared and used. In recent years, there has been particular interest in methods that make it easier to prepare, store and sell taro as a food commodity.

TABLE 1. Preparation and storage of taro

Methods		Plant part			
General elements	**Specific elements**	**Corms (central & side)**	**Petiole**	**Blade**	**Comments**
FOOD PREPARATION					
Physical treatment	a. peel	o	o	na	Before or after cooking; peel is never eaten
	b. de-rib leaf	na	na	o	Main ribs (i.e. primary and secondary veins)
	c. remove tip(s) of blade	na	na	o	Common in Polynesia; said to reduce acridity
	d. beat incompletely	o	–	–	Compressive force, only to flatten and break open partly
	e. chip	o	na	na	With metal knife; twist & snap produces rough fractures
	f. slice/dice	o	o	o	With metal knife
	g. grate or macerate	o	–	o	Manual grater or food processor; 'macerate' suggests more fine than 'grate'
	h. mash or pound	o	–	o	Compressive force; 'pound' suggests more force than 'mash'
	i. strain	o	–	–	e.g. to remove lumps while making *poi*, after pounding
	j. stir	o	–	–	e.g. in a cooking pot
	k. mill	o	–	–	to make flour
	i. extrude	o	na	na	A recent experimental report
Heat treatment	a. grill or roast	o	–	–	i.e. direct dry heat, under oven element or over charcoal
	b. bake	o	–	o	i.e. even, less-direct heating
	c. fry	o	o	–	e.g. with butter, ghee or various vegetable oils
	d. boil	o	o	o	e.g. simmer, parboil, boil in pure water or in sauce or soup with other ingredients
	e. steam	o	–	o	e.g. in earth oven over hot stones
	f. microwave	o	–	–	May be common, but too recent for records

o =observed or recorded
na = not applicable (not technically possible)
– = technically possible, but not observed or recorded

Methods		Plant part			
General elements	**Specific elements**	**Corms (central & side)**	**Petiole**	**Blade**	**Comments**
Chemical treatment	a. wash in cold water	o	o	o	Shorter/more active treatment than b
	b. soak/leach in cold water	o	o	–	longer/more-passive treatment than a
	c. make acidic	o	o	–	e.g. add lemon, to modify colour, taste, texture & viscosity
	d. make alkaline	o	–	–	e.g. add sodium bicarbonate, to soften
	e. add salt	o	o	o	Adds flavour, also hardens
	f. pickle	–	o	–	Chemical details not known
Fermentation	a. aerobic (yeasts + lactobacilli)	o	–	–	e.g. *poi*
	b. semi-anaerobic	o	–	–	e.g. *ma*, masi
STORAGE					
Temperature control	a. store at ambient temp.	o	o	–	e.g. in locations with moderate ambient temperatures (c.10–20°C); living or fresh parts
	b. store at less than ambient temp.	o	o	o	e.g. pit, shade, or refrigeration in tropics & in summer of temperate areas; living or fresh parts
	c. store at more than ambient temp.	o	–	–	e.g. in pit, or insulated mound, in winter of temperate areas; living corms
	d. freeze	o	–	o	e.g. for raw corms, or partially processed corms and leaves; parts neither living nor fresh
Water control	a. dehydrate	o	o	o	e.g. by solar drying; parts neither living nor fresh
	b. humidify	o	–	–	To reduce dehydration of living corms
Hygiene control	a. pack in +/– airtight condition	o	–	–	e.g. canned sweet desert from corms; corms or poi in bags; parts neither living nor fresh
	b. sterilize surface	o	–	–	With chemical or heat (fresh peeled corms)

Note: The elements shown here are not mutually exclusive categories; different elements are combined in many different ways in practice.

Modern food culture and crop development

Internationally, most efforts to develop crops through research have been guided by prospects for improving their commercial value. At the same time, there has been a tendency to neglect the many non-commercial values associated with plants in human societies. Rather than arguing for or against the expansion of taro as a commercial crop, my aim here is to shed light on the complex connections between modern food culture and crop development. These are illustrated in linked discussions of storage, trade, changing tastes, market development, chemical ecology and plant breeding.

Storage and trade

The storage and transport of taro and other edible aroids is a problem due to their bulk and susceptibility to physical damage (O'Hair and Asokan 1986; Matthews 2002). Physical damage is of concern because it opens the internal starchy tissues to microbial invasion and rotting. Plant chemical defences involved in protection against microbial invasion and rotting are likely to have been favoured by human selection, but they are not invariably successful. For long periods of storage (more than a few days, for example), it is necessary to control temperature, water conditions and hygiene (Table 1).

Historians have observed that rice, in contrast to root crops, is a dry and mobile commodity, easily divided and measured, and therefore most suitable for trade economies (Barrau 1966, cited by Chang 1970). The historical significance of the contrast with rice is confirmed by the manner in which taro has become an international trade commodity, in recent years. Modern long-distance trade depends on various combinations of energy-intensive processing, transport and storage (see Plucknett 1979; Nip 1997). Trucks, ships and aircraft are now used for rapid, long-distance transport of fresh taro from the Pacific tropics to temperate Australia, New Zealand and the USA; from Africa and Cyprus to major cities in Western Europe, and from China to Japan. Refrigerated containers have been used to maintain the quality of fresh taro sent to New Zealand (Watson 1979), and frozen corms from Thailand have been sold in New Zealand since at least the mid-1990s (author's observation).

Many countries have been recorded as sources for fresh or frozen taro imported by the USA (Hodge 1954; Hollyer 1990). The surprising fact that non-producing countries such as Finland and France are included implies that taro (most probably frozen) is being stored and traded by middle parties. Small side corms—fresh or peeled and frozen at -18°C—are exported from China to Japan in large quantities, and are then repackaged in 500-g lots for retail. Taro flour was developed as a commercial product in Hawaii by 1910 (Barrett 1910) and is now also produced in China and exported (packets seen in Australia). The main market for this product may be for Chinese cooking (mashed-taro croquettes) and various sweet desserts in Asia. Taro chips (thin slices of corm fried and salted) are now produced in mainland USA and exported to Hawaii (packets seen in Honolulu, 1997). The modern trade succeeds by either overcoming the constraints of weight, indivisibility and short durability, or transforming taro into a lighter, more divisible or more durable products. There is also an emphasis on uniformity.

Before modern storage and transport methods were available, fresh corms or leaves could easily become rotten or lose nutritional value during long-distance transport (in warm conditions the problem for planting materials is less severe; rotting corms often remain viable owing to the presence of numerous buds). An early example confirms the need for rapid transport when distances are great: in 1618 (late Ming period) taro and other delicacies of the Yangtze region or further south were

given priority for barge transport on the Grand Canal, to supply the imperial palace in Peking (Mote 1977:215).

Simple and traditional methods for dehydrating taro have been reported. Corm slices were dried in Hawaii (Pollock 1992:92); cubes were dried in the sun in the Ellice Islands, packed into coconut shells, and could be kept indefinitely in this manner (Kennedy 1931); small corms and side corms were dried (without prior peeling or cutting) over a fire in Taiwan (Matsuyama 1973). In Nigeria, small corm pieces were dried in the sun for the production of flour (Oke and Ojofeitimi 1980). To make this flour, cormels (side corms) were peeled, cut into small pieces, dried in the sun and then pounded with a mortar. In Uganda, cocoyam (taro) leaves were steamed, dried and made into a powder that is stored "up to any length of time", only to be used in mixture with groundnuts and sesame when required (i.e. as part of a condiment) (Semambo 1970). In Japan, the peeled and dried petioles of taro (*C. esculenta* and *C. gigantea*) are commercial products that can be stored for months or years. They can be traded over long distances, but are economically and nutritionally very minor in importance.

Changing tastes and new markets

In Australia, the Americas and elsewhere, there are still many locations and regions where taro is not grown, despite suitable temperate to tropical conditions. There is thus a great potential for developing new production areas and new markets. The main limitations are economic and cultural. Taro is still an unfamiliar food for most Europeans and their descendants. Global warming may improve conditions for growing taro, overall, and production can be mechanized to a degree, but will people continue to use taro if other foods become easier to produce and obtain? How are tastes for taro changing? It is not really possible to generalize, given the huge geographical range of the crop, and the lack of food culture research involving taro.

A common observation in modern industrial societies is that, while food safety and available diversity have increased, the organoleptic (sense-stimulating) qualities of specific crops have declined. In Japan, for example, the poor taste and mouth-feel of mass-produced and stored taro is very evident. This is undoubtedly one reason why the relatively local production and sale of fresh taro continues, on a small scale at least. Lower-quality taro may be tolerated for three reasons: low cost, convenience and inexperience. There are always customers who will try something new at least once, or who have not learned how to distinguish the different grades of taro offered for sale, but such people cannot be relied upon to develop a new market.

How might new markets be developed? This will also depend on food culture research. Throughout an immense geographical range, traditional ways of preparing taro remain largely unrecorded. Methods must exist that are suitable for almost every cuisine. Strategically directed research and promotion could significantly enlarge the markets for taro in many countries, in large urban populations with mixed geographical origins, and among Europeans or others who have inherited European tastes.

Chemical ecology, plant breeding and the future

In this review, I have said very little about the cultural and dietary significance of taro in different societies—as a food for feasts and religious rituals, as a reserve food, as a main or daily food, or as a condiment. My main focus has been the physical nature of taro as a food, and how this relates to culinary knowledge.

The present survey confirms the importance that Johns (1990) and others have placed on plant chemical ecology and plant-processing techniques in the domestication of food plants. Taro is an example par excellence of a crop in which practical culinary knowledge is needed to deactivate or remove chemical components that deter consumption. To date, most studies of plant chemicals in taro have been focused on their initial identification and quantification, their nutritional significance, and their stability during food processing. There has been no sustained effort to explore their genetic basis, natural ecological functioning or agricultural significance. Nor has there been any systematic, wide-ranging study of human responses (physical and cultural) to phytochemicals in taro.

In recent years, agriculturalists have become increasingly aware of the compromises that must be made when attempting to control the direction of crop development, through plant breeding, agronomic research and extension. In the case of taro, if the phytochemicals involved in defence are modified through plant breeding, to make taro more palatable and digestible, this could increase the costs of production and storage by making the crop more susceptible to attack by herbivores and microorganisms.

Johns and Kubo (1988) suggested that detoxification provides a means for exploiting toxic plants that may be vigorous and high yielding because of their defensive capacity. Although domesticated forms of taro are much more easily prepared for eating than wild-type taro, selection for greater palatability and digestibility has probably been constrained by the value of having a crop with its own defensive capacity. A major achievement of early domestication of taro was probably selection leading to an increase in starch yield, since the basic techniques that let people eat taro must have preceded close management and cultivation of the plant. Over time, the acridity of taro was also reduced, but not uniformly across all cultivars. Taro is at present a very heterogeneous crop (see Lebot *et al.* 2010 this volume, Mace *et al.* 2010 this volume, and Yoshino 2002), and there is undoubtedly great potential for modifying its phytochemistry through plant breeding. If the present phytochemical qualities of taro are a result of opposing selection pressures, then it follows that plant breeders might pursue different goals in apparent conflict with each other. This point is illustrated below by proposing two possible scenarios. Cultural responses that may help resolve the conflict are suggested.

Plant breeders in the future might want to increase the edibility of taro by reducing acridity, in corms or leaves, but an alternative response would be to explore culinary methods that maximize the positive qualities of existing cultivars. In a reverse scenario, if herbivorous pests become increasingly serious for taro, then plant breeders might want to increase acridity in corms or leaves, but an alternative response would be to explore production systems that make this unnecessary. By investing more effort in cultural approaches to crop development, it may be possible to avoid situations in which new cultivars are heavily promoted at the expense of existing cultivars with important cultural values, physical properties or economic potential.

There is currently no pressing need to develop taro as a large-scale industrial crop, but there is a pressing need to preserve the plant in all its diversity, as a globally distributed food resource. Fundamental historical research is needed, in the natural and human sciences, to inform the development of new cultivars, production areas and markets.

Agricultural institutions can help by encouraging the preservation of cultivars and culinary knowledge in existing taro-producing areas. Since different cultivars are used in different ways, preserving culinary knowledge is essential for preserving genetic diversity in the crop, and vice versa: without the one, the other may become irrelevant.

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Assessment of cultivar diversity and agronomic characteristics of cocoyam (*Xanthosoma sagittifolium*) in Ghana through ethnobotanical documentation

Carol Markwei, S.O. Bennett-Lartey and Eric Quarcoo

Introduction

Cocoyam (*Xanthosoma sagittifolium*), a member of the Araceae, is found mainly in the high-humidity areas of the tropics It is a native of Central and South America and was introduced into Ghana by West Indian missionaries in 1843 (Wright 1930). It was originally used as a cover crop for cacao and as a consequence followed the spread of cacao into the various geographical zones of the forest belt. It is cultivated in areas with mean annual rainfall of about 1200 mm (Dickson and Benneh 1988).

Different cultivars of cocoyam have been noted in Ghana. Wright (1930) described a cultivar called 'amankani kyirepe' which had a sweet white corm that needed to be boiled for prolonged periods to get rid of poisonous constituents before being eaten. Karikari (1971) indicated that Wright (1930) had described five cultivars of cocoyam. These were referred to as *amankani pa/amankani kokoo*, *amankani fufuo*, *amankani fita*, *amankani Serwaa* and *amankani kyirepe*.

Cocoyam was the second major root crop in the country after cassava (*Manihot esculenta*), but its position has now been taken over by yam (*Dioscorea* spp.). In 1997 estimated production levels were 1535.5 x 10^3 metric tons (Ministry of Food and Agriculture data). The underground cormels are used as a high-energy food while the leaves are used as spinach. Liefstingh (1963) indicated that the leaves had a protein content of 22.17 g/100 g dry weight. In spite of its nutritional importance, cocoyam cultivation has remained largely in the hands of resource-poor, smallholder farmers in the rural areas who still use traditional cultivation methods. Research on the crop has been rather scanty in Ghana. Thus the farmers who form the major group of custodians of the cocoyam germplasm of the country are also the main owners of knowledge about the crop. As the popularity of the crop decreases there is the possibility of loss of some cultivars as farmers select the more marketable varieties for cultivation.

The last published information on cocoyam germplasm in Ghana is more than 30 years old (Karikari 1971). This study, initiated to assess the state of cocoyam germplasm in Ghana and also to collect indigenous knowledge on the agronomic and pathological characteristics of the crop through ethnobotanical studies, is therefore very important for both germplasm conservation and the root and tuber crop improvement programme of the country.

Area of study and methodology

Two geographical regions were randomly selected from the six cocoyam-growing regions in the country. Two districts were then randomly selected from each region and in each district five villages were randomly selected for the studies. In each village, ten randomly selected farmers were interviewed. A structured questionnaire was used to conduct individual interviews with 200 farmers.

There were also visits to randomly selected farms to see the cocoyam crops. Table 1 contains the names of the regions, districts and villages in which the survey was carried out.

TABLE 1. Regions, districts (and their capitals) and villages surveyed

Region	District	Capital	Villages
Eastern	Fanteakwa	Begoro	Adjeikrom, Akoradarko, Amoafoasu, Nkankama, Feyiase
Eastern	East Akim	Kibi	Akim Juaso, Akim Adukrom, Asikam, Potroase, Odumase
Ashanti	Asante- Akim South	Juaso	Odubi, Sarbo, Atiemo, Kwarbeng, Asuboa
Ashanti	Ahafo-Ano North	Tepa	Oldmankrom, Dormaa, Boagyaa 1, Mfante, Akrofonso

Results and discussions

Gender and ethnicity of cocoyam farmers in study

In all the districts surveyed there were both male and female cocoyam farmers; however, male farmers were in the majority (Figure 1). In the Fanteakwa, East Akim and Asante-Akim South districts, 15–31% of farmers interviewed were female. A higher percentage (42%) was recorded in the Ahafo-Ano North district. The lack of current demographic data for the regions made it impossible to explain the gender ratios obtained. In both regions studied cocoyam farmers interviewed were of different ethnicities, although some ethnic groups formed the larger group (Figure 1). In the Fanteakwa district, Krobos formed the major ethnic group (52.5%) followed by the Akims (29.5%). In the East Akim district, on the other hand, the Akwapims were in the majority (32.5%) with the Krobos and the Akims being the second major group (27.5%). All three major ethnic groups are native to the Eastern region of the country. Asantes formed the major ethnic group in both the Asante-Akim South (44.5%) and the Ahafo-Ano North (57.0%) districts. This is not surprising since the area originally belonged to the Asantes before the political regions were created after the country's independence. Akwapims and Kwahus each made up 20% of the farmers interviewed in the Asante-Akim South district while in the Ahafo-Ano North district none of the non-Asante tribes exceeded 9% of the farmers interviewed. There were more farmers from northern Ghana in the Ahafo-Ano North district than in the other districts due, obviously, to the proximity of this district to the northern part of the country. This district also had the largest number of Fantis (9.0%) recorded in the study. The Akwapims were the only ethnic group identified in all four districts. The variation in farmer ethnicity, however, did not influence the farmers' knowledge of cocoyam.

Description of cocoyam germplasm

Most farmers interviewed could not tell the origin of the cocoyam germplasm on their farms because in most farms there had been spontaneous sprouting of cocoyam corms or cormels buried in the soil, after the land had been slashed and burnt in preparation for planting. In all four districts surveyed

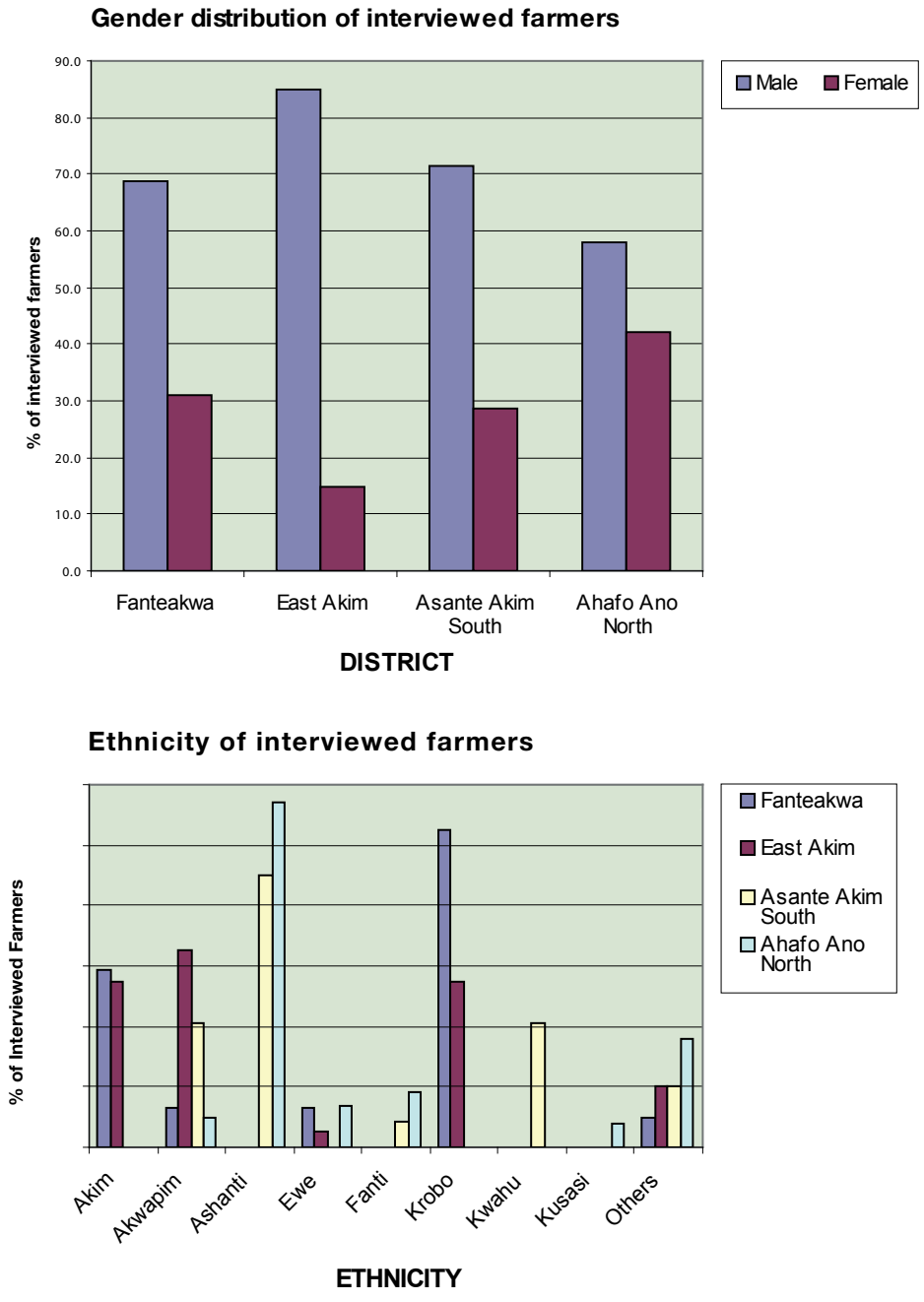

Figure 1. Distribution of gender and ethnicity of interviewed farmers

three cultivars of cocoyam were grown by farmers (Table 2). These were the *amankani kokoo*, the *amankani fita* and the *amankani Serwaa*. The major classification characteristics were the colour of the flesh of cormels and the texture of cooked cormels. The *amankani Serwaa* and the *amankani fita* cormels had white flesh. However, boiled cormels of *amankani Serwaa* were very soft, while those of *amankani fita* were firm. The *amankani kokoo* cormels had pale purplish flesh and were as firm as the *amankani fita* when cooked. Farmers also insisted that the *amankani kokoo* tasted better than the other two cultivars. Karikari (1971) described five cultivars in his study. However, the present study identified only three cultivars. *Amankani fita* and *amankani fufuo*, which Karikari (1971) listed as different cultivars, seem to be the same cultivar with different local names. One farmer in the Fanteakwa district indicated that there used to be a cultivar with yellow-fleshed cormels that was no longer available in the area. Both Wright (1930) and Karikari (1971) mentioned *amankani kyirepe* as a cocoyam cultivar; however, this cultivar was not encountered in the current study. Wright (1930) indicated that this cultivar of cocoyam formed sweet, edible corms, which however required a long period of cooking in order to get rid of poisonous contents. It is therefore possible that there has been selection against it over the years. In all the districts surveyed a majority of farmers (>50%), had two cultivars growing in their fields (Table 2). These were mostly the *amankani kokoo* and the *amankani fita*. Only a few farms contained all three cocoyam cultivars. *Amankani Serwaa* was always only a minor component of the crop. It seems that farmers consciously did not grow this cultivar because of a lower market demand for it. The proportion of farmers with fields containing *amankani kokoo* (Table 3) clearly indicates a preference for this over the other two cultivars. The bias of farmers for *amankani kokoo* could eventually lead to loss of the *amankani fita* and *amankani Serwaa* germplasm. The results suggest that the latter germplasm might disappear faster. The percentage of farmers growing this cultivar was less than 20%.

TABLE 2. Percentage of farmers with indicated number of cultivars in their fields

Number of cultivars	Eastern Region		Ashanti Region	
	East Akim	Fanteakwa	Ashanti-Akim South	Ahafo-Ano North
1	9.1	2.0	4.7	9.8
2	54.5	68.8	79.0	82.9
3	36.4	29.2	16.3	7.3

TABLE 3. Cocoyam cultivars occurring in farmers' fields

Cocoyam cultivar	Eastern Region		Ashanti Region	
	East Akim	Fanteakwa	Ashanti-Akim South	Ahafo Ano North
Amankani kokoo	84.2	94.5	100.0	94.2
Amankani fita	65.7	68.5	100.0	57.1
Amankani Serwaa	18.3	18.6	9.1	5.7

Germplasm conservation

Table 4 shows methods that farmers have adopted for cocoyam germplasm conservation. Both the corms and cormels were utilized as conservation units by the farmers interviewed. Cocoyam corms were the major units of conservation of the plant in the Eastern region. In both the East Akim and Fanteakwa districts more than 80% of the cocoyam farmers interviewed conserved their cocoyam germplasm as corms. In the Ashanti region the percentage of farmers using corms as the conservation units was lower, about 62% and 48%, respectively, for Asante Akim South and Ahafo Ano North. Corms were conserved *in situ*, in farmers' fields buried under leaves or under a thin layer of soil in the shade. Some farmers (>20% in the East Akim, Eastern Region and >30% in the Asante Akim South district) stored the corms for a few months at home either in jute bags or in open cribs under shady conditions. Fewer farmers in the Eastern region conserved their germplasm as cormels. Only about 20–30% of the farmers interviewed indicated that they conserved their cocoyam germplasm as cormels. In the Ashanti region, however, the percentage using cormels as conservation units was higher: 38.1% for the Asante Akim South and 55% for the Ahafo Ano North district. Farmers preferred the corms because plants from corm segments formed cormels earlier than those from cormel segments.

Growth characteristics of cocoyam

Cocoyam plants usually sprouted as multiple shoots, which were then thinned out. This was especially the case when plants grew spontaneously after land preparation by slashing and burning. Farmers believed that these fallow lands were old abandoned farms on which cocoyam had grown previously and that dormant cocoyam corms were activated for growth by the fire. Planted corm setts generally resulted in the formation of single shoots since the sett would normally have only a single bud. The majority of farmers (72–94%, Table 5) have noticed flowering in cocoyam although they agreed that only a few of the plants on their farms flowered. Flowering generally occurred in plants that were 6–12 months old. Soil fertility was considered by the majority of these cocoyam farmers—between 66 and 91% of the farmers interviewed—as the most important factor influencing flowering. Factors such as plant vigour, age and the type of planting material were of little importance in determining whether or not a plant will flower. In both regions farmers noticed flowering during the harmattan season (November–February), the same period during which cormels were harvested. There are no published studies on dormancy of cocoyam corms or factors influencing flowering under natural conditions in farmers' fields.

TABLE 4. Methods of germplasm conservation by cocoyam farmers

Method of conservation	Percentage of interviewed farmers carrying out procedure			
	Eastern Region		Ashanti Region	
	East Akim	Fanteakwa	Asante-Akim South	Ahafo-Ano North
Fresh corms buried on farm	69.2	82.5	31.0	47.5
Fresh cormels buried on farm	23.0	19.3	35.7	27.5
Fresh corms stored at home	27.7	8.9	30.9	0.0
Fresh cormels stored at home	7.7	5.3	2.4	27.5

TABLE 5. Assessment of the flowering characteristics of cocoyam by farmers

Details of flowering	Percentage of farmers which made observation			
	Eastern Region		Ashanti Region	
	East Akim	Fanteakwa	Asante-Akim South	Ahafo-Ano North
Flowering detected	94.4	89.8	91.9	72.3
Flowering not detected	5.6	10.2	8.1	27.7
Age of plant at flowering:				
6-8 months	32.4	22.1	35.6	43.8
12 months	64.7	57.6	28.9	34.4
18 months	2.9	18.6	4.4	0.0
24 months	0.0	5.1	8.9	9.4
Factors affecting flowering:				
Soil fertility	90.9	66.7	91.3	81.8
Plant age	0.0	8.3	0.0	4.5
Vigour of plant	1.9	16.7	8.7	9.1
Flowering season:				
Rainy season	19.4	7.5	4.4	3.1
Harmattan	80.6	90.6	91.2	96.9

Cocoyam crop management by farmers

Cocoyam plants in farmers' fields were from three sources: corm setts with one or two buds, spontaneously sprouted plants, or whole corms. The first two sources were the major means by which farmers produced cocoyam crops. Between 70 and 96% of farmers in every district planted corm setts. In the Eastern region 63–80% of farmers interviewed also had plants that had spontaneously sprouted on the farms, while in the Ashanti region 74–75% had spontaneously sprouted plants on their farms (Table 6). In all the areas surveyed farmers relied solely on rainfall for the cultivation of their cocoyam crop. This was not surprising since farming in Ghana is rain-fed agriculture. Cocoyam was cultivated without any fertilizer input by the majority of farmers surveyed. A small minority (5%) in the East Akim district, however, fertilized their crop with organic manure. Cocoyam is among the first crops to grow on land that has been left fallow for a long period. These lands are quite fertile and farmers, therefore, did not find the need to use fertilizers. Opoku (1963), in his study on fertilization effect on cocoyam, did not record any increase in yield as a result of fertilizer application. Karikari (1971) suggested that this lack of response to fertilization might be due to high soil fertility, so that cocoyam plants were giving their maximum yield in that soil and additional fertilization did not increase yield. Cocoyam was cultivated mainly in a mixed cropping system by 80–98% of all farmers in the survey. A few farmers in each region studied grew cocoyam

as a monocrop. In the Ashanti region and the East Akim district of the Eastern region fewer than 10% of the farmers monocropped their cocoyam, while in the Fanteakwa district of the Eastern region 17% cultivated it as a monocrop.

TABLE 6. Farmers' response to various cultural practices

Agronomic practice	Percentage of farmers using technique			
	Eastern Region		Ashanti Region	
	East Akim	Fanteakwa	Asante Akim South	Ahafo Ano North
Propagation unit:				
Whole corm	17.5	21.3	22.4	14.0
Corm sett	70.0	96.1	83.8	88.0
Cormel minisetts	0.0	0.0	12.2	8.0
Spontaneously sprouted plants	80.0	63.9	75.4	74.0
Crops irrigated	0.0	0.0	0.0	0.0
Rain-fed crops	100.0	100.0	100.0	100.0
Crops fertilized	5.0	0.0	0.0	0.0
Crops not fertilized	95.0	100.0	100.0	100.0
Cocoyam monocropped	7.4	17.4	2.4	6.4
Cocoyam mixed with other crops	92.6	82.3	97.6	93.6

Cassava (*Manihot esculenta*) and plantain (*Musa* sp.) are the major crops grown in association with cocoyam (Table 7). Other crops found mixed with cocoyams are yams (*Dioscorea* spp.), pepper (*Capsicum annuum*), oil palm (*Elaeis guineensis*), maize (*Zea mays*), and eggplant (*Solanum* spp.). The minor species grown in association with cocoyam varied among the districts surveyed. In the Fanteakwa district more than 10% of farmers grew maize and water yams together with cocoyam, while in the East Akim district very few (<10%) farmers had crops other than cassava and plantain in the same field as cocoyam. In the Asante-Akim South district yam was the third most important crop on cocoyam farms (about 10% of farmers). In the Ahafo Ano North district 14.9% of farmers interviewed planted cocoyam as a cover crop for the young cacao seedlings.

Light conditions required for growth

Farmers interviewed indicated that the growth of cocoyam plants was affected by both light regime and soil type. In the East Akim, Asante Akim South and Ahafo Ano North districts, the majority of farmers in the survey (66–72.7%) noticed that plants grew more vigorously under partial shade than in full sunlight (Table 8). In the Fanteakwa district, however, farmers were equally split between vigorous growth under partial shade and full sunlight. In all the regions, however, there was agreement that deep shade was not suitable for cocoyam growth.

TABLE 7. Species intercropped with cocoyam

Crop	Percentage of farmers using species in mixed cropping			
	Eastern Region		Ashanti Region	
	East Akim	Fanteakwa	Asante Akim South	Ahafo Ano North
Cassava	60.0	69.5	93.9	85.1
Eggplant	2.9s	3.6	0.0	0.0
Maize	8.6	14.4	4.1	6.3
Okra	5.8	5.4	0.0	0.0
Oil palm	2.9	0.0	0.0	0.0
Pepper	3.6	5.8	0.0	2.1
Plantain	88.6	75.0	100.0	91.5
Water yam	5.7	10.8	2.0	0.0
Yam	3.4	14.2	10.2	0.0

TABLE 8. Farmers' response to light requirements of cocoyam plants

Environmental factor	Percentage of farmers indicating requirement			
	Eastern Region		Ashanti Region	
	East Akim	Fanteakwa	Asante Akim South	Ahafo Ano North
Light regime:				
Full sunlight	24.2	48.2	26.5	30.0
Partial shade	72.7	46.0	69.4	66.0
Deep shade	3.1	5.8	4.1	4.0

Harvesting of cocoyam

Both the cormels and foliage (i.e. leaf blades) of cocoyam are harvested for food by most farmers. The time course for harvesting the different plant parts varied significantly in the two regions studied (Table 9). In the Eastern Region cormels and foliage were commonly harvested at the same time. Between 89% and 95% of the farmers in the survey harvested cormels and foliage together in the East Akim and Fanteakwa districts. In the Ashanti region 70% of farmers harvested cormels and foliage together, while a lower percentage of farmers (53%) did this in the Ahafo Ano North district. In both regions farmers indicated that the time required for cormel maturity was dependent on soil fertility. Cocoyam plants were often harvested in batches and the remaining cormels were

left underground until farmers were ready to sell them. Foliage was harvested before cormels, but only after plants were six months old, in both districts of the Eastern region. In the Ashanti region, however, a few farmers—21% in the Asante-Akim South district and 28% in the Ahafo-Ano North district—harvested foliage from plants less than six months old. In all the districts only a few leaves were harvested at a time, mainly for home consumption. Farmers maintained that very few cormels formed when there was continuous foliage harvest. Since cormels had a higher market value, extensive foliage harvest was not practised. The regional difference in the timing of first foliage harvest may reflect different eating habits in the two regions. Cocoyam foliage is used to prepare many more dishes in the Ashanti region than in the Eastern Region. In every district, a small minority of farmers (<10%) did not harvest the foliage of their cocoyam plants at all.

TABLE 9. Harvesting regimes for cocoyam crops

Harvesting practice	Percentage of farmers employing regime			
	Eastern Region		Ashanti region	
	East Akim	Fanteakwa	Asante Akim South	Ahafo Ano North
Foliage and cormels harvested together	94.6	89.3	70.8	53.1
Foliage and cormels harvested separately	5.4	10.7	29.2	46.9
Age of plant at 1st foliage harvest:				
<6 months	0.0	0.0	20.5	27.9
6-8 months	30.3	34.6	35.8	37.2
12 months	60.6	53.8	35.9	32.6
24 months	3.0	3.8	0.0	0.0
Foliage not harvested	6.1	7.8	7.8	2.3
Age of plant at cormel harvest:				
6-8 months	15.4	5.9	22.2	15.2
12 months	69.2	86.3	66.7	60.9
18 months	15.4	3.9	2.2	13.0
24 months	0.0	3.9	8.9	10.9

Cooking properties of cocoyam cormels

The cormels of the cultivars *amankani kokoo* and *amankani fita* are firm when boiled and of a sticky consistency when pounded. They are both used to prepare the *fufu* dish in all districts except the East Akim district where, because the pounded *amankani fita* looked like pounded cassava, the cultivar was not used for preparing this dish because pounded cassava was considered food for the poor. The two cultivars are also boiled and eaten. *Amankani Serwaa* was softer when boiled and required less cooking time than the other two cultivars. It was used mainly for a porridge dish. All three cultivars were also fried or roasted as snacks.

Other uses of cocoyam

Farmers used various parts of the plant for treating different ailments. In all four districts, scrapings of the uncooked corm or cormel flesh were applied to cuts to stop bleeding of wounds. A few older farmers mentioned other medicinal uses. In the Fanteakwa district, corm and cormel flesh scrapings were used as anti-tetanus and anti-poison agents. In the latter case they were used specifically to treat tarantula, scorpion and snake bites. In this district and also in the Ahafo-Ano North district, corm and cormel flesh scrapings, as well as mashed fresh leaves, were used to treat toothache. In the Asante Akim South district fresh cocoyam leaves were bandaged on the swollen navels of children to shrink them. In the Eastern Region some farmers also used the cocoyam foliage as poultry feed.

Conclusion

The study indicates that there has been some loss of certain cocoyam cultivars, e.g. *amankani kyirepe*, mentioned by both Wright (1930) and Karikari (1971). The market preference for *amankani kokoo* has resulted in the neglect of the other two surviving cultivars. *Amankani fita* and *amankani Serwaa* may face the risk of being lost unless they are deliberately cultivated for purposes other than human consumption. Medicinal uses could be expanded for these cultivars, and the production of industrial starch is a possibility.

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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).

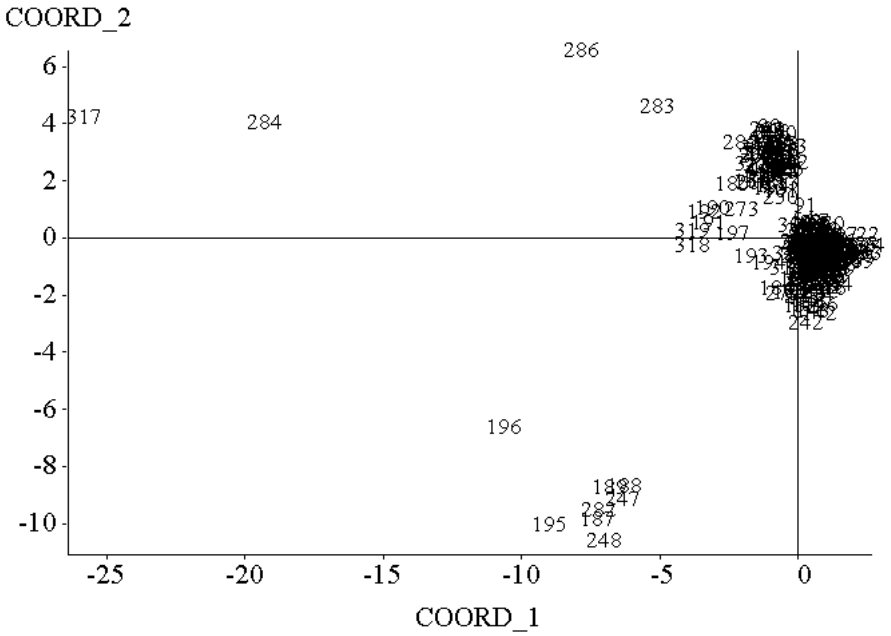

Figure 1. Distribution of 319 taro zymotypes in plane of a principal components analysis of isozyme variation in 2081 cultivars originating from seven countries of Southeast Asia and Oceania. Two major zymotypic groups are identified by cluster analysis using DICE coefficient and UPGMA on the matrix 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

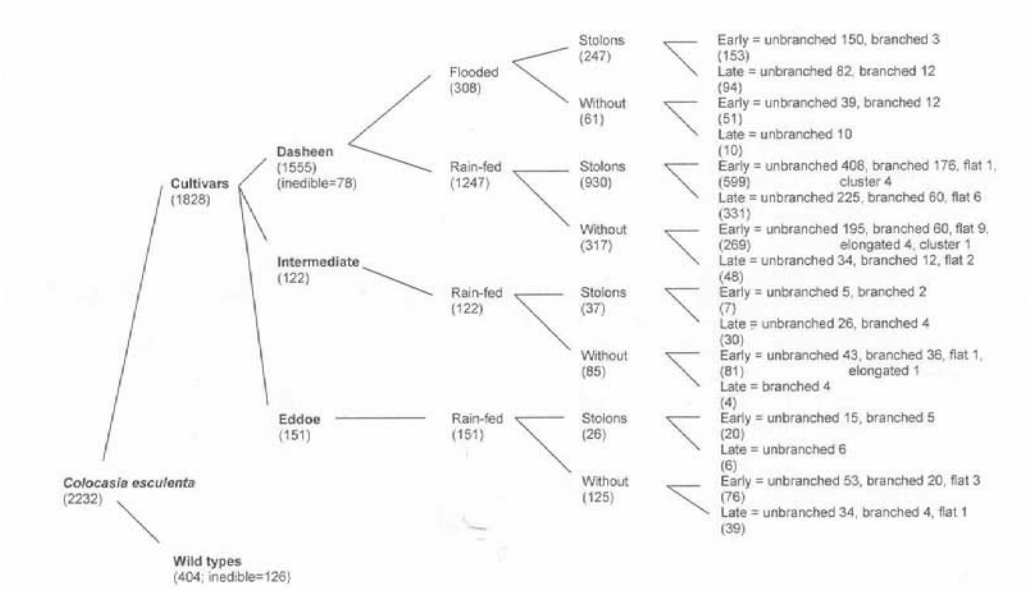

Figure 2. Branching method used to classify accessions in taro germplasm collections.

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

This study would not have been possible without the support of the Taro Network for Southeast Asia and Oceania (TANSAO), a project funded by the INCO-DC programme of the European Commission, Directorate General XII, Contract number ERBIC18CT970205.

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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'.]

A study on isozyme variation in the Indonesian taro (*Colocasia* spp.) germplasm collection

M.S. Prana, S. Hartati and T.K. Prana

Introduction

As an indigenous crop, taro (*Colocasia esculenta*) is no doubt one of the oldest staple food crops for people living in the more humid or wet parts of the Indonesian archipelago. Apart from the Papua Province (formerly known as Irian Jaya), remnants of its utilization as a staple can be observed in the Mentawai islands (Siberut, Pagai, etc.), Toraja district (South Sulawesi), Sangir and Talaud islands (North Sulawesi), and even in Java in districts such as Banten (West Java) and Magetan (East Java), where rice is cultivated and consumed by the majority of the population.

Since Indonesia is part of the centre of origin for taro, and is also a centre of domestication/ cultivation, it is quite natural to expect a wide range of genetic diversity among taros in this country. Such diversity can be easily expected by looking at the morphological variation in corm, stolon, leaf and floral characters. It has been estimated that the existing collection in Cibinong, in Bogor, contains no less than 200 distinct morphotypes. Moreover, after studying isozyme variation among taros from Southeast Asia (including some from Indonesia) and the Pacific islands, Lebot and Aradhya (1991) concluded that morphological characters did not necessarily link with isozymic characters. It was therefore of interest to do a similar study with more extensive sampling from within the Indonesian archipelago in order to discover whether or not the previous conclusion is relevant to Indonesia.

Materials and methods

The 677 samples used in the present study were collected during 1998–2000, from various parts of Indonesia, namely Java, Sulawesi, Sumatra, Kalimantan, Bali, Lombok and Flores. They were all grown at a field station belonging to the R&D Center for Biotechnology–LIPI, at Cibinong in Bogor.

Six enzymes were analyzed, namely MDH (Malate dehydrogenase), PGI (Phosphogluco isomerase), 6-PGD (6- Phosphogluconate dehydrogenase), IDH (Isocitrate dehydrogenase), ME (Malic enzyme) and SDH (Shikimic dehydrogenase).

Sample preparation and electrophoresis were carried out as described by Hartati and Prana (1999), who modified the methods described by Lebot and Aradhya (1991). The modifications mostly concerned sample preparation and buffer composition.

Results and discussion

Isozyme profiles

Isozyme profiles were identified using letters, exactly as employed by Lebot and Aradhya (1991). New profiles were given new designations. All the profiles are shown in Figure 1. From our survey it appears that some of the profiles previously reported in other regions were absent in Indonesia (profile E of 6-PGD, C of IDH, and D, F of ME). No new profiles were observed for SDH, indicating

that five is perhaps the maximum number of profiles (variation) for this enzyme. However, 10, 4, 7 and 4 new profiles were recorded for MDH, PGI, 6-PGD and ME, respectively. These new records of variation are not at all surprising, since only 50 Indonesian samples were included in the survey by Lebot and Aradhya (1991).

The number of profiles obtained varied greatly among the enzymes (Figure 1). The degree of genetic diversity associated with each enzyme is presumably proportional to the number of different profiles displayed. The biggest number of profiles was observed for 6-PGD (16), followed by MDH (14), PGI and ME (8 each), and IDH and SDH (5 each).

Plant zymotypes

The plant samples were not originally collected in the field in a completely random manner. Nevertheless, since the overall collection was very large, and represented many locations, it may be quite representative for the whole country. The most common zymotype (8.57%) was AADAAA (found in cultivar 'Bentul'), followed by zymotype HADAAA (6.50%, mostly found in cultivar 'Sutera') and finally zymotype AADAAC (6.35%, found mostly in cultivar 'Bogor'). Surprisingly, 17.43% of the zymotypes were represented in the collection by only one sample. This indicated the fragility of their condition from a conservation view point. The cultivar concerned could be lost at any time.

Geography

Wide isozyme (genetic) variation was observed among the samples from West Java, South Sulawesi, and Lampung (southern Sumatra). This is not surprising for at least two reasons. Firstly, those places are well known as taro-growing areas, and secondly, more samples were obtained owing to more intensive collecting efforts in those places. If similarly intensive exploration and collection could be carried out in Irian Jaya/Papua, North Sulawesi, Jambi and Riau, a similar amount of variation might be found.

Morphology and isozyme variation

Some samples that appeared identical on the basis of morphological characters, and that were believed to belong to a certain cultivar, were found to display different isozyme profiles. Conversely, some samples that were morphologically quite distinct showed exactly the same isozyme profiles. These results confirmed the findings of Lebot and Aradhya (1991) and Prana *et al.* 1999,

that morphological characters are not necessarily correlated or linked with the isozymic characters. This further suggests that either geneflow has happened quite freely among the taro cultivars or that somatic mutation is a common phenomenon in this particular plant, or perhaps both. Prana (1984) suggested that interspecific crosses are possible in taro.

Zymotypes and cultivars

Based on similarity coefficients derived from the isozyme profiles for all six enzymes, a dendrogram was constructed for all 192 zymotypes (Figure 1). Most samples of both *C. esculenta* and *C. gigantea* show a relatively high degree of similarity (over 76%), but a few zymotypes (nos. 176, 178, 191, 180, 105 and 166) were more or less well separated from the others (similarity coefficient <68%). Interestingly, these all had a single representation in the collection, and at least two of them (105 and 178, represented by accessions 265 and 266 respectively) were obtained from the southeastern part of Jakarta, which indicated that they are both valuable (distinct zymotypes) and vulnerable (may be

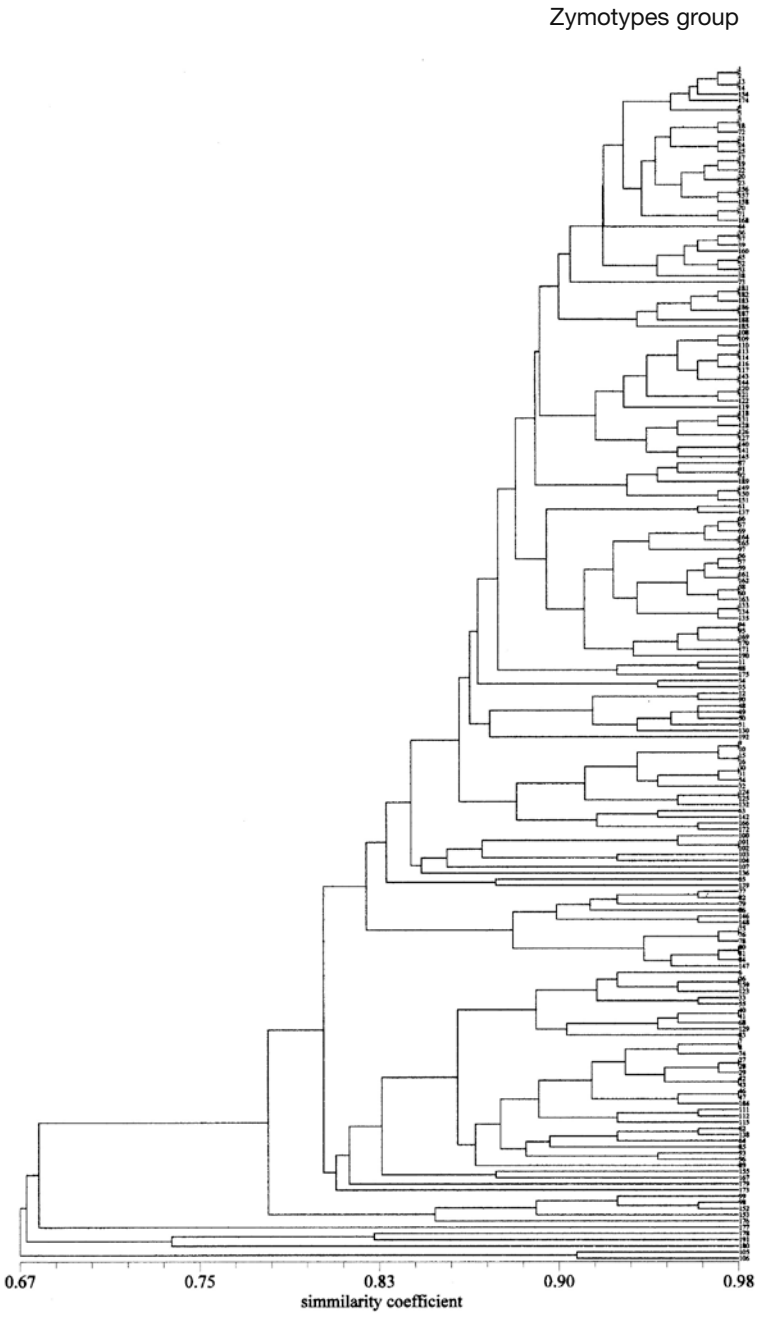

Figure 1. Dendrogram of taro based on six enzyme systems.

lost at any time because there is limited gardening space in such a big city, and people do not normally grow more than five taro plants in each yard).

Urgency of germplasm collecting

For various reasons Indonesians have shifted from their traditional diets, in whichdiverse staple foods were consumed, and are consuming mainly rice. People in the drier parts of Java, for example, used to eat maize, sweet potato or cassava. In the wetter areas of Sumatra, Kalimantan, Sulawesi, certain parts of Java, Maluku and Papua (Irian Jaya) they lived on either taro or sago, or both. However, most people have gradually come to believe that rice is not only more nutritious, but also much more prestigious than traditional foods. Therefore all those traditional staple foods have been gradually replaced by rice. The process is still going on even today. For taro, one consequence has been a decrease in the acreage of cultivation in many places, and this has led to a loss of cultivars and genetic diversity. Meanwhile in the taro-growing centres, especially in Java, where taro is grown as a cash crop, commercially superior varieties have been replacing the inferior ones. In Bogor and Sumedang (both in the West Java province), for example, 'Bentul' and 'Semir' respectively are the dominant cultivars grown. In Malang (East Java) 'Bentul' cultivars are preferred by the locals. For these reasons in Indonesia, taro has been undergoing serious genetic erosion.

From the foregoing discussion and our present results, it is quite obvious that collecting of diverse taro cultivars is urgently needed, while they are still available. Most taro-growing areas have been covered by the collecting mission using the project budget, but some important areas are yet to be explored (North Sulawesi–especially Sangir and Talaud islands, Riau–the islands group, Jambi, North Sumatra, Maluku, and perhaps also Irian Jaya/Papua).

Acknowledgement

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Taro germplasm collection in Vietnam

Nguyen Thi Ngoc Hue, Nguyen Van Viet, Vu Linh Chi and M.S Prana

Introduction

Vietnamese farmers have been growing taro for generations in a wide range of agroecological conditions in the country, from 8°N to 23°N latitude and from 102°E to 110°E longitude, from lowlands to highlands, with altitudes from 5 to 1800 m asl.

In Vietnam, taro is common in both home gardens and the larger agricultural production systems of the middle hills because it is easy to cultivate, propagate and store. The keeping quality of taro tubers is much greater than that of any other tuber crop. In the field, taro is mostly found as a mixed crop with upland rice, ginger and maize in a shifting cultivation system in mountain areas, and as an intercrop with sweet potato, legume or vegetables in lowlands and mid-elevations. Taro is used either as food for humans and animals, and for medicinal or ornamental purposes.

Taro cultivation in Vietnam is mainly practised by the smallholders. It is cultivated in two cropping seasons: the main crop is planted in paddy fields in October–December, after summer rice is harvested. Planting of the second crop starts during the wet season, in April or May, mainly in upland areas. No official statistics are available for the production of taro in Vietnam, but it plays an important role in household food security. Every household maintains and grows a few varieties of taro. It is estimated that the extent of taro cultivation reaches approximately 15 000 ha per annum, ranking fourth after sweet potato, cassava and potato. The average corm yield is about 10 t ha^{-1}.

In the past, taro was a much more important crop. With the development of modern agriculture and the green revolution, taro production started to decline, mainly because of a lack of processing facilities and markets. This root crop, which is widely consumed and valued for both nutrition and commerce, has been relatively neglected in research and conservation. Because taro is mainly vegetatively propagated and its commercial importance is largely local, less attention has been paid to the collecting and conservation of taro genetic resources. With increased crop improvement of other crop species, resulting in the release of new crop cultivars, and commercialization in food and vegetable production, the loss of taro germplasm is becoming more and more serious.

In Vietnam there has been no research on taro genetic resources, or any work to understand how taro genetic resources are affected by the different systems used for growing and using taro. Therefore, since 1994, taro genetic resources have been collected throughout the country and collections of taro have been established. The objectives of the study are to characterize the Vietnamese taro accessions by their morphological and agronomical traits, and to determine the extent of genetic variation, geographical distribution of genetic diversity and breeding value, and to select core samples for the establishment of a taro core collection in Vietnam. This report presents the research results on the taro germplasm collection.

Materials and methods

This study included 350 taro accessions collected throughout Vietnam in the period 1994–96. These accessions were described during 1997–99 using IPGRI descriptors (IPGRI 1999). The susceptibility

of accessions to Taro Leaf Blight (TLB) was measured under natural inoculum pressure and favourable conditions for disease development. Accessions were electrophoresed on starch gels and six enzyme systems: malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), isocitrate dehydrogenase (ICD), 6-phosphogluconate dehydrogenase (PGD), malic enzyme (ME) and shikimate dehydrogenase (SKDH). 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. The data gathered were analyzed using appropriate statistical software.

Results and discussion

Characterization and evaluation of germplasm collection

The morphological characterization of 350 accessions was conducted to identify accessions and eliminate duplicates in the collection. The morphological variation of 350 accessions was scored for important highly heritable traits. The range of variation for these characteristics in the taro collection is presented in Table 1.

Characterization results indicate that these accessions show wide variability with regard to morphological traits such as colour, shape and size of tuber, petiole length and colour, and stolon formation. Most accessions can be clearly differentiated as dasheen (39.8%) or eddoe types (35.7%) and 24.5% were found to be intermediate. Vietnamese like both the eddoe and dasheen types and both types are cultivated and sold in markets.

The characteristics of lamina orientation, L/W ratio of lamina, maturity and lamina colour have a narrow range of variation, while variation was higher for traits such as junction colour, petiole colour, corm shape, L/W ratio of corm and eating quality of corm. These characteristics can be used for classifying the accessions into varietal groups.

The distribution of taro accessions, based on eight important characteristics, as presented in Table 2, also demonstrates the presence of wide genetic variability in Vietnamese taro. The results of the characterization were used for rationalization of the collection. However, the collecting site, as recorded in the passport data, was used to ensure that accessions, which were collected at distant locations but were identical to others according to morphological characterization, were not eliminated.

Classification of accessions based on the different approaches was conducted. Table 3 lists the different genetic resources identified. We believe that these data will be useful to taro breeders for selecting desirable genetic material for different uses.

Results of isozyme study

A total of 201 taro accessions in Vietnam were electrophoresed. Two distinct groups, including 74 zymotypes, were identified based on the isozyme profiles obtained after analysis. Distribution of the accessions based on the zymotypes shows the AACAAA zymotype in up to 20 accessions, followed by the AACAAC zymotype (17 accessions). Some zymotypes include 8–10 accessions. Forty-eight zymotypes had only one accession each. The resultant dendrogram, illustrating genetic similarity of taro accessions, shows that there is significant genetic diversity existing in the taro germplasm collection of Vietnam. Statistical analysis (STATITCF) of 201 accessions, based on 15

main morphological characteristics, identified 75 phenotype groups. From these results, possible duplicates in the collection were first identified.

The results of analyzed isozymes were compared with the morphological characters. The comparison showed that zymotype variation was not fully parallel with the morphological one. Many taro cultivars representing distinct morphotypes appeared to have identical zymotypes. No correlation was found between morphological patterns and isozyme banding patterns. Results are similar to those of Lebot and Aradhya (1991) and Li Xixiang *et al.* (1998). Gene expression and regulation, and enzyme production are all complicated processes.

TABLE 1. Range of variation of some important characters in taro collection

Character	Min.	Max.	Mean/Mode	CV%
Growth habit	1	2	1.27	–
Stolon formation	1	3	1.15	–
Plant height (cm)	30	169	78.8	31.3
Leaf lamina shape	1	4	3	–
Lamina orientation	3	6	4	–
Lamina colour	3	4	3	–
Outline of the sinus	1	4	2	–
Vein junction colour	1	8	4.99	–
Petiole colour	1	6	2.31	–
Flowering formation	1	3	1.29	–
Maturity period	2	5	2.98	23.6
Corm shape	1	9	6	–
Corm/cormel weight	1	3	1.3	–
Corm flesh colour	1	8	1.2	–
Eating quality	1	6	4	–
Reaction to TLB	2	5	3.77	–
Ratio L/W of lamina	1.0	1.8	1.39	8.1
Ratio L/W of corm	0.09	5.1	1.24	45.8

Based on evaluation data, the five best taro varieties were selected: VN183 and Vn117 (eddoe), VN3 (intermediate) and VN29 and VN125 (dasheen). The general characteristics of the five promising taro varieties are presented in Table 4.

TABLE 2. Distribution of taro accessions based on the important characters

Character		No. accessions	Percent	Examples
Growth habit	Erect	260	74.3	VN003, VN 005
	Semi-erect	90	25.7	VN002, VN009
Stolon formation	Absent	318	90.9	VN017, VN019
	Partly absent	20	5.7	VN188, VN190
	With stolon only	12	3.1	VN189, VN196
Plant height	Dwarf	45	12.9	VN007, VN009
	Medium	263	75.1	VN024, VN025
	Tall	36	10.3	VN017, VN005
	Very tall	6	1.7	VN196
Petiole colour	Light green	28	8.0	VN044, VN067
	Lt. green + upper purple	110	31.8	VN042, VN59
	Dark green	16	4.5	VN135, VN153
	Dk. green and darker top	3	1	VN82
	Dk. green + dk. green lines	3	1	VN127
	Dark green + purple lines	113	32.3	VN009, VN024
	Red purple	5	1.5	VN57
	Lt. purple + lt. green lines	5	1.5	VN174
	Dark purple	40	11.4	VN021, VN049
	Dk. purple + lt. green lines	5	1.5	VN122
	Dk. purple + dk. green lines	12	4.0	VN103, VN167
	Dk. purple + purple lines	2	0.5	VN046
	Brown-purple + dk. green lines	5	1.5	VN170, VN180
Vein junction colour	Whitish	1	0.3	VN140
	Yellow	50	14.3	VN082, VN055
	Light green	63	18.0	VN070, VN050
	Dark green	11	3.1	VN036, VN220
	Light purple	80	22.9	VN138, VN106
	Dark purple	51	14.6	VN191, VN239

Character		No. accessions	Percent	Examples
Vein junction colour	Red	1	0.3	VN190
	Not uniform	93	26.5	VN151, VN153
Eating quality	Not edible	9	2.6	VN201, VN196
	Poor quality	15	4.3	VN031, VN052
	Acceptable	107	30.5	VN012, VN025
	Good	171	48.9	VN022, VN023
	Very good	40	11.4	VN010, VN005
	Excellent	8	2.3	VN047, VN054
Corm shape	Unbranched round	39	11.1	VN019, VN055
	Unbranched dumb-bell	16	4.6	VN189, VN180
	Unbranched conical	57	16.3	VN003, VN004
	Unbranched elliptical	57	16.3	VNO20, VN029
	Unbranched cylindrical	8	2.3	VN064, VN158
	Branched	145	41.7	VN008, VN006
	Branched head	7	2.0	VN070, VN068
	Elongate	4	1.1	VN089, VN167
	Flat	16	4.6	VN018, VN059
Maturity period	Early	78	22.3	VN183, VN121
	Intermediate	214	61.1	VN179, VN180
	Late	54	15.1	VN190, VN189
	Very late		1.1	VN195, VN201

The genes determining enzyme production may be different from those affecting morphological character. There was no distinction between the eddoe and dasheen types based on zymotype analyses.

According to Dr Made Sri Prana, Vietnam has 43 unique zymotypes (pers. comm.) that are not found in other Southeast Asian countries. Once again, these results show the significance of genetic diversity in taro accessions in Vietnam.

The richness in genetic variability displayed by the phenotypic variation and occurrence of characters, is an indication of a wild type retained in some of the cultivars. This, plus the occurrence of the true wild types of taro, support the idea put forward by Lebot and Aradhya (1991) that Indonesia, Thailand and Vietnam could well be the centres of origin of taro.

TABLE 3. Accession classification based on the different approaches

Approach	No. of accessions	% of total
Germplasm type		
Cultivated	329	94.0
Wild	3	0.86
Feral	18	5.14
Undetermined	0	0
Growing conditions		
Flooded	207	59.14
Rain-fed	143	40.86
Not determined	0	0
Altitude		
Lowlands	207	59.14
Mid-elevation	115	32.86
High altitude	26	7.43
Not determined	2	0.57
Maturity period		
Early (4–8 months)	222	63.5
Late (8–11 months)	57	16.2
Not determined	1	0.29
Based on the reaction to TLB		
Immune	78	22.29
Resistant	144	41.14
Tolerant	122	34.86
Susceptible	6	1.71

Given the isozyme analysis results a core taro collection can now be established in Vietnam.

Study of geographical distribution of accessions

Taro is grown throughout Vietnam. The data presented in Table 5 show the location and number of distinct taro accessions collected so far from each of the major regions of the country. Table 5 also presents the geographical distribution of accessions exhibiting the 74 distinct zymotypes. The number of zymotypes was highest in Caobang province, followed by Son la, Lai chau, Lao cai, Hoa binh and Quang ninh provinces. It can be observed that the northern mountainous zone

has greater taro diversity than other zones in the country. Provinces located in the subtropical zone have a swidden farming system, which alternates between subsistence farming to market-oriented system. The cultivars collected from this region—managed by the Tay, H'mong, Dao, Thai and Xa people—were shown to have the greatest diversity. They were generally planted in swidden, rain-fed uplands, irrigated paddies, home gardens or orchards, mainly in mountain areas. Perhaps the diverse microecological conditions, geographical isolation, and selection based on people's hobbies and cultures affected the development of taro diversity and distribution in this hilly area of Vietnam.

TABLE 4. Promising taro varieties and their general characteristics

Varietal information	Eddoe		Intermediate	Dasheen	
	VN183	VN117	VN3	VN29	VN125
Local name	Khoai lui som	Mon do	Hau bac	Hau pe	Khoai mon
Corm yield† (t/ha)	15.54	17.52	16.14	20.22	18.24
Dry matter content (%)	24.5	31.4	30.5	26.6	23.0
Maturity period (months)	5	6	6	6-7	6-7
Reaction to TLB (score)	6.3	5.3	5.3	6.0	5.5
Reaction to leaf spot (score)	2.8	1.5	4.8	3.0	2.0
Growth habit	Dwarf, erect absent stolon	Medium, erect, absent stolon	Medium, erect, absent few stolons	Medium erect with few stolons	Medium, erect, with few stolon
Foliage colour	Dark green with purple lines petiole, light green junction colour	Dark green with purple line petiole. Not uniform junction colour	Dark green with purple lines petiole, light purple junction colour	Dark purple petiole with light purple junction	Light green petiole, light green junction colour
Eating quality	4.3	4.7	4.3	4	5
Corm shape	Round	Round	Round	Conical	Conical
Ratio L/W	0.88	1.02	1.08	1.30	1.35
No. of corms/plant	13.7	12.7	18	17.8	11

† Under medium input conditions. CV=24.7%, LSD= 4.2 (T/ha).

A study of the distribution of different taro zymotypes in the provinces of Vietnam has shown that duplicate accessions can be limited. The data show that there are 28 duplicates among the 201 accessions that were analyzed.

TABLE 5. Number of zymotypes, number and location of collected accessions

Agroecological zone/Ethnic minority	Province	No. accessions	No. zymotypes
North west mountain: Thai, H'mong,	Hoabinh	13	11
Dao, Tay, Nung, Muong, Xa, Kinh	Sonla	16	14
	Laichau	18	12
	Yenbai	10	7
	Laocai	12	11
North east mountain: Kinh, H'mong,	Hagiang	4	3
Dao, Sandiu, Hoa, Tay, Thai, Nung,	Tuyenquang	2	2
Cao lan, Muong	Caobang	21	18
	Bacthai	6	6
	Langson	6	6
Midlands: Kinh, Tay, Thai, Nung	Habac	8	7
	Ninhbinh	1	1
	Quangninh	11	11
	Vinhphu	3	3
North central: Kinh, H'mong, Dao,	Thanhhoa	3	3
Vankieu, Catu, Muong	Nghean	6	6
	Quangbinh	8	3
	Hatinh	4	7
	Quangtri	10	6
	Hue	4	2
South central: Kinh	Quang nam	4	3
	Quang ngai	3	1
	Binh dinh	3	2
Highlands: Kinh, Khome	Lam dong	3	2
	Gia lai	2	2
Red river delta: Kinh	Hanoi	4	3
	Nam dinh	3	3
	Thai binh	2	2
Mekong river delta: Kinh, Khome	Tiengiang	3	3
	Tayninh	2	1
Total		201	74*

Conclusion

In Vietnam, the indigenous taro collection contains 350 accessions. Taro accessions were characterized using IPGRI descriptors (IPGRI, 1999). Characterization results indicate that these accessions possess a wide variation with regard to morphological traits such as colour, shape and size of tuber, petiole length and colour, stolon formation. The taro collections were classified into phenotypic groups based on the results of the morphoagronomic characterization.

The genetic diversity of 201 Vietnam taro accessions was assessed using a 6-enzyme system. A total of 74 zymotypes including 43 unique zymotypes were recorded. At the zymotype level, there was significant allelic diversity among accessions. The zymotype variation was not fully comparable to the morphological one and no correlation could be found between morphological patterns and isozyme banding patterns.

The distribution of zymotypes in different agro-ecological zones indicated that the northern mountain zone had greater taro diversity than other zones in the country. Using both morphological and isozyme data, 28 duplicates were identified among 201 accessions.

In general, a wide variation of taro was observed within the collection, indicating its potential for further varietal improvement through breeding programmes.

Different genetic resources were identified from the taro collection. Some promising clones were evaluated for their yield, disease and pest resistance, dry matter content, eating quality, etc. The five best clones (2 of eddoe, 1 of intermediate type and 2 of dasheen) were singled out as choice material and should be further tested for their use on farmer's fields.

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Morphological and isoenzyme variability of taro (*Colocasia esculenta* L. Schott) germplasm in Cuba[3]

Arlene Rodríguez Manzano, Adolfo A. Rodríguez Nodals, María I. Román Gutiérrez, Zoila Fundora Mayor and Leonor Castiñeiras Alfonso

Introduction

The Global Plan of Action for the Conservation and Sustainable Use of Plant Genetic Resources (GPA) holds *ex situ* conservation among its high priority activities. In addition, the GPA emphasizes the need for studies concerning characterization, evaluation and development of core collections, as these studies are important for the effective classification of the collections and allow users to access their information needs (FAO 1996a).

There are 6000 *Colocasia* accessions around the world; the six largest germplasm collections are in Malaysia (22% of the total), Papua-New Guinea (13%), India (11%), USA (8%), Indonesia (7%) and the Philippine Islands (6%) (FAO 1996b). At the Research Institute on Tropical Roots and Tubers (INIVIT), Cuba, a collection of introduced, collected and genetically improved clones of taro [*Colocasia esculenta* (L.) Schott] has been held since 1967. Particular attention is paid to the maintenance and introduction of economically important species in Cuba, among them the root and tropical tuber crops that play an important role in human nutrition.

One of the first attempts to classify and identify taro germplasm in Cuba was carried out by Roig (1913) at the former Agronomic Experimental Station of Santiago de las Vegas (now INIFAT). Roig characterized and identified clones belonging to the genera *Xanthosoma* and *Colocasia* whose identification was ambiguous, and emphasized the importance of the corm, cormel, leaf and petiole characters in the evaluation and identification of the genetic mixtures, as not all the clones have inflorescences.

Rodríguez Nodals (1971, 1979) made several taxonomic studies based on morphological characters. Rodríguez Manzano *et al.* (1994, 1998) described the germplasm during 1989–91, taking into account passport descriptors and morphological traits, including subterranean, leaf and inflorescence characteristics, as well as cytogenetic and biochemical aspects. This led to a better understanding of the systematics of this genus, although the lack of a statistical analysis reduced the effectiveness of the study.

More recently, Rodríguez Manzano *et al.* (1999a, 1999b) used multivariate statistical analysis to study the diversity of *Colocasia esculenta* existing in Cuba. In crops such as beans, chickpeas, onions and peanuts, multivariate analysis of agronomic and morphological characters have been used for the characterization, evaluation and classification of germplasm in Cuba (Castiñeiras 1992; Fraga *et al.* 1996; Shagarodsky *et al.* 1996; Fundora *et al.* 1997).

3 Reprinted with permission from Plant Genetic Resources Newsletter, 2001, No. 126:31–40.

In this work, subterranean and leaf morphological characteristics were used, along with esterase and peroxidase isozyme analysis, to establish a list of minimum descriptors for characterization, genotype identification and formation of a core collection, and to verify that there were no duplicates in the germplasm collection of *Colocasia esculenta (*L.) Schott in Cuba.

Materials and methods

Materials

Forty-two clones introduced from Asia, Africa and America (Table 1), collected in different Cuban regions (Figure 1), and genetically improved, were used. These clones belong to the national collection of *Colocasia esculenta* L. Schott maintained by INIVIT, Santo Domingo municipality, Villa Clara province, Cuba. Each clone was stored *ex situ* in a four-row plot, totalling 80 rows. The planting distance was 0.90 m between rows and 0.35 m between plants in a row. The two central rows of each plot were evaluated (40 plants).

Morphological traits

Plants were harvested 10 months after planting and two consecutive years were evaluated. Sixteen descriptors were used to evaluate corm, cormel and root characteristics, as well as quality, and twenty-six descriptors to evaluate the leaf characteristics (Table 2). The characteristics and modalities used were those reported by IPGRI (1999), IBPGR (1980) and Rodríguez Manzano *et al.* (1999a,b).

The characterization results enabled the selection of a minimum descriptors list to study the Cuban clones of *Colocasia esculenta*. For this purpose, principal component analysis (PCA) was carried out, starting from a standardized correlation matrix analysing independently for the underground and foliar plant organs (*n*=16 and *n*=26).

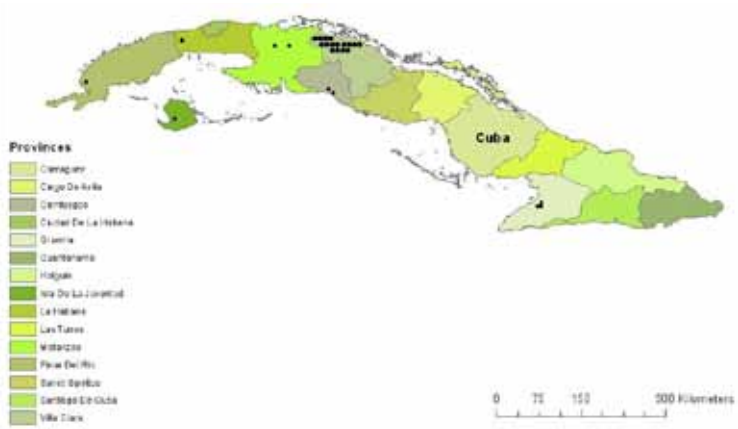

Figure 1. Geographic distribution of local and advanced clones of the Cuban collection.

TABLE 1. Passport of the clones of *Colocasia esculenta* (L.) Schott studied

Name	Origin	Source
1. Isleña Blanca # 2	Cuba (Villa Clara)	Prospecting
2. Isleña Mulata # 1	Cuba (Villa Clara)	Prospecting
3. Isleña Mulata # 2	Cuba (Villa Clara)	Prospecting
4. Isleña Mulata # 3	Cuba (Villa Clara)	Prospecting
5. Isleña Rosada # 1	Cuba (Villa Clara)	Prospecting
6. Isleña Rosada Escambray	Cuba (Cienfuegos)	Prospecting
7. Isleña Rosada Jibacoa	Cuba (Villa Clara)	Prospecting
8. Isleña Rosada Mayajigua	Cuba (Sancti Spíritus)	Prospecting
9. Isleña Rosada Sancti-Spiritus	Cuba (Sancti Spíritus)	Prospecting
10. Isleña Violácea	Cuba (Villa Clara)	Prospecting
11. Isleña Japonesa	Cuba (Isla de la Juventud)	Prospecting
12. Isleña China	Cuba (Cienfuegos)	Prospecting
13. Madere Graines	Guadalupe (Domaine Duclos)	Introduction
14. Madere Soufre	Guadalupe (Domaine Duclos)	Introduction
15. Selección Herradura	Cuba(Pinar del Río)	Prospecting
16. Camerun 2	Camerun	Introduction
17. Camerun 8	Camerun	Introduction
18. Camerun 9	Camerun	Introduction
19. Camerun 14	Camerun	Introduction
20. Camerun 22	Camerun	Introduction
21. Camerun 23	Camerun	Introduction
22. Isleña Miranda	Cuba (Villa Clara)	Selection of somatic mutations
23. Isleña Rosada # 2	Cuba (Villa Clara)	Prospecting
24. Isleña Rosada Habana	Cuba (C. Habana)	Selection of somatic mutations
25. Madere Blanc	Guadalupe (Domaine Duclos)	Introduction
26. Isleña Blanca # 1	Cuba (Villa Clara)	Prospecting
27. Isleña Mulata # 4	Cuba (Matanzas)	Prospecting
28. Isleña Rosada Sabanilla	Cuba (Matanzas)	Prospecting
29. CEMSA 75-11	Cuba (Villa Clara)	Selection of somatic mutations

Name	Origin	Source
30. México 1	México (Tabasco)	Prospecting
31. México 2	México (Tabasco)	Prospecting
32. México 3	México (Veracruz)	Prospecting
33. Rosada CEMSA	Cuba (Villa Clara)	Selection of somatic mutations
34. MC-2	Cuba (Villa Clara)	Selection of somatic mutations
35. Isleña Cienfueguera	Cuba (Cienfuegos)	Prospecting
36. Isleña Yabú	Cuba (Villa Clara)	Selection of somatic mutations
37. Francesa	Viet Nam	Introduction
38. Sao Tomé	Saotomé y Príncipe	Introduction
39. Isleña Rosada Bayamo	Cuba (Granma)	Prospecting
40. Isleña Bayamesa	Cuba (Granma)	Prospecting
41. Isleña Granma	Cuba (Granma)	Prospecting
42. Panameña	Panamá (Chiriquí)	Introduction

Characteristics that contributed most to variability were determined on the basis of those original variables with greater influence on the components (C1 at C5), according to the following approach. The mean values from the highest and lowest eigenvectors were used as the threshold for the selection of the most contributing variables (Fundora *et al.* 1992). Associations between the factors reported by Rodríguez Manzano *et al.* (1999a, 1999b) were also taken into account.

To establish the core collection, an additional PCA was carried out using the characteristics selected in the first analysis in order to select those contributing most to the variability.

According to the C1–C2 interaction, groups of representative clones were formed. In order to select the significant associations between the characteristics of the leaf and subterranean organs, confidence intervals of n=40 df and a significance of 0.001% (Sigarroa 1985) were used.

Isozyme analysis

To study peroxidase and esterase isozyme variation, the techniques of Gonzáles and Román (1982) and Gonzáles (1989) were used for electrophoresis and preparation of leaf extracts.

From the zymogram results of the peroxidase and esterase isozyme systems (Rodríguez Manzano *et al.* 1998) similarities between clones were calculated using the MAT–GENE statistical programme (Sigarroa and Cornide 1995) based on Jaccard's similarity index. Data were recorded according to the presence or absence of bands.

TABLE 2. Descriptors for the subterranean and leaf organs of clones

Palatability (PAL)
Consistency (CON)
Corm dry matter percentage (DMC)
Cormel dry matter percentage (DMS)
Corm shape (CS)
Corm weight (CW)
Corm flesh colour of the central part (CCF)
Fiber degree (FD)
Number of cormels (NC)
Percentage of cormels under 50 g (PCV)
Percentage of cormels over 100 g (PCO)
Percentage of cormels between 50 and 100 g (PCE)
Shape of cormel (CLS)
Flesh colour of cormel (CFL)
Bud colour (BC)
Root colour (ROC)
Growth habit (GH)
Shoots after 5 months (S5M)
Shoots after 6 months (S6M)
Shoots at harvest time (SHT)
Plant height (PH)
Petiole/lamina length ratio (PSR)
Maturity at harvest time (MHT)
Leaf blade margin colour (CSE)
Leaf lamina length to width ratio (SLW)
Leaf lamina (SS)
Leaf blade colour—upper (SCU)
Leaf blade colour—lower (SCL)
Petiole junction pattern (upper surface of leaf) (LPU)
Petiole junction pattern (lower surface of leaf) (LPL)
Colour of petiole junction pattern (upper surface of leaf) (CUP)

Colour of petiole junction pattern (lower surface of leaf) (CLP)
Colour of V vein pattern (upper part of leaf) (VCV) (Figure 2)
Colour of I vein pattern (lower part of leaf) (VCI) (Figure 2)
Colour of A–B vein pattern (lower part of leaf) (VAB) (Figure 2)
Petiole colour (PC)
Leaf sheath colour in outer part (CAO)
Leaf sheath colour in inner part (CAI)
Colour of the petiole to corm insertion point (CI)
Wax in the petiole (WP)
Petiole transversal section (PTS)
Ratio of sheath length to total petiole length (PLR)

Data on the similarity matrix were introduced in the programme database and processed using cluster analysis in order to represent the phenetic relationships between clones by means of a dendrogram.

The number of loci and alleles per locus, as well as the percentage of polymorphic loci and the number of alleles per polymorphic locus, were determined in both the isozyme systems, according to the following formulae:

$$\text{Percentage of polymorphic loci} = [\text{Number of polymorphic loci}/\text{Total number of loci}] \times 100$$

Mean number of alleles per polymorphic locus = [Number of alleles per polymorphic locus/ Number of polymorphic loci] × 100

Results and discussion

Morphological and statistical traits

From the results of the matrix of eigenvectors and values for the subterranean organ characteristics, the major descriptors influencing 70.9% of the total variability were accumulated until the fifth component could be selected (Table 3). Root colour (ROC), bud colour (BC) and cormel flesh colour (CFL) showed the greatest variability in the first component. Although the colour of the corm flesh (CCF) had a high value, it was not selected for integration in the list of minimum descriptors, as Rodríguez Manzano *et al.* (1999a) had demonstrated that it was significantly and positively correlated with CFL. Consequently, it was sufficient to record CFL for the characterization, because the cormels can be easily removed from the plant and their colour remains the same, irrespective of plant age. It should be emphasized that the characteristics selected in the first component are qualitative in nature. Most of these traits are determined by one or a few genes and have a discrete distribution. They can be easily identified and are modestly affected by the environment, although sometimes their expression may be altered by the action of modifying genes (Gálvez 1997).

Corm dry matter percentage (DMC), corm shape (CS) and cormel shape (CLS) were the characteristics that showed the greatest variability in component 2. Cormel dry matter percentage (DMS) was not selected as it is significantly and positively correlated with DMC (Rodríguez Manzano *et al.* 1999a).

The characteristics showing greater influence in C3 were the percentage of cormels under 50 g (PCV) and percentage of cormels over 100 g (PCO), as well as the palatability (PAL). The percentage of cormels between 50 and 100 g (PCE), the corm weight (CW) and the total number of cormels (NC) were selected when variables in components 4 and 5 were analyzed.

TABLE 3. Matrix of eigenvectors and values of the principal components for the subterranean characteristics

Principal components					
	C1	**C2**	**C3**	**C4**	**C5**
Variance	4.8831	1.9492	1.6611	1.6132	1.2338
% total	30.5	12.2	10.4	10.1	7.7
% accumulated	30.5	42.7	53.1	63.2	70.9
Eigenvectors					
CS	–0.0018	0.4596	–0.0217	–0.3450	–0.3662
CW	–0.1532	0.1717	0.0931	0.2906	–0.1716
CCF	–0.3399	–0.1783	–0.2354	–0.3403	0.0895
FD	0.1565	0.1763	–0.1275	–0.0500	0.1266
NC	0.2471	–0.1265	0.1372	–0.1449	–0.4620
PCV	0.1181	–0.0966	–0.4888	0.2169	–0.4796
PCE	–0.0978	–0.1434	0.3384	0.5082	0.1645
PCO	0.0455	0.0121	0.4776	–0.5064	0.1168
CLS	–0.1310	0.4572	0.0981	0.0849	–0.2847
CFL	–0.3436	–0.1800	–0.3018	–0.2653	–0.0749
BC	–0.3468	–0.1573	–0.1173	0.0212	–0.1013
ROC	–0.3763	–0.1284	–0.0038	–0.0016	–0.1768
PAL	–0.3362	–0.0790	0.3432	0.0795	–0.2210
CON	–0.3268	–0.1401	0.2571	–0.0252	–0.2495
DMC	–0.2610	0.4606	–0.1115	0.0616	0.1479
DMS	–0.2510	0.3602	–0.1018	0.0813	0.2590

Table 4 shows the matrix of eigenvectors and values from the principal component analysis for leaf characters in *Colocasia esculenta* L. Schott, and the descriptors influencing more than 64.9% of the variability accumulated up to the fifth component.

The most important descriptors for clone identification were petiole junction pattern in the upper part of the leaf (LPU), colour of the petiole junction pattern in the upper part of the leaf (CUP), and colour of the vein pattern from A to B in the lower part of the leaf (VAB) (Figure 2). VAB showed a higher value than the colour of the veins in the V-shape (VCV) and I-shape (VCI) parts described by IPGRI (1999) (Figure 2). Rodríguez Manzano *et al*. (1999b) reported a significant association among VCV, VCI and VAB. In the second component, the colour of the petiole to corm insertion point (CI), shoots at harvest time (SHT), plant height (PH) and petiole colour (PC) presented the greatest variability.

Rodríguez Manzano *et al*. (1999b) reported a significant and positive association between the shoots at harvest time (SHT) and shoots at 5 (S5M) and 6 months (S6M). Hence only SHT was used which, together with plant height, is an important agronomic characteristic for predicting yield per plant.

Considering up to the fifth component, where about 69.4% of the total variability for leaf organs was accumulated, the ratio of sheath length to total petiole length (PLR), maturity at harvest time (MHT), petiole to lamina length ratio (PSR), leaf blade colour in the lower part (SCL), and leaf sheath colour in the inner part (CAI) for C4, as well as leaf blade margin colour (CSE) and leaf lamina length to width ratio (SLW) for C5, were included. Thus, CAI showed variability important for the characterization and identification of clones of this genus in the collection studied, although this was not reported by IPGRI (1999).

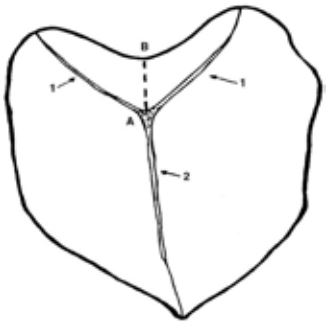

Figure 2. Vein by the sheet lower part. 1: V part, 2: I part, AB part.

TABLE 4. Matrix of eigenvectors and values of the principal components for the leaf characteristics

Principal components					
	C1	**C2**	**C3**	**C4**	**C5**
Variance	8.8275	3.8515	2.1860	1.7738	1.3883
% total contribution	34.1	14.8	8.4	6.8	5.3
% accumulated	34.1	48.9	57.3	64.1	69.4
Eigenvectors					
GH	0.2432	–0.0934	–0.0125	0.1875	0.0133
S5M	–0.0087	–0.2619	0.3852	0.3064	–0.1102
S6M	–0.0060	–0.2968	0.3813	0.1517	0.0920
SHT	0.0951	–0.3339	0.1861	0.2542	0.1214
PH	0.0050	–0.3181	0.1938	–0.0661	–0.1009
CSE	0.1790	–0.2023	–0.0447	–0.1209	–0.3481
CLW	–0.1621	–0.0778	0.1730	0.2089	–0.2757
SS	0.1780	–0.2199	–0.3070	–0.0868	–0.1068
SCU	0.2084	–0.1783	–0.2212	0.1565	0.2634
SCL	0.2275	0.0115	–0.1118	0.2719	0.1091
LPU	0.2997	0.1265	0.0731	0.0237	–0.1501
LPL	0.2549	0.1852	0.1477	–0.1872	–0.1901
CUP	0.2948	0.1531	0.1368	0.0466	–0.0677
CLP	0.2685	0.0963	0.1809	0.0622	0.0301
VCV	0.2263	0.1642	0.1575	–0.0788	–0.1257
VCI	0.2404	0.1107	0.1010	–0.1509	–0.1068
VAB	0.2800	0.1391	0.1225	–0.0067	–0.1376
PC	0.2127	–0.2778	–0.1381	–0.2096	0.0868
CAO	0.2459	–0.2158	–0.1166	–0.2118	0.0465
CAI	0.1540	–0.2474	0.0140	–0.2644	0.2121
WP	0.2626	0.0469	–0.0918	0.2088	0.0924
CI	–0.0396	0.3383	0.2783	–0.0756	0.2241
PTS	0.0877	0.0217	0.3293	–0.0771	0.3653
MHT	–0.0731	–0.1635	0.2724	–0.3227	–0.1906
PSR	0.1684	0.1490	–0.0020	0.2931	0.2642
PLR	0.0629	0.0450	–0.1278	0.3743	–0.4483

TABLE 5. Minimum descriptors for characterizing *C. esculenta* (L.) Schott clones in Cuba

Root outer colour (ROC)
Bud colour (BC)
Flesh colour of cormel (CFL)
Corm dry matter percentage (DMC)
Corm shape (CS)
Shape of cormel (CLS)
Percentage of cormels under 50 g (PCV)
Percentage of cormels over 100 g (PCO)
Percentage of cormels between 50 and 100 g (PCE)
Palatability (PAL)
Corm weight (CW)
Number of cormels (NC)
Shoots at harvest time (SHT)
Plant height (PH)
Leaf blade margin colour (CSE)
Leaf lamina length to width ratio (SLW)
Leaf lamina surface (SS)
Leaf blade colour - lower part (SCL)
Petiole junction pattern (upper surface of leaf) (LPU)
Colour of the petiole junction pattern (upper surface of leaf) (CUP)
Colour of the vein pattern (A–B vein in the lower part) (VAB) (Figure 2)
Petiole colour (PC)
Leaf sheath colour in the inner part (CAI)
Colour of the petiole to corm insertion point (CI)
Petiole transversal section (PTS)
Maturity at harvest time (MHT)
Petiole to leaf lamina length ratio (PSR)
Ratio of sheath length to total petiole length (PLR)

A list of 28 minimum descriptors for the correct characterization and evaluation of the Cuban collection of this genus was established (Table 5), 12 of which represent the subterranean organs and 16 refer to leaf characteristics. These include both quantitative and qualitative characters, and permit coverage of an important part of the existing diversity. Rodríguez Manzano and Rodríguez Nodals (unpublished data) selected five characteristics of the inflorescences to include in the minimum descriptors for the morphological characterization.

Table 6 shows the correlations among 28 characteristics included in the minimum descriptor list. Forty-nine significant correlations were obtained, although for this work only the 20 correlations among the subterranean and leaf characters were taken into account, as the associations within the leaf and subterranean organs considered independently, were studied by Rodríguez Manzano *et al*. (1999a, 1999b).

Four subterranean organ characteristics were involved in the correlationexercise: CFL, ROC, DMS, and NC. Four leaf characteristics showed significant correlations: SHT, SS, PC and SCL. Tanimoto and Matsumoto (1986) did not report significant correlations between characteristics of different organs, perhaps because fewer characteristics were studied. Thus, it seems to be important to use a large number of characteristics in biometric studies of germplasm collections.

The highest correlation (0.949) was recorded between the colour of petiole to corm insertion point (CI) and bud colour (BC), as in all clones both organs are the same colour, either pink or white, with the exception of 'Madere Soufre' whose buds were white and the insertion point was pink. Incidentally, this clone is the only one with yellow flesh in the corms and cormels.

TABLE 6. Correlations among the most viable subterranean and leafy organs. Underlined values are significant at the 0.001% probability level.

	SHT	PH	CSE	SLW	SS	SCL	LPU	CUP	VAB	PC	CAI	CI	PTS	MHT	PSR	PLR	CS	CW	NC	PCV	PCE	PCO	CLS	CFL	BC	ROC	PAL	DMC
SHT	1.000																											
PH	0.155	1.000																										
CSE	0.148	0.070	1.000																									
SLW	0.032	0.009	-0.183	1.000																								
SS	0.279	-0.172	0.492	-0.405	1.000																							
SCL	0.181	0.116	0.333	-0.369	0.321	1.000																						
LPU	0.122	-0.172	0.493	-0.419	0.369	0.576	1.000																					
CUP	0.114	-0.215	0.352	-0.362	0.264	0.585	0.923	1.000																				
VAB	0.117	-0.305	0.421	-0.364	0.301	0.441	0.896	0.852	1.000																			
PC	0.382	-0.170	0.486	-0.276	0.692	0.310	0.371	0.340	0.354	1.000																		
CAI	0.359	0.084	0.314	-0.200	0.383	0.203	0.148	0.185	0.111	0.663	1.000																	
CI	0.390	-0.110	-0.361	0.000	-0.474	-0.105	0.056	0.122	0.133	-0.437	-0.190	1.000																
PTS	0.090	-0.064	0.000	-0.143	-0.039	0.020	0.225	0.281	0.222	0.188	0.160	0.289	1.000															
MHT	0.102	0.054	0.155	0.121	-0.199	-0.258	-0.191	-0.239	-0.189	-0.003	0.114	-0.122	-0.024	1.000														
PSR	0.102	0.264	0.000	-0.245	-0.044	0.397	0.466	0.503	0.432	0.093	0.114	0.163	0.178	-0.337	1.000													
PLR	0.038	0.054	0.110	0.101	0.181	0.154	0.217	0.169	0.140	-0.006	-0.100	-0.174	-0.141	-0.239	0.265	1.000												
CS	0.014	-0.181	0.123	-0.053	-0.129	-0.019	0.182	0.209	0.202	0.071	0.080	-0.039	0.210	0.267	0.099	-0.209	1.000											
CW	-0.365	0.057	0.033	-0.142	-0.186	-0.121	0.041	-0.025	-0.047	-0.198	-0.197	0.104	-0.060	0.229	-0.147	-0.279	0.028	1.000										
NC	0.694	0.153	0.015	-0.030	0.060	0.045	-0.037	-0.081	0.014	0.228	0.206	-0.167	0.110	0.152	0.161	-0.267	0.091	-0.151	1.000									
PCV	0.282	-0.011	-0.155	-0.053	-0.076	0.052	-0.305	-0.310	-0.264	-0.022	0.097	-0.196	-0.028	0.192	-0.032	-0.119	-0.027	0.025	0.263	1.000								
PCE	-0.241	0.150	0.086	-0.245	0.131	0.006	0.170	0.132	0.052	0.018	-0.049	0.190	0.063	-0.240	0.030	-0.013	-0.341	0.240	-0.123	-0.213	1.000							
PCO	0.046	0.030	-0.085	-0.011	-0.173	-0.142	0.147	0.184	0.340	-0.044	-0.102	0.135	0.078	0.026	0.170	-0.066	0.191	-0.070	0.245	-0.485	-0.122	1.000						
CLS	-0.145	-0.131	0.328	-0.105	0.040	0.089	0.235	0.118	0.129	0.068	0.227	0.133	0.292	0.041	0.018	-0.100	0.388	0.202	-0.149	-0.132	0.032	-0.086	1.000					
CFL	-0.564	-0.322	-0.383	0.329	-0.453	-0.445	-0.358	-0.303	-0.274	-0.538	-0.460	0.606	0.085	0.040	-0.352	-0.008	-0.045	-0.114	-0.378	-0.075	-0.076	-0.089	-0.014	1.000				
BC	-0.411	-0.116	-0.333	0.003	-0.421	-0.067	-0.000	0.051	0.013	-0.426	-0.160	0.949	0.264	-0.103	0.084	-0.154	-0.096	0.121	-0.256	-0.052	0.235	-0.176	0.160	0.639	1.000			
ROC	0.551	0.156	-0.319	0.097	-0.526	-0.348	-0.236	-0.246	-0.287	-0.627	-0.289	0.707	0.136	0.000	-0.051	-0.148	-0.018	0.221	-0.371	-0.138	0.192	-0.114	0.256	0.625	0.745	1.000		
PAL	0.465	0.239	0.037	0.046	-0.352	-0.062	0.148	0.143	0.078	-0.425	-0.319	0.556	0.211	0.022	0.261	-0.014	0.000	0.268	-0.208	-0.255	0.332	0.073	0.212	0.338	0.537	0.621	1.000	
DMC	-0.479	-0.402	-0.186	-0.053	0.004	-0.161	0.000	0.057	-0.021	-0.260	-0.274	0.235	-0.023	-0.182	-0.115	-0.200	0.267	0.348	-0.422	-0.178	0.016	-0.130	0.407	0.321	0.276	0.305	0.261	1.000

CI positively correlated not only with bud colour, but also with root colour (ROC), flesh colour of the cormels (CFL), palatability (PAL), and consistency (DMC).

Rodríguez Manzano *et al*. (1999a) reported significant correlations between cormel flesh, bud and root pigmentation, and palatability, and that 100% of the clones with pink pigmentation in these three organs possessed a delicious, or at least good, palatability. Therefore, the association of these characteristics with the pigmentation in the petiole to corm insertion point will permit the use of this descriptor for indirect selection in taro breeding programmes. This will permit early selections for quality before harvest.

Number of shoots at harvest time (SHT) showed a positive correlation with number of cormels (NC). Thus, it can be used as a selection index in yield prediction. It is negatively correlated with cormel flesh (CFL), and bud (BC) and root colour (ROC), as well as with the corm dry matter percentage (DMC). That is, when the dry matter percentage in corms decreases, and when the cormel flesh, buds and roots are white, the number of shoots per plant increases.

Pandey *et al.* (1996) studied the correlations between eight subterranean characteristics influencing yield, and pointed out that the mother corm and cormel weight can be used as selection criteria for yield. However, the correlations obtained in the current study have a great practical importance since the crop cycle is long and leaf correlations would allow prediction of future qualitative and quantitative characteristics of corms and cormels.

The matrix of eigenvectors and values for the minimum descriptors set (Table 7) shows the leaf and subterranean organ traits influencing 61.1% of the variability accumulated up to the fifth component.

For the first three components, which accounted for 46.4% of the total variability, the most important characteristics accountable for clone differentiation were bud colour, flesh colour in the cormels and petiole colour in C1; petiole junction pattern in the upper part of the leaf, vein pattern colour from A to B in the lower part of the lamina, colour of the petiole to corm insertion point, and palatability in C2; and dry matter percentage in corms and plant height in C3. Other important components of variation were number of cormels and corm shape in C4, and corm weight in C5.

Although the percentages for different cormel weights (<50 g, 50–100 g and >100 g) contributed more than corm weight (CW) in C5 (Table 7), they were not included in the list, since these traits are greatly influenced by the environment. Inclusion of number of cormels was found to be sufficient for the formation of core collections.

Among the characteristics contributing most to the variability in leaf organs analysis, petiole colour (PC) was less important than LPU, CUP, VAB, CLP, CI, SHT, PH and S6M, for the first three components (Table 4). However, when combining the most important leaf characteristics with those of subterranean organs (Table 7), petiole colour had the third highest value among all leaf characteristics, thus, it is very important for clone identification due to its great variability (Figure 3). This result is supported by Rodríguez Nodals (1979) and Rodríguez Manzano *et al.* (1994, 1999b). Other important attributes in C1 were bud colour (BC) and cormel flesh colour (CFL).

TABLE 7. Matrix of eigenvectors and values of the principal components resulting from the interactions of the most important subterranean and leaf characteristics

Principal components					
	C1	**C2**	**C3**	**C4**	**C5**
Variance	6.2582	4.3889	2.3252	2.2031	1.9147
% total contributions	22.4	15.7	8.3	7.9	6.8
% accumulated	22.4	38.1	46.4	54.3	61.1
Eigenvector					
SHT	–0.2524	–0.1750	0.1592	0.2274	0.0129
PH	–0.0038	–0.0879	0.3496	0.0371	0.3679
CSE	–0.2249	0.1051	–0.1936	–0.0601	0.1533
SLW	0.1415	–0.1854	0.0620	0.0337	–0.2411
SS	–0.2753	0.0250	–0.1845	–0.2727	0.0645
SCL	–0.2193	0.1743	0.1185	–0.0925	0.1255
LPU	–0.2208	0.3634	0.0119	–0.0032	–0.0587
CUP	–0.1999	0.3634	0.0463	0.0269	–0.1196
VAB	–0.2039	0.3375	0.0160	0.0656	–0.2005
PC	–0.3160	0.0079	–0.1658	0.0045	0.0778
CAI	–0.2169	–0.0215	–0.1040	0.1498	0.2594
CI	0.2477	0.2644	0.1482	0.1695	0.0293
PTS	–0.0137	0.1775	–0.0032	0.3222	0.1004
MHT	0.0270	–0.1539	–0.2107	0.3089	0.0989
PSR	–0.1136	0.2218	0.3592	0.1074	0.0251
PLR	–0.0713	0.0387	0.2192	–0.3346	–0.1999
CS	–0.0348	0.0856	–0.2813	0.3831	–0.1816
CW	0.1149	0.0831	–0.2292	–0.0037	0.2431
NC	–0.1521	–0.1567	0.1777	0.3860	0.0728
PCV	–0.0163	–0.2153	–0.0194	0.0970	0.2469
PCE	0.0323	0.1605	0.0894	–0.2411	0.3528
PCO	–0.0317	0.0727	0.1661	0.2662	–0.3721
CLS	0.0087	0.1935	–0.3411	0.1474	0.1627
CFL	0.3262	0.0427	–0.0750	–0.0267	–0.1675

Principal components					
	C1	**C2**	**C3**	**C4**	**C5**
ROC	0.2601	0.2372	0.0882	0.0846	0.1472
BC	0.3272	0.1407	0.0376	0.0545	0.1792
PAL	0.1958	0.2637	0.1285	0.0608	0.1689
DMC	0.1580	0.1728	–0.3631	–0.0994	–0.1106

In addition to the 28 minimum descriptors, including subterranean and leafy organs, needed to identify the genotypes of the whole collection, 13 characteristics of more variable attributes (Table 8) were included in a list of useful descriptors for the establishment of a core collection. Among them: chromosome number and presence or absence of inflorescences. Twenty clones were selected as representatives of this variability. Such a list is helpful for quickly accessing information on variability during collecting missions, on uncharacterized genebank accessions, or on different regions where *in situ* conservation in home gardens is taking place (Esquivel and Hammer 1994; Esquivel *et al.* 1994b; Castiñeiras *et al.* 2000).

These results can be used for identifying variability present in each geographic niche. For example the clone 'Isleña Rosada Sabanilla' collected by Rodríguez Nodals in February of 1975

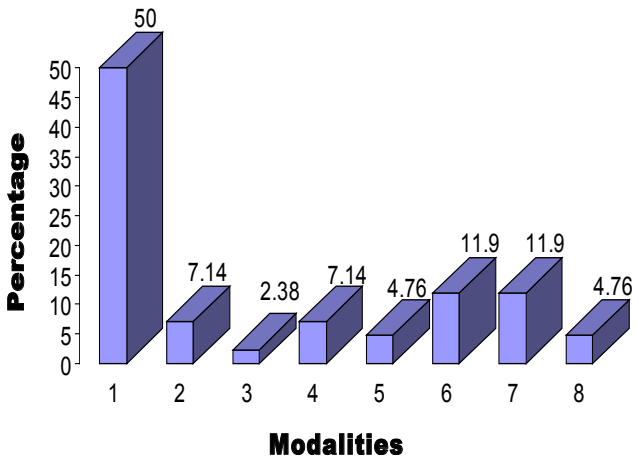

Figure 3. Petiole colour (1) Green, (2) green with light violaceous tint, (3) pinkish-green with purple tint, (4) verde violaceous with pink tint, (5) green violaceous with white edges, (6) green violaceous, (7) violaceous-green with strips, (8) violaceous-greenish with uniform colour.

in Matanzas Province was found again near the place where it was originally collected (Rodríguez Manzano *et al*. 2000).

Characteristics contributing more to the variability in C1 were bud colour and cormel flesh colour, as well as petiole colour; in C2 they were colour of the petiole junction pattern in the leaf upper part, and the colour of the vein from A to B in the leaf lower part. Based on these traits, three large groups and eight subgroups were formed, taking into account the interaction of these characteristics (Figure 4). It is important to note that clones with a pink root were located at the right side of the X-axis, except clones 11, 15 and 22, which were placed at the left side, due to the strong influence of petiole colour.

TABLE 8. LIST OF DESCRIPTORS FOR CREATING CORE COLLECTIONS

Bud colour (BC)
Flesh colour of the cormels (CFL)
Corm dry matter percentage (DMS)
Corm shape (CS)
Corm weight (CW)
Number of cormels (NC)
Palatability (PAL)
Colour of the petiole to corm insertion point (CI)
Plant height (PH)
Petiole junction pattern (upper surface of leaf) (LPU)
Colour of the vein pattern (A–B pattern) (VAB)
Petiole colour (PC)
Petiole to lamina length ratio (PSR)
Inflorescence formation
Chromosome number

Groups I and II included clones exhibiting a green pigmentation in the vein from A to B in the lower part of the leaves, while group III showed a violet pigmentation. The descriptions of the groups are as follows:

Group I. This group includes 13 clones whose buds and cormel flesh are white, except 'Isleña Rosada Bayamo' (39 in Figure 4), in which they are pink. Nevertheless, this clone belongs to the group due to the high incidence of white root colour. Only clone 38 showed pigmentation in some lower leaves while the others did not exhibit violet pigmentation in the petiole junction pattern in the upper part of the leaf. Vein colour from A to B was always green and root colour was white in all cases.

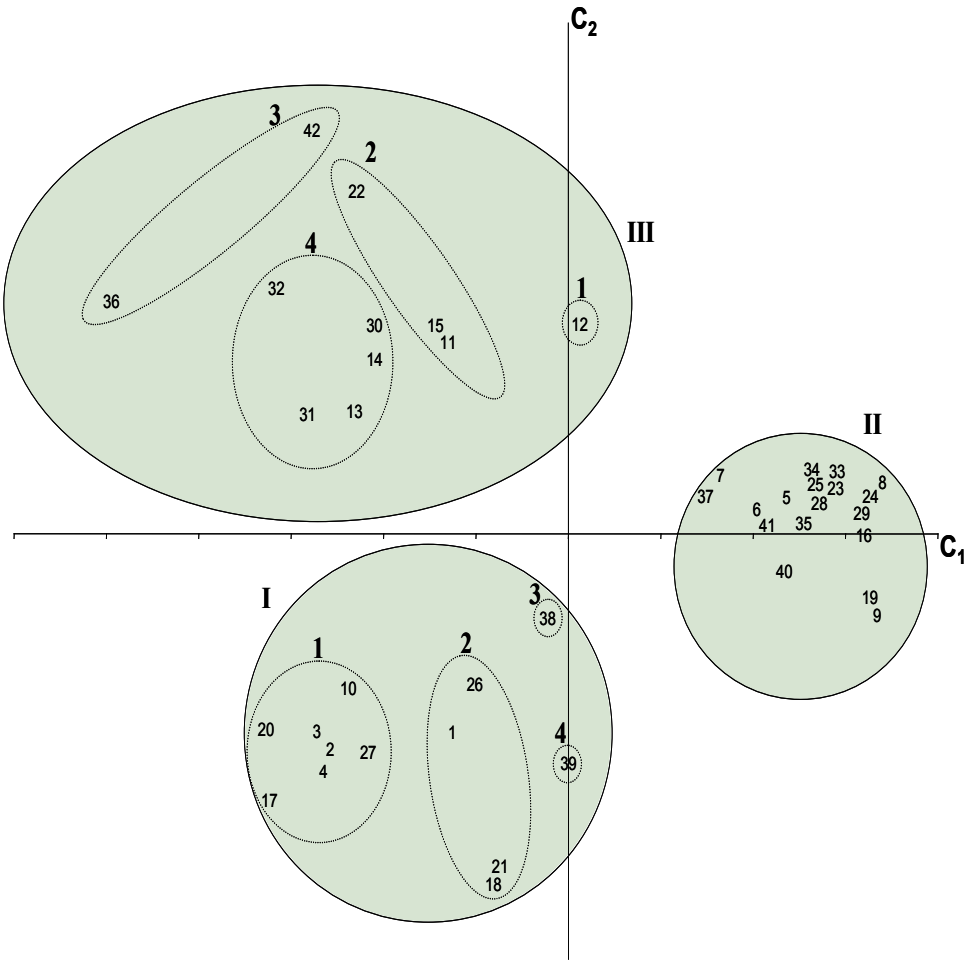

Figure 4. Groups formed from the principal component analysis combining attributes from the subterranean and leafy organs in components 1 and 2.

Subgroup 1 comprised seven clones with violet–green petioles; four clones (2, 3, 17 and 27) had stripes and the other three (4, 10 and 20) almost uniform colour.

Subgroup 2 is made up of four clones with green petioles (26, 1, 21 and 18).

Subgroup 3 is formed by only one clone (38), exhibiting green petioles and violet pigmentation in the limb–petiole insertion point in the upper part of some lower leaves.

Subgroup 4 includes one clone showing green petioles as well as pink buds and pink cormel flesh, located here because of the strong incidence of the white colour of the root.

Group II. This group contains 18 clones with pink buds and pink cormel flesh, except 'Francesa' (37), which was white, but is located in this group because of the strong incidence of the pink root colour. As in the clones of Group I, Group II did not show violet pigmentation in the petiole junction pattern in the upper part of the leaf, although some clones exhibited such pigmentation in some lower leaves: 'MC-2' (34), 'Isleña Bayamesa' (40) and 'Isleña Granma' (41). The petiole colour (PC) was green in all the clones, with the exception of 'Isleña Rosada Habana' (24) and 'MC-2' (34) which were green with a light violet tint. All the clones had pink roots.

Group III. This group consists of 11 clones with both buds and roots white, or with both organs pink. Pigmentation was purple or intense purple in the petiole junction pattern in the upper part of the leaf and veins from A to B, with different variations (Rodríguez Manzano *et al.* 1999b). All clones had white cormel flesh, except 'Isleña China' (12) and 'Madere Soufre' (14) which had white flesh with violet and yellow tints, respectively.

Subgroup 1 includes only 'Isleña China' (12), the only clone having cormel flesh with violet pigmentation, pink buds and green–violet petioles. Its root was white.

Subgroup 2 is formed by three clones (11, 15 and 22), with pink buds and white cormel flesh. Petioles were green—violet with a pink tint. Their roots were pink.

Subgroup 3 includes five clones with pink buds and white roots. Four of them (13, 30, 31 and 32), however, had white cormel flesh and green–violet petioles, while the fifth, 'Madere Soufré', had green–rose petioles with a violet tint and yellow cormel flesh.

Subgroup 4 includes two clones (36 and 42) with white buds, cormel flesh and roots, and green–violet petioles with whitish edges.

Isozyme analysis

Peroxidase analysis did not differentiate all the clones, and only seven bands were visually detected. However, the esterase banding patterns of all cultivars were determined and all showed a characteristic banding pattern. Within this system, 40 different bands were found.

Table 9 shows the result of the quantitative analysis of zymograms of the 42 clones. Great variation among individuals was observed, since each enzyme showed 100% of polymorphic loci, with an average of 2.25 alleles per polymorphic locus.

The grouping of the clones on the basis of esterase and peroxidase isozyme patterns is shown in Figure 5, showing the associations among individuals, and possible genetic proximity among them.

Clones were grouped in ascending order in six clusters, and subgroups within groups III, IV and VI were formed. 'Isleña Rosada Escambray' (6) and 'Isleña Rosada Sancti Spiritus' (9) clustered in isozyme Group I. These clones were collected in zones that were very close together in the central region, and are diploid clones (Rodríguez Manzano *et al*. 1998). In contrast, 'Isleña China' was placed in isozyme Group II**;** it did not associate with other accessions and was the only one with violet pigmentation in the flesh of corms and cormels.

Group III was composed of 12 clones collected in Cuba and one introduction from Panama, and was divided into three subgroups. Subgroup "a" included three clones collected in the eastern region and one in the central region. Subgroup "b" had only one clone, 'Isleña Miranda' (22), which was obtained through selection from 'Isleña Japonesa' (11), which was located in subgroup "c". In subgroup "c" there were seven clones—six collected in Cuba and one introduced from Panama. Within this subgroup, four clones had very marked morphological similarities: 'Isleña Japonesa' (11), collected in Isla de la Juventud and probably introduced from Japan (Rodríguez Nodals, pers. comm.); 'Selección Herradura' (15) collected in southern Pinar de Rio province; 'Isleña Yabú' (36), a mutation from 'Isleña Japonesa' and 'Panameña' (42), very similar to clone 11 with respect to leaf characteristics.

The clones 'Isleña Blanca #1' (26), 'Isleña Blanca #2' (1) and 'Isleña Violácea' (10) were collected in the Punta Felipe municipality, Villa Clara province, in the central region, and differed from other clones within the group in leaf and petiole colour.

Clones of this group could have a common origin in Asia, and could have been introduced directly from Japan or from the Canary Islands, developing a great variability since their introduction. These results would confirm the suggestion that *C. esculenta* in Cuba is a plant of Asiatic origin (Hammer and Esquivel 1994). This could be further demonstrated by the fact that there are several clones from this species in the western and central regions of Cuba, but in the eastern part there are more clones from *Xanthosoma* spp. (Rodríguez Nodals 1984; Esquivel *et al*. 1994b; Castiñeiras *et al*. 2000).

TABLE 9. Quantitative analysis of the 42 clones zymograms

Isozymatic system	No. of loci	No. of alleles	No. of rare alleles	Polymorphic loci percentage	Allele avg. no. per polymorphic locus
Peroxidase	3	7	0	100%	2,3
Esterase	9	20	20	100%	2,22
Total	12	27	20	100%	2,25

Group IV consisted of eight clones; three collected in Mexico (30, 31 and 32), four with striped petioles from Cuba (2, 3, 4 and 27) and one introduction from Asia (37). There is much evidence of the introduction of many Asian plants in Mexico from the Philippines Islands through the route from Manila to Acapulco, and the exchange between Mexico and Cuba from Veracruz to Havana was also very intense in the colonial period. Thus it is not difficult to assume the probable common origin of these clones (Figure 5). Four clones with striped petioles (2, 3, 4 and 27), named 'mulatos' by Rodríguez Nodals (1979), and a clone from Asia (37) belong to subgroup "a", while subgroup "b" contains three clones collected in Mexico with morphological similarities. These are the only clones with white cormel flesh, pink buds and white roots, except for 'Madere Graines', which has a greater African influence**.**

Group V contained only one clone (38). This clone was recently introduced into Cuba from Sao Tomé and Principe, and showed no association with any of the other clones studied. Thus, during the colonial period, no germplasm was introduced into Cuba from the West African Islands.

Group VI consists of 18 clones, six of which were from continental West Africa (16, 17, 18, 19, 20 and 21), three introduced from Guadeloupe (13, 14 and 25) and the other nine collected or obtained by selection of somatic mutations in Cuba from clones of African origin. The germplasm coming from Africa has a strong influence in this group, since it gave rise to the other clones from the French Antilles and Cuba by the selection of somatic mutations.

It is likely that several *Colocasia* clones were introduced from tropical Africa, a secondary centre of genetic variation of the Asian taro, through the slave trade and also with the Spaniards from the Canary Islands. These results confirm the hypothesis of Gonzalo Oviedo (cited in Esquivel *et al.* 1994a), who claimed this crop was related to African customs, because it was prepared in home gardens in Cuba using 'pilones'—traditional instruments used for the slave trade (Esquivel *et al.* 1994a, Tirado and Martínez 1994).

Within this group there are three subgroups. Subgroup "a", consists of 'Madere Graines' (13). Subgroup "b", is formed by 'Camerun 2' (16). Subgroup "c", includes 16 clones, five of which came from West Africa (Cameroon), two from the French Antilles, six collected in Cuba and the other six obtained through clonal selection from spontaneous mutations of some of the clones included in this subgroup.

No association was found between the ploidy level reported by Rodríguez Manzano *et al.* (1998) and the isozyme groups. These results confirm those published by Tanimoto and Matsumoto (1986) and Lebot and Aradhya (1992).

These earlier authors found no correspondence between the zymotypes and the morphological characteristics studied. However, in this study a correspondence was found between some groups of clones based on morphological characters and subgroups from the cluster analysis formed on the basis of the isozyme analysis. This is the case with clones with striped petioles with a probable Asian origin (Group IV, subgroup "a") and those from Mexico (Group IV, subgroup b), which are the only ones with white cormel flesh and roots and pink buds.

Conclusions

Twenty-eight descriptors, 12 for subterranean organ traits and 16 for leaf characteristics, have been included in the list of minimum descriptors for taro [*Colocasia esculenta* (L.) Schott]. These descriptors enable correct characterization and evaluation of collections of this species in Cuba, covering most of the existing diversity.

PCA for the interaction of the 28 descriptors used in characterizing the clones showed that colour of the limb to petiole insertion point and the distribution of pigmentation in the upper part of the leaf, vein colour from A to B in lower part of the blade, bud colour, colour of the cormel flesh and petiole colour were the descriptors contributing most to the total variability and were determinant in the formation of the groups of clones.

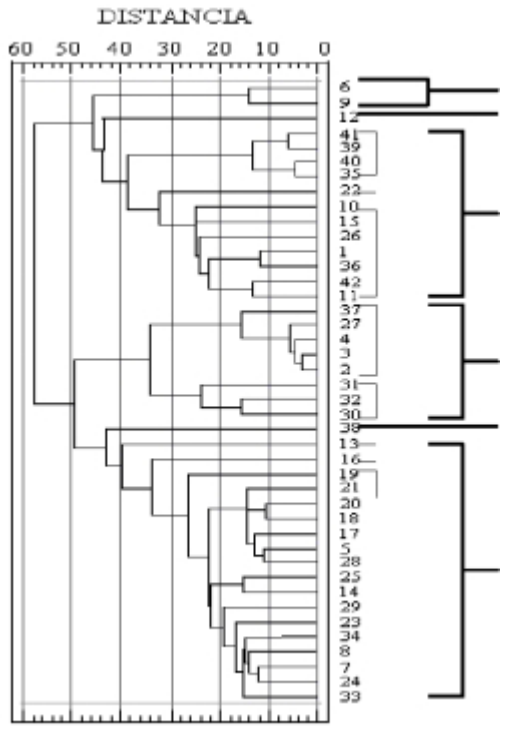

Figure 5. Dendrogram indicating genetic similarities among 42 clones from *Colocasia esculenta*; this was generated by cluster analysis of the peroxidase and esterase isozymatic systems.

Twenty significant correlations among the characteristics of the leaf and subterranean organs were found. Some of them are important for indirect selection in taro improvement programmes.

On the basis of the esterase and peroxidase isozyme analysis, clones were classified in six groups and several subgroups.

Clones with striped petioles, as well as those with white cormel flesh, pink buds and white roots were grouped together in the dendrogram derived from the cluster analysis of the esterase and peroxidase isozymes, and possibly originate from introductions from Asia.

Twelve polymorphic loci with 27 alleles, 20 of which were rare alleles, were found for the esterase isozyme system. The average allele number per polymorphic locus was 2.25.

A taro core collection can be formed using only the proposed 13 descriptors that contributed to the variability, and should be composed of clones representing the three main groups of variability and the eight subgroups within them. Thus, information on the variability of the accessions collected or maintained *in situ*, as well as on the uncharacterized accessions in the genebank can be obtained quickly.

The Cuban taro collection has a strong African and Japanese influence, as well as influence from the centre of origin in Southeast Asia and the Philippines.

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Genetic diversity assessment of taro collections in China using RAPD assays

Li Maolin, Li Rugang, Zhou Mingde, Eyzaguirre P., Ayad W.G.

Abstract

In China, taro (*Colocasia esculenta* (L.) Shott) collections have a large number of traditional cultivars distributed all over the country. However, the most abundant taro genetic resources are located in the Yunnan province, in southwest China. The present study is based on RAPD analysis of accessions collected from 7 counties within the Yunnan province and 7 ethnic groups in China. Accessions were donated by the Kunming Institute of Botany. RAPD assays were used to analyze the accessions, and assess the extent and distribution of genetic diversity within and between farmer communities. Thirty three DNA primers were selected and used for RAPD assay across 46 accessions. A total of 193 DNA bands were produced and used in Cluster Analysis. Results indicate high genetic diversity among taro accessions. The 46 accessions clustered as two groups, one comprising the wild types and another the cultivated accessions. The dendrogram also shows that taro accessions could be divided into both diploid and triploid types.

The cluster analysis showed that taro intra-species genetic diversity of these accessions was related to the selection pressure applied by the ethnic group during the course of its domestication and cultivation and indicated that ethnobotanical selection is one of the most important strategies to use for the maintenance of taro genetic diversity in germplasm collections.

Materials

One hundred and four taro accessions, received from the Kunming Institute of Botany, were planted at the Institute of Vegetables and Flowers, CAAS, Beijing. These accessions were collected from 7 ethnic groups belonging to 7 counties of the Yunnan province in southwest China. Samples of young leaves from 46 selected accessions (Table 1) were harvested from each individual plant and placed in a nylon bag. Tissue was freeze-dried and stored at -20°C until use.

Genomic DNA extraction

Tissue grinding followed the protocol outlined by Colosi and Schaal (1993) with 0.06-0.1g of freeze-dried tissue in 1.5 ml microfuge tubes. Immediately after grinding, genomic DNA was extracted using CTAB buffer according to the protocol by Hillis and Mortilz (1990). The final DNA pellet was dissolved in 100 l TE solution (10 mM Tris-HCl, pH 7.5 and 0.1 mM EDTA), and the DNA concentration was determined using a DU-70 spectrophotometer (Beckman).

TABLE 1. List of taro accessions used

Accession No.	Native Name	Ethnic group	Collecting Site
97103	Gou Gua Yu	Han	Yiliang
97105	Qing Yu(Mao Tou Yu)	Han	Yiliang
97106	Wan Gen Yu	Han	Jiangchuan
97107	Pobule	Jinuo	Jinuo Shan, Jinghong
97108	Bulena	Jinuo	Jinuo Shan, Jinghong
97109	Bulece	Jinuo	Jinuo Shan, Jinghong
97118	Byong Ma Za	Hani	Mengsong , Jinghong
97120	Byong Ma Ni Zong	Hani	Mengsong , Jinghong
97121	Byong Ma A Yo	Hani	Mengsong , Jinghong
96002	Chang Bi Yu	Hani	Mengsong , Jinghong
97122	Bi Ge A Na (Hei Yu)	Hani	Mingzhishan,Jiangcheg
97124	A Chi Chi Biu (Tian Yu)	Hani	Mingzhishan,Jiangcheg
97125	Jiang Bian Yu	Hani	Mingzhishan,Jiangcheg
97126	Ku Biu (Hong Yu)	Hani	Mingzhishan,Jiangcheg
97128	Bi Ka La Ka	Hani	Mingzhishan,Jiangcheg
97129	Mi Biu (Jiu Tou Yu)	Hani	Mingzhishan,Jiangcheg
97135	Wild Yu	Dai	Jinpin
97113	Mian Hua yu	Jinou	Jinuo Shan, Jinghong
97133	Sang zang yu	Hani	Jiahe, Jiangchen
98006	Ma guan ye yu		Gu Lin Qing, Ma Guan
98022	Daziyu	Han	CaoJian Yunlong
98023	Chengayeyu		CengGa, LuShui
98024	Heigengyeyu	LiLi	CengGa, LuShui
98025	Baiyu	LiLi	CengGa, LuShui
98026	Kua mo mei(baiyu)	Nu	PiHe,FuGong
98028	Mei na(heiyu)	Nu	PiHe,FuGong
98029	Lugengziyu	LiLi	ShangPa,FuGong
98030	Baigengziyu	LiLi	ShangPa,FuGong
98041	zhongbaoyu	Han	MangKuan,BaoShan
98042	Yeyu		YunHua,TengChong

Accession No.	Native Name	Ethnic group	Collecting Site
98044	Duoyalugengyeyu		JiuCheng, YingJiang
98058	Yinjiangcaiwenyeyu		TongBi,YingJiang
98063	Dayinjiangyeyu		DaYingJiang,YingJiang
98068	Yaoguanyeyu		YaoGuan,ShiDian
98087	Dishuiyu(haiyushu)	Han	MengTing,GengMa
98093	Ziyu	LaKu	ShanYun,LangChang
98094	Hongyu	LaKu	MaKa,LangChang
98095	Ziyu	LaKu	MaKa,LangChang
98096	Hongxiangyu	LaKu	MaKa,LangChang
98105	Byong ma a yo piu	Hani	Mengsong , Jinghong
98108	Byong ma byong na	Hani	Mengsong , Jinghong
98114	Huo pe	Dai	DaMengLong,Jinghong
98124	Xiangyu	Han	GuanPing,Jinghong
98127	Xiaoluyu		Jinuo Shan, Jinghong
98129	Wenwan(Tianwanyu)	Dai	LuLinTian,YuanJian
98130	Wen mei(mawanyu)	Dai	LuLinTian,YuanJian

Method of DNA amplification

Polymerase chain reaction (PCR) was used to amplify accession DNA. PCR reaction mixture (25 l) contained 10 mM (ph 8.3) Tris-HCl, 2.5 mM KCL, 2.5 mM $MgCl_2$, 0.1% Triton-X100, 0.2 mM each of dATP, dCTP, dGTP, dTTP (Sino-American Biotechnology Company), 0.64 M of a single decamer primer (Biotechnology Laboratory, the University of British Columbia , Canada), approx 25ng of genomic DNA and 0.625 unit of DNA *Taq* polymerase (Beijing Agricultural University). PCR reaction mixtures were incubated in a PTC-100 Programmable Thermal Controller (MJ Research, Inc.) programmed for 45 cycles at 94°C for 1 min, and annealing temperature of 38°C (for Method 1) or 45°C (for Method 2) for 5 min, a 3-min ramp to 72°C and 2 min at 72°C.

Electrophoretic analysis

Amplified products were analysed by gel electrophoresis in 1.5% agarose in 1×TAE buffer. A DNA molecular weight marker (Sino-American Biotechnology Company) was used to estimate sizes of amplified products. The gels were stained with ethidium bromide and photographed with black and white film under UV light.

Screening of RAPD primer

Three accessions from different geographic regions (No.98058, No. 97117, No. 98092) were chosen to screen the primers. Accession 98058, 97117 and 98092 were respectively collected from the

TongBi and YingJiang County, from Mensong in the Jiangcheng County, and accession from ZhuTang in LangChang. The criteria for selecting useful primers are: 1) the amplified DNA fragments were very clear on the gel; 2) more polymorphisms were displayed among the tested samples; 3) amplified fragments were reproducible. Based on these conditions, 33 primers were selected out of a total 700 primers. The sequences of these primers are presented in Table 2.

TABLE 2 List of decamer oligonucleotides selected and their sequences

Primer Identification UBC No.	Sequence (5' to 3')	Primer Identification UBC No.	Sequence (5' to 3')
136	TAC GTC TTG C	443	TGA TTG CTC G
137	GGT CTC TCC C	451	CTA ATC TCG C
301	CGG TGG CGA A	454	GCT TAC GGC A
303	GCG GGA GAC C	435	CTA GTA GGG G
305	gct ggt acc c	633	CGT TGT ATC C
324	ACA GGG AAC G	635	GGG ATA TCG C
328	ATG GCC TTA C	636	CCC TAA AGC G
333	GAA TGC GAC G	638	GCG GTG ACT A
334	ATG GCA AAG C	643	ATA AGC GGT G
341	CTG GGG CCG T	645	TAC AGC GTT G
345	GCG TGA CCC G	657	GTC CTT TAG C
347	TTG CTT GGC G	664	GCC TGA AAA C
409	TAG GCG GCG G	665	GAC GCT TTT C
414	AAG GCA CCA G	666	CTT AAC ACG C
415	GTT CCA GCA G	692	ACA TTG GGG G
417	GAC AGG CCA A	695	GCT AAT CAG C
439	GCC CCT TGA C		

PCR amplification

The polymorphic bands with method 1 (annealing temperature 38°C) were less specific than that of method 2 (annealing temperature 45°C). Hence, further analysis using 33 primers on 46 accessions were carried out at an annealing temperature of 45°C. The 33 primers produced 139 reproducible polymorphic bands across 46 accessions (Fig. 2).

Data analysis

The bands were recorded as present (1) and absent (0). Pairwise Euclidean distances were calculated between samples and Ward's method was used for cluster analysis.

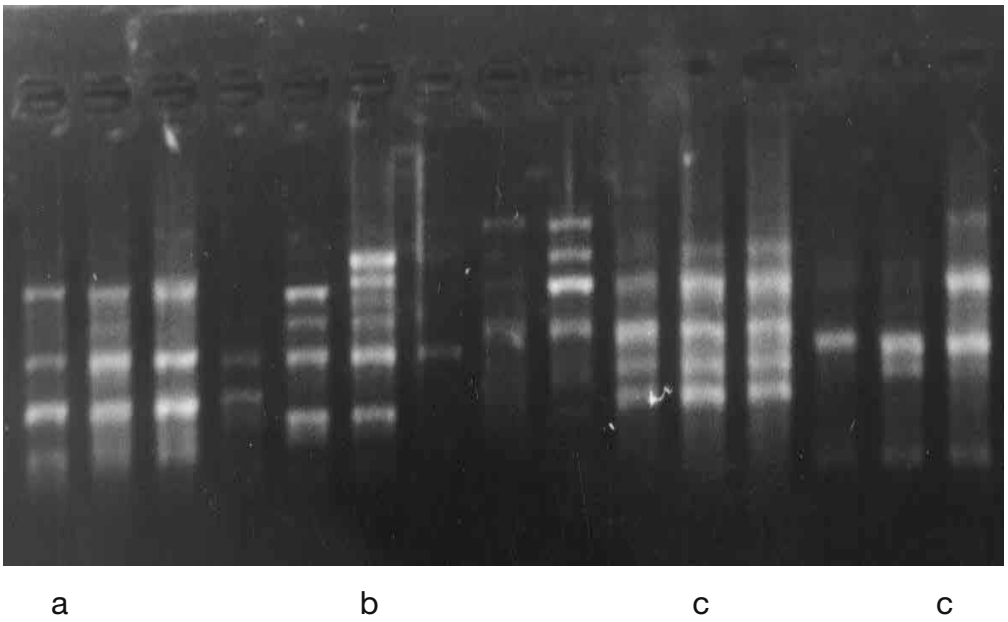

Figure 1. Amplification patterns across accessions 98058(a), 97117(b) and98092 (c) using five primers.

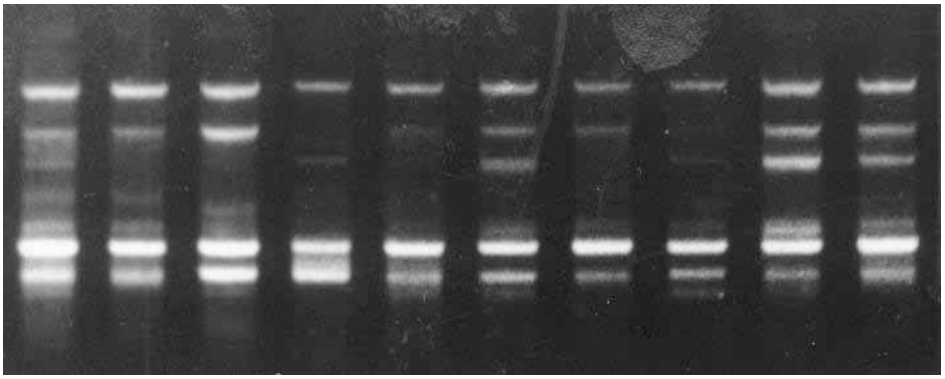

Figure 2. An example of an amplification pattern across different accessions generated by a primer

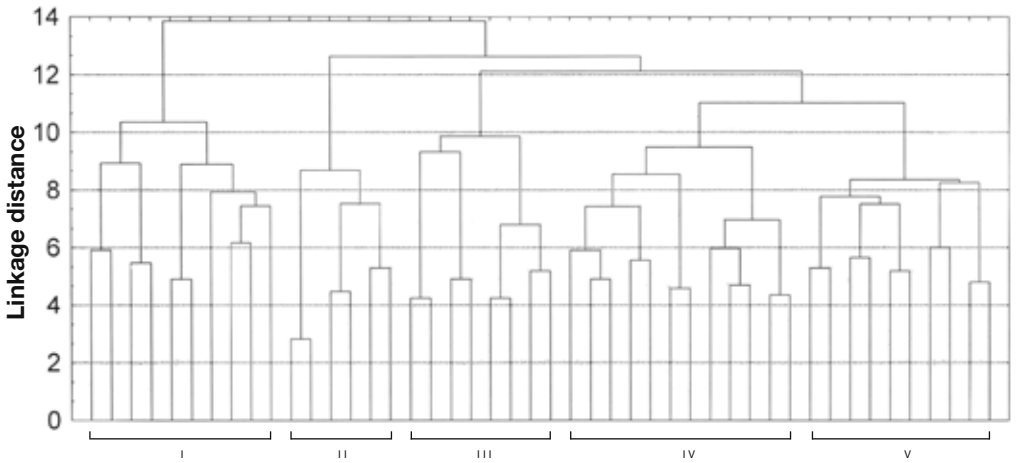

Figure 3. Dendrogram showing genetic relationship among 46 taro accessions (Ward's method was used for cluster analysis based on the presence or absence of bands). Notes: a=Nu; b=Hani; c=Laku; d=LiLi; e=Jinuo; f=Dai; g=Han; o=No ethnic groups

Results

Between-accession variation

The dendrogram based on the genetic distance (Euclidean distance) and Ward's method of clustering for 46 accessions is presented in Fig. 3. Results indicate the existence of substantial variation among taro accessions. The accessions were clustered as five groups, Group I comprising the wild accessions and groups II, III, IV and V the cultivated accessions. As expected, all the group V accessions are triploid, while the other accessions are diploid.

Impact of ethnobotanical grouping on the genetic diversity of taro

The dendrogram also highights a relationship between genetic diversity of accessions and their ethnic origin. Among the cultivated taro groups, groups IV and V comprise 14 accessions from the Hani ethnic group and 5 from the Han. Group III includes 4 accessions each from the laku and Jinuo ethnic groups. Group II includes 2 accessions from the Nu ethnic group and 4 from the LiLi group. These results suggest that ethnobotanical selection was an important factor in maintaining taro genetic diversity.

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Ethnobotany and genetic diversity of taro in Yunnan, China – analyses of diversity using multiple techniques

Li Xixiang, Shen Di, Zhu Dewei, Yang Yongping, Xu Jianchu, Zhou Mingde, P. Eyzaguirre and W.G. Ayad

Introduction

Taro (*Colocasia esculenta*) is an important tuber crop that can be cultivated in rain-fed uplands, in forests, in home gardens and paddy fields. Some taro types are particularly well adapted to difficult lands, such as swamps. Taro is a staple of developing countries, mainly Africa, the West Indies, the Pacific region and Asia. Different parts of the taro plant—corms, cormels, leaves, petioles and even flowers—are consumed as vegetables or used for other purposes, depending on the cultivars and cultures. As taro is mainly vegetatively propagated and difficult to preserve, its commercial importance is largely local, so farmers have been the main users and custodians of taro genetic diversity. Less attention has been paid to the collection and conservation of taro germplasm, or to variety improvement. But, with human population growth, industrialization and the growing dependence on a few global staples to meet the world's needs, the loss of local taro varieties becomes increasingly serious. Global strategies and effective local measures are needed to protect this crop that is well adapted to many uses and environments in developing countries. This is especially so for developing countries undergoing rapid economic growth and changes in food culture and consumption.

China has a large number of traditional cultivars of taro distributed all over the country (Zhang 1984). The Yunnan province, which lies in SW China, at the periphery of the Asian centre of diversity for many crops, is not only the main distribution area of taro germplasm, but is also the major production site in China under diverse farming systems. Taro is the major staple food for many ethnic minorities in Yunnan (Pei 1985). This might, at least in part, explain the extent of genetic diversity of taro and its condition in this particular geographic and ethnic region.

Plant genetic diversity and the evolution of taro can be studied mainly through four aspects: morphology, cytology, protein and DNA. Morphological markers representing different phenotypes are usually few, difficult to detect and are easily affected by environment. Cytological markers are also few and difficult to discern (Xu 1995).

Isozyme markers are the products of gene expression and the phenotypes in biochemistry. They are co-dominant, more so than morphological markers, and easier to detect. But isozymes may fail to detect diversity within some species, due to plant organ specificity, developmental stage, experiment condition on the enzyme expression, post-translation modification of enzyme and poorer resolution than molecular techniques (Hillis and Dixon 1991). However, isozyme analyses have been widely used in such aspects as plant systematic classification, genetics and breeding (Bousquet *et al.* 1987; Pasteur 1988; Doebley 1989; Murata and Kawahara 1994). In taro, isozyme markers have been of limited use in identifying taro cultivars that have arisen from somatic mutations (Moore

and Durham 1992). In genetic improvement, isozyme markers are often poorly correlated with the morphological characters of interest to crop breeders. Nevertheless, greater isozyme variation was demonstrated both in identifying taro cultivars, wild and related species (Kuruvilla and Singh 1981; Tanimoto and Matsumoto 1986; Lebot and Aradhya 1991; Moore and Durham 1992; Yoshino 1994; Isshiki *et al.* 1995; Nguyen *et al.* 1998). Furthermore, it is postulated that the higher the order of organisms, the fewer differences in their genetic structure, and the differences between their phenotypes are more determined by the systematic expression and regulation of existing structural genes (Zhou *et al.* 1993). Therefore, isozyme markers may provide a better understanding of the distribution of genetic diversity among populations of traditional taro cultivars.

Molecular markers at DNA level can reflect the differences in DNA structure. They are more steady and effective, and easy to detect on a large scale. They are not affected by environment or the phenology of the plant. DNA as the medium of heredity directly reflects the relatedness between and within collections (Li 1995). The spacer region of ribosomal DNA was reported to be highly variable, and has been used to understand genetic relationships of species and cultivars (Kresovich *et al.* 1994). Many different rDNA patterns were found in taros by digesting total DNA with *Taq* I and *Hinf* I and using taro rDNA probes (Matthews *et al.* 1992).

Plant evolution and the distribution of genetic diversity within crops is closely related to human activities and environmental factors within crops. The study of this interaction between people, plants and the environment is called ethnobotany. It encompasses the study of human societies, ecology, evolution and symbols, and the dynamic nature of the interrelations between them.

In this study, ethnobotanical survey and documentation methods, and biochemical and molecular biological analysis, were combined to illustrate how taro genetic diversity is structured and managed by farmers in different communities under distinct socioeconomic conditions, ecological environments and farming systems. A second objective was to find a way to protect this crop from further erosion and promote the use and conservation of taro and other vegetatively propagated crops.

Evolution of taro in Yunnan and phylogenetic relationships

The centre of taro origin and domestication has been debated (Kuruvilla and Singh 1981; Coates *et al.* 1988; Matthews *et al.* 1992; Yoshino 1994). From the aspect of the great number of wild, escaped and cultivated taro germplasm, and their distribution and diversity as detected by molecular methods, it can be deduced that Yunnan and southern parts of China are important origin and domestication areas of taro.

The taxonomy of taro has been studied for a long time (Kumazawa *et al.* 1956). There has been some research on variation between taro genera, species, varieties, and even cultivars (Lebot and Aradhya 1991; Tanimoto and Matsumoto 1986). According to the China Plant Records, *Colocasia* includes 13 species distributed mainly in tropical and subtropical Asia. There are at least four distinct species in China: *C. esculenta* Schott*, C. fallax* Schott*, C. formosana* Hayata, and *C. gigantea* (Blume) Hook.f.*,* while others named may be synomous with *C. esculenta (C. antiquorum* Schott*, C. tonoimo* Nokai*, C. konishii* Hayata) or have been misidentified as *Colocasia* (*C. kotoensis* Hayata*).* It is thought that the original taro in China grew in low, wet regions. As the water level rose and fell, the plant form changed. Taro varieties growing in both water and uplands evolved from water taros;

further, upland taros were formed from the former through natural action and human cultivation and selection over a long time. Various upland taros became differentiated as the leaves and corms changed. Taros used for the leaf came into being as the petiole thickened and its acridity lessened. The corm also evolved: as the old and coarse mother corm of wild taro became edible, a head-like form with creeping stolons was produced. Eventually, the creeping stolons became shorter and closer to the mother corm. Multi-cormel taros appeared, with many child-corms set on the mother corm. Finally, multi-head taros evolved through fusion, when mother corms and child-corms became shorter and not easily distinguished (Chinese Vegetable Cultivation 1987).

In this study, the plants of wild taro accessions were small, without corms or with corms undeveloped. Taro accessions growing in water or near the water have stolons. Included in this study were cultivated accessions with head-like corms, multiple cormels and multiple heads, and a variety of corm and cormel shapes. Although some useful morphological data was obtained, it was not enough for taxonomic classification.

Most wild taro accessions could be grouped together, but some wild taros and some cultivated taros were clustered together. They were far removed from other wild taros. The reasons may be that different cultivars evolved from different wild taros, or the wild taro accessions were at different stages of evolution, or some wild taros were not true wild taros and may have escaped temporarily from cultivation. Some varieties with stolons could not be distinguished by either isozyme or RAPD methods. This may be because the primers selected were too few to detect all mutations, or because these characters were easily affected by the environment.

C. gigantea Hook (accession 98008 in Table 1) was close to *Alocasia macrorrhiza* (accession 98087 in Table 1). They were far away from all other taro accessions. According to the crossing experiments made by Yoshino (1994) and Nguyen *et al.* (1998), *C. gigantea* may be produced from natural crossing between *Alocasia macrorrhiza* and *C. esculenta.* Our results may prove that deduction.

The effects of geographical location and human activities on taro genetic diversity in Yunnan

Yiliang, situated in a subtropical agroecological zone in central Yunnan, has a market-oriented farming system. No wild taros were collected there. The genetic base revealed in the studies reported here was narrow. Jingcheng, Jinghong and Maguan—located in the southern subtropics and tropics agroecological zone—have a swidden farming system, in transition from a subsistence to a market-oriented-system. The taros there, managed by Jinuo, Hani and Han Chinese people, displayed the greatest diversity. They were generally planted in swidden, rain-fed upland, irrigated paddy, home gardens or orchards, mainly on mountain areas. The diversity among cultivated accessions was greater than that of wild and escaped accessions. Above all, the special geographical environment in different communities, such as geographical isolation, diverse microecological condition, and human selection based on people's interests and their cultural communication habits had great effects on the development of taro diversity and distribution.

Materials and methods

Field survey and germplasm collection

In 1996, an exploratory survey was carried out in Xishuangbanna, Jiangcheng and some counties around Kunming. In 1997, a repeated sampling was undertaken in these three regions. In 1998, more than 100 samples were collected in some remote, poorly known and marginal regions within Yunnan. Methods used are listed below.

Informal interviews: basic information gathered in the villages , e.g. planting history, usage, processing methods and customs.

Participatory surveys: local people were invited to participate in sample collection, in identification of characters and assessment of local environments.

Market surveys: market surveys were carried out in the local marketplaces to obtain information about yield, people's preferences and prices.

Sample collection: samples were collected from different plants in one taro plot cultivated by a household. If samples had to be taken from the same plant, this was noted. Usually, 10 taro tubers with buds were collected, and stored with moist mosses in the field.

Morphological survey: if possible, basic morphological characters were recorded at time of sampling. Some morphological characters were identified when samples were planted in the experimental plot at the Institute of Vegetables and Flowers, CAAS.

Materials used for isozyme and RAPD analysis

Taro samples (n=103) in the form of tubers with buds were received from the Institute of Kunming Botany, Chinese Academy of Science during 1997–98. They were cultivated in the experiment field and managed normally at the Institute of Vegetables and Flowers, CAAS in Beijing. Some did not germinate, some died at an early stage; 72 accessions that germinated and grew normally were used in isozyme analysis (Table 1); later only 28 saved accessions were used in RAPD analysis. The same suitable parts of all plants in an accession were collected and mixed.

ISOZYME ANALYSIS

The polyacrylamide gel vertical electrophoresis with Tris-Gly electrode buffer was employed for our study. In our preparatory experiment, seven isozymes (POD, EST, SOD, -AMY, COD, PPOD and ME) were screened from 12 enzyme systems including peroxidase (POD), superoxidase (SOD), esterase (EST), polyphenoloxidase (PPOD), cytochrome oxidase (COD), -amylase (AMY), malic enzyme (ME), malate dehydrogenase (MDH), alcohol dehydrogenase (ADH), 6-phosphogluconate dehydrogenase (6-PGD), isocitrate dehydrogenase (IDH) and shikimic dehydrogenase (SKDH). Because most taro germplasm did not produce tubers and the variance of ME isozyme between different varieties was low, five isozymes except for -AMY and ME were tested.

TABLE 1. The sources and ethnobotanical information of taro accessions.

Code	Local name	Collecting location	Ethnicity	Status†	Agroecological niche	Use	Part used
97101	Kai hua yu	Yiliang, Xiaoma	Han	C	Home garden, paddy field, orchard	edible	Flower, cormel
97110	Bu le ne	Jinghong, Jinuo yaruo	Jinuo	C	—	—	—
97117	Byong ma ne	Jinghong, Jinuo situ	Hani	C	home garden, swidden field	edible	cormel
97118	Byong ma za byong	Jinghong, Mengsong	Hani	C	rain-fed upland, paddy, swidden	edible	cormel
97120	Byong ma ni zong	Jinghong, Mengsong	Hani	C	home garden, swidden field	edible	cormel
97121	Byong ma a yo	Jinghong, Mengsong	Hani	C	paddy, swidden, home garden	forage	leaf
97122	Bi ge a na	Jinagcheng, Jiahe	Hani	C *	home garden, rain-fed upland	edible	cormel
97123	Bi ge bi na	Jinagcheng, Jiahe	Hani	C	home garden, rain-fed upland	edible	cormel
97126	Ku biu	Jiangcheng, Jiahe	Hani	C *	home garden, rain-fed upland	edible	cormel
97133	Shang zhan yu	Jiangcheng, Jiahe	Hani	C	home garden, rain-fed upland	edible	cormel
98001	Baishan da tou yu	Baoshan, Market	Han	C *	home garden, rain-fed upland	edible	multi-head corm
98003	Yimen qing yu	Yimen, Market	Han	C *	home garden, rain-fed upland	edible	cormel
98004	Jinpin kui yu	Jingping, Market	Han	C	home garden, rain-fed upland	edible	multi-head corm
98005	Maguan bai yu he	Maguan, iaotou, Market	Han	C	home garden, rain-fed upland	edible	multi-head corm
98006	Maguan ye yu	Maguan, Gulinqing		W *	margin	not used	
98007	Mengzi hong yu	Mengzi, He kou		E C	margin	edible	flower, cormel
98008	Caiyu he	Maguan, Gulinqing	Han	C *	home garden, rain-fed upland	edible	multi-head corm
98019	Ye yu	Yunlong, Caojian		W *	margin	not used	

Code	Local name	Collecting location	Ethnicity	Status†	Agroecological niche	Use	Part used
98022	Da zi yu	Yunlong, Caojian	Han	C	rain-fed upland	edible	cormel, corm
98023	Chengga ye yu	Lushui, Chengga		W	margin	not used	
98024	Hei geng yu	Lushui, Chengga	Lili	C *	home garden, rain-fed upland	edible	cormel
98025	Bai yu	Lushui, Chengga	Lili	C *	home garden, rain-fed upland	edible	cormel
98026	Kua mo mei	Fugong, Pihe laomudeng	Nu	C *	home garden, rain-fed upland	edible	cormel
98028	Mei na	Fugong, Pihe laomudeng	Nu	C	—	—	—
98029	Lugeng zi yu	Fugong, Shangpa	Lili	C	rain-fed upland	edible	petiole
98030	Baigeng zi yu	Fugong, Shangpa	Lili	C *	rain-fed upland	edible	petiole
98033	Honggeng zi yu	Fugong, Shangpa	Lili	C *	rain-fed upland	edible	cormel, corm
98034	Hong yu	Fugong, Shangpa	Lili	C	rain-fed upland	edible	cormel, corm
98035	Heigeng ye yu	Baoshan, Mangkuan		W	magin	not used	
98036	Lugeng ye yu	Baoshan, Mangkuan		W	margin	not used	
98039	Hong yu	Baoshan, Mangkuan	Han	C	honegarden	edible	flower, cormel
98040	Xiao lu yu	Baoshan, Mangkuan	Han	C *	home garden,	edible	cormel
98041	Zongbao yu	Baoshan, Mangkuan	Han	C	—	—	—
98042	Ye yu	Tengchong, yunhua		E *	margin	not used	
98044	Duoya lugeng yeyu	Yingjiang, jiucheng		E *	margin, home garden	not used	
98045	Jiucheng wangenyu	Yingjiang, jiucheng		E	margin	edible/ forage	flower, petiole, leaf
98052	Tuan yu	Yingjiang, Nabang	Han	C	swidden field	edible	cormel, corm
98053	Tuan yu	Yingjiang, Nabang		E *	swidden field	edible	cormel, corm

Code	Local name	Collecting location	Ethnicity	Status†	Agroecological niche	Use	Part used
98054	Tongbiguan angenyu	Yingjiang, Nabang	Jingpo	C	margin	edible/ forage	flower, petiole, leaf
98057	Chushui yu	Yingjiang, Nabang	Han	C *	swidden field	edible	cormel, corm
98061	Hongya yu	Yingjiang, Mangyunsishe	Han	C *	swidden field	edible	cormel, corm
98064	Husan ye yu	Longchun, Husan		W	margin	not used	
98066	Shui yu	Shidian, Youwang	Han	C	paddy field	edible	cormel, corm
98069	Hong yu	Shidian, Xiaohanzhuang	Han	C *	home garden	edible	flower, cormel
98070	Rentou yu	Yongde, Yongdian	Han	C *	home garden, rain-fed upland	edible	cormel, corm
98075	Caiwen ye yu	Zhenkang, Xiaoshuijing		W *	forest	not used	
98078	Shidian wangengyu	Shidian, Wandian		W	margin	not used	
98080	Bai yu	Shidian, Wandian	Han	C	home garden	edible	cormel
98083	Bai yu	Gengma, Mengding nuo e	Wa	C *	home garden	edible	cormel
98084	Lu yu	Gengma, Mengding nuo e	Wa	C *	home garden	edible	cormel
98086	Shui yu	Gengma, Mengding moya	Han	C	irrigated upland	edible	cormel
98087	Dishuiyu (Alocacia)	Gengma, Mengding	Han	C *	home garden	edible	petiole
98088	Lu yu	Gengma, Mengding shidui	Han	C	rain-fed upland	edible	cormel
98089	Zi yu	Gengma	Han	C *	rain-fed upland	edible	cormel
98090	Hongya yu	Cangyuan, Tuanjie xiaohei	Han	C	home garden	edible	cormel
98093	Zi yu	Lancang, Shangyun	Lahu	C	rain-fed upland	edible	cormel
98094	Hong yu	Lancang, Makadi	Lahu	C	rain-fed upland	edible	cormel
98095	Zi yu	Lancang, Makadi	Lahu	C	rain-fed upland	edible	cormel
98096	Hongxiang yu	Lancang, Makadi	Lahu	C	rain-fed upland	edible	cormel

Code	Local name	Collecting location	Ethnicity	Status†	Agroecological niche	Use	Part used
98099	Zi yu	Menghai, Mengzhe	Dai	C	home garden	edible	petiole, corm
98100	Xiaobai yu	Menghai, Mengzhe		E	roadside	edible	cormel
98102	Byong ma ne	Jinghong, Mengsong	Hani	C	swidden field	edible	cormel, corm
98105	Byong ma a yo piu	Jinghong, Mengsong	Hani	C	swidden field	edible	cormel, corm
98106	Byong ma za byong	Jinghong, Mengsong	Hani	C	swidden field	edible	cormel, corm
98108	Byong ma byong na	Jinghong, Mengsong	Hani	C *	swidden field	edible	cormel, corm
98114	Huo pe	Jinghong, Damenglong	Dai	C *	home garden	edible	cormel
98115	Ye yu	Jinghong, Mengsong		W	margin	forage	petiole, leaf
98116		Jinghong, Mengsong		C	swidden field	edible	cormel
98119	Bu le ne	Jinghong, Jinuo	Jinuo	C	home garden, swidden field	Edible	cormel, corm
98120	Bu le ce	Jinghong, Jinuo	Jinuo	C	swidden field	Edible	cormel, corm
98121	Bu le na	Jinghong, Jinuo	Jinuo	C *	swidden field	Edible	cormel, corm

† C = cultivated; W = wild; E = escaped. * = accessions used for RAPD analysis.

The acrylamide and bisacrylamide concentration suitable for different enzymes is not identical. The separating gels with the total concentration (T) of acrylamide and bisacrylamide of 8–9% and bisacrylamide concentration (C) of 4% and the concentrating gels with T=4% and C=20% were used for POD and COD. The suitable gel concentration for SOD and EST is T=10%, C=4% for separating; T=4%, C=20% for concentrating. T=7.5%, C=4% in separating gels and T=3.5%, C=20% in concentrating gels are best for PPOD.

Because the electrophoretic band of POD in roots was clear and the band number was highest, roots were used for POD isozyme analysis. The acceptable extract concentration is 1:3 of root to extract buffer weight, with the extract volume of 20 µl. Young developed leaves were used for EST analysis, as EST is most active in that plant part. The suitable extract concentration is 1:4 of sample to extract buffer weight and the extract volume is 20 µl. Young leaves were used in SOD analysis The extract can be prepared as 1:4 of the sample to extract buffer weight. In COD analysis, roots were used. The extract was prepared as 1:1 of the sample to extract buffer weight, with 35 µl of the

extract acceptable. PPOD was present in all leaves but separated badly whereas the bands in roots, tuber and petiole all separated clearly, so roots were used. The acceptable extract concentration is 1:1, with 35 µl of the extract for analysis.

All samples were put into an ice-box and taken to our laboratory. Isozyme extract was prepared using modified Bousquet buffer (Bousquet *et al.* 1987) through grinding and centrifuging at 5000 rpm. All operations were carried out under low temperature.

Electrophoresis was run at 4°C for 2.5–4 hr at 2.0–2.5 V/cm. After electrophoresis, the gels were stained using different methods according to different enzymes (Vallejos 1983; Pasteur 1988).

RAPD ANALYSIS

Young leaves were harvested from individual plants and placed in a nylon bag. The tissue was then freeze-dried and stored at –20°C until use. Three accessions (97101, 98008 and 98075) were chosen to screen primers.

Genomic DNA extraction: Tissue grinding followed the protocol of Colosi and Schaal (1993) with 0.06–0.1 g of freeze-dried tissue in 1.5 ml micro-refuge tube. Immediately after grinding, genomic DNA was extracted using the SDS buffer (100mM Tris-HCl pH 8.0, 80mM EDTA pH 8.0, 500mM NaCl, 2% SDS, 10mM β-sulphydryl ethanol) and phenol-chloroform-isoamyl alcohol (25:24:1) according to the protocol of Wang and Fang (1998). The required amounts of protein enzyme K and Rnase were added for DNA purification. The final DNA pellet was dissolved in 100 l TE solution (10mM Tris-HCl, pH 8.0 and 0.1mM EDTA pH 8.0), and the DNA concentration was determined by DU-70 spectrophotometer (Beckman) and diluted to 40 ng/ l for PCR reaction.

DNA amplification: Reaction mixture (20 l) contained 2 l 10×PCR buffer (500mM KCl, 100mM tris-HCl pH 9.0, 10% Triton-x100, 20mM MgCl), 0.4 l 2.5mM of dATP, dCTP, dGTP, dTTP each (Pharmacia Bioteck products from Sino-American Biotechnology Company), 2.0 l 8pmol of Operon primers (Sino-American Biotechnology Company), 40 ng genomic DNA, 1 unit of DNA *Taq* polymerase (2 U/ l, Promega Corporation products from Sino-American Biotechnology Company) and 14.1 l of PCR-grade water. PCR reaction mixtures were incubated in a PTC-200 Programmable Thermal Controller (MJ Research, Inc.) programmed as: Step 1, 94°C, 2 min; Step 2, 92°C, 20 sec; Step 3, 36°C, 40 sec; Step 4, 72°C, 80 sec; Step 5, GOTO Step 2, 39 cycles; Step 6, 92°C, 20 sec; Step 7, 36°C, 60 sec; Step 8, 72°C, 2 min; Step 9, 4°C, 7 min.

Electrophoretic analysis: Amplified products were analyzed on DYY-34A horizontal electrophoresis trough by gel electrophoresis in 1.0% agarose in 1×TBE buffer. A DNA marker (λDNA/ EcoR +Hind (Sino-American Biotechnology Company) was used to estimate the sizes of amplified products. The gels were stained with ethidium bromide, EXPOSED TO uv LIGHT and photographed with black and white film.

In data analysis, the electromorphs in isozyme or RAPD analysis were considered as characters with presence scored as 1 and absence scored as 0. Distinct electromorphs in each accession using each method were used as descriptors for accessions. If two cultivars were different for one electromorph, they were considered to exhibit two different zymotypes. Euclidean distances were

calculated between any two accessions. Unweighted Pair-group Averages (UWPGA) were used for cluster analysis on the raw data of electromorphs.

Isozyme variation within the locations, the status and ethnicity of the community where the sample was taken were determined by calculating the percentage of electromorphs not common to all accessions (percent of dissimilarity = 100 – percent of identical electromorphs). For the comparison of isozyme, RAPD and integrated methods, all electromorphs for an accession from isozyme and RAPD analyses were considered as the character descriptors of this accession. From the data matrix constructed from all electromorphs and 28 landraces, Euclidean distances were calculated between any two accessions. Unweighted Pair-group Averages (UWPGA) were used for cluster analysis on the raw data.

Results and analysis

Germplasm sources and identification of taro accessions

Seventy-two taro accessions came from 34 different areas of 21 counties with 9 ethnic groups and 7 agroecological niches (Table 1). Jinghong was richest in taro accessions. Among 72 accessions, 9 were wild, 6 were escaped, and 57 were cultivated varieties. With regard to their uses, 55 taro accessions were edible, 2 could be used as forage, 2 could be used as both food and forage, and 10 were not used as food. Information for three accessions was lacking.

Concerning the parts used, cormels of 26 accessions, cormels and corms of 16 accessions, corms of 1 accession, and multi-head corms of 4 accessions were used. The corms and flowers of 6 accessions, flowers and petioles of 2 accessions, petioles and leaves of 1 accession, petioles and corms of 1 accession could be used. Petioles of 1 accession and leaves of 1 accession could be consumed (Table 1).

In fact, the petioles of all wild and cultivated taros in Yunnan could be used as forage and, in some cases, also the corms and cormels. Human consumption focused on *Colocasia gigantea*, another species of this same genus, which is widely cultivated to collect petioles. Most wild and cultivated taro plants were usually not used by local people for vegetables. The petioles of most taro cultivars are edible following suitable treatment. Kaihuayu (flowering taro) was widely planted as monoculture in the central part of Yunnan. Local inhabitants traditionally cooked the flower stalk with eggplant.

The cultivars with good-tasting mother tubers belong to the single-corm morphotype. Among them, the most famous cultivars include *Jinpingkuiyu* (Jinping big taro), *Baoshandayutou* (baoshan big taro) and *Binglangyu* (betelnut-like taro). The cormels of most cultivars of this type are also edible, but owing to low cormel yields, they are seen as less significant.

Cultivars planted for cormels mostly belong to the multi-cormel morphotype. Because of human selection for centuries, most plants of this type produce many cormels with good taste, but the mother corms are not much favoured. Most cultivars of this type are in Yunnan, and they include *Qingyu* (Green taro), *Baiyu* (White taro), *Hongyu* (red taro) and *Ziyu* (purple taro). In some cultivars with multiple cormels, the corm and cormels are not distinct because corm and cormel are closely connected. Both cormel and corm taste good. *Gouzhuayu* (dog's claw taro) is the best known example of this type.

Some morphological characters are presented in Table 2. Because most taro cultivars rarely flower under cultivation, flowers were seldom used as diagnostic characters in this study. The botanical taxonomy of all accessions could not be performed in detail, but folk taxonomy of all accessions was described according to the criteria used commonly by local people in Yunnan. It can be seen that the colours of leaf veins and petioles in different accessions were diverse. The tillering in most accessions was weak. Some varieties had 5–7 plantlets produced from their mother plant. Most wild taros and water taros had stolons.

TABLE 2. Major morphological characters of 72 taro accessions.

Code	Leaf shape	Leaf colour†	Vein colour†	Petiole colour†	Leaf surface	Plant height	Flowering/ tillering	Stolon
97101	Narrow	Purple	Red	Purple	Smooth	Short	No/no	No
97110	Broad	Green	LG	Green	Smooth	High	No/3	—
97117	Narrow	Green	Red	Purple	Smooth	High	No/1	No
97118	N/A ‡	N/A	N/A	N/A	N/A	N/A	N/A	N/A
97120	Narrow	Green	LG	Purple	Smooth	High	No/1	No
97121	Broad	LG	LG	Purple	Smooth	High	Yes/no	—
97122	Broad	Green	LG	Green	Smooth	High	No/no	No
97123	Broad	SP	Red	Dull red	Smooth	Short	No/no	No
97126	Broad	Green	SP	Purple	Smooth	High	Yes/6	No
97133	Narrow	LG	LG	LG	Smooth	Short	No/no	—
98001	Broad	LG	LG	LG	Smooth	High	No/no	No
98003	Broad	Green	LG	LG	Smooth	High	No/1	No
98004	Broad	LG	LG	LG	Smooth	Short	No/1	—
98005	Narrow	Green	LG	Dull red	Smooth	Short	No/no	—
98006	Narrow	Green	LG	Dull red	Smooth	Short	No/no	Yes
98007	Broad	Green	LG	Purple	Smooth	High	No/1	No
98008	Broad	LG	LG	Green	Smooth	High	No/5	No
98018	Narrow	Green	LG	Purple	Smooth	Short	No/no	No
98019	Narrow	Green	LG	LG	Wrinkled	Short	No/1	Yes
98022	Broad	Green	LG	Dull red	Smooth	High	No/1	No
98023	Narrow	LG	LG	LG	Wrinkled	Short	No/1	Yes
98024	Narrow	LG	LG	Dull red	Smooth	High	No/2	No
98025	Broad	Green	LG	LG	Smooth	High	No/5	No

Code	Leaf shape	Leaf colour†	Vein colour†	Petiole colour†	Leaf surface	Plant height	Flowering/ tillering	Stolon
98026	Narrow	LG	LG	LG	Smooth	Short	No/1	No
98028	Broad	Green	Red	Purple	Smooth	Short	No/2	—
98029	Narrow	Green	Green	Green	Smooth	High	No/2	—
98030	Broad	Green	LG	LG	Smooth	Short	No/no	Yes
98033	Broad	Green	LG	Dull red	Smooth	High	No/2	No
98034	Narrow	LG	LG	Dull red	Smooth	Short	No/no	—
98035	Narrow	Green	Red	BP	Smooth	Short	No/no	—
98036	Narrow	Green	LG	LG	Smooth	Short	No/no	Yes
98039	Broad	LG	LG	Dull red	Smooth	Short	No/no	No
98040	Broad	Green	LG	Green	Smooth	High	No/7	No
98041	Narrow	LG	LG	LG	Smooth	Short	No/no	—
98042	Braod	Green	LG	LG	Smooth	Short	NO/no	—
98044	Narrow	LG	LG	LG	Wrinkled	Short	No/no	Yes
98045	Broad	LG	LG	LG	Smooth	Short	No/5	Yes
98052	Broad	LG	LG	LG	Smooth	Short	No/no	—
98053	Broad	LG	LG	LG	Wrinkled	Short	No/2	No
98054	Narrow	Green	LG	LG	Wrinkled	Short	No/no	Yes
98057	Broad	Green	LG	Dull red	Smooth	High	No/1	No
98061	Narrow	Green	Green	Purple	Smooth	High	No/3	No
98064	Narrow	DG	Green	DG	Smooth	Short	No/no	Yes
98066	Narrow	LG	Red	Dull red	Wrinkled	High	No/1	No
98069	Narrow	Green	Red	Light red	Smooth	High	Yes/no	No
98070	Narrow	DG	Green	DG	Smooth	High	No/no	No
98075	Narrow	Green	Green	Green	Wrinkled	Short	No/1	Yes
98078	Narrow	Green	Green	Green	Smooth	Short	No/no	Yes
98080	Narrow	DG	DG	DG	Smooth	High	No/2	No
98083	Narrow	Green	LG	Green	Smooth	High	No/no	No
98084	Narrow	Green	Green	LG	Smooth	Short	No/1	No
98086	Broad	Green	Green	LG	Wrinkled	Short	No/no	No
98087	Broad	Green	Green	Green	Smooth	High	No/3	No

Code	Leaf shape	Leaf colour†	Vein colour†	Petiole colour†	Leaf surface	Plant height	Flowering/ tillering	Stolon
98088	Narrow	Green	Green	DG	Smooth	High	No/no	—
98089	Narrow	Green	Green	Purple	Smooth	High	No/no	No
98090	Broad	Green	Green	DG	Smooth	Short	No/no	No
98093	Narrow	Green	Green	Purple	Smooth	High	No/no	No
98094	Broad	Green	Green	Dull red	Smooth	High	No/1	No
98095	Narrow	Green	Green	Purple	Smooth	High	No/no	—
98096	Broad	Green	Green	LG	Smooth	High	No/3	No
98099	Narrow	DG	Red	Dull red	Wrinkled	High	No/no	No
98100	Narrow	LG	Green	LG	Smooth	High	No/1	No
98102	Broad	DG	Red	Dull red	Smooth	High	No/no	No
98105	Narrow	DG	Red	Purple	Smooth	Short	No/no	No
98106	Narrow	LG	LG	LG	Smooth	High	No/3	—
98108	Narrow	Green	Red	BP	Smooth	High	No/no	No
98114	Narrow	LG	Green	Purple	Smooth	Short	No/2	No
98115	Narrow	Green	Green	Green	Smooth	Short	No/no	Yes
98116	Narrow	Green	Green	Purple	Smooth	High	No/no	No
98119	Narrow	Green	Green	Dull red	Smooth	High	No/no	No
98120	Broad	Green	Green	Dull red	Smooth	Short	No/no	No
98121	Broad	Green	Red	BP	Smooth	High	No/no	No

† LG-= light green, DG = dark green, BP = black purple.
‡ not available (information not provided).

Isozyme polymorphism among 72 taro accessions

Two zones of EST activity could be seen. Bands in the anodal zone were stronger than those in the cathodal zone. Twenty-one EST bands were observed; no bands were common to all the accessions. Among 72 accessions there exist 45 EST zymograms, which showed great diversity.

Most active bands of POD were distributed in the cathodal zone. Seventeen bands from all accessions were obtained, one of which was common to all accessions. In all, 46 zymograms were shown. There was great diversity among accessions.

The COD enzymes are similar to those of POD, but their bands have different migration rates. There were 11 bands, and 37 zymograms in all accessions.

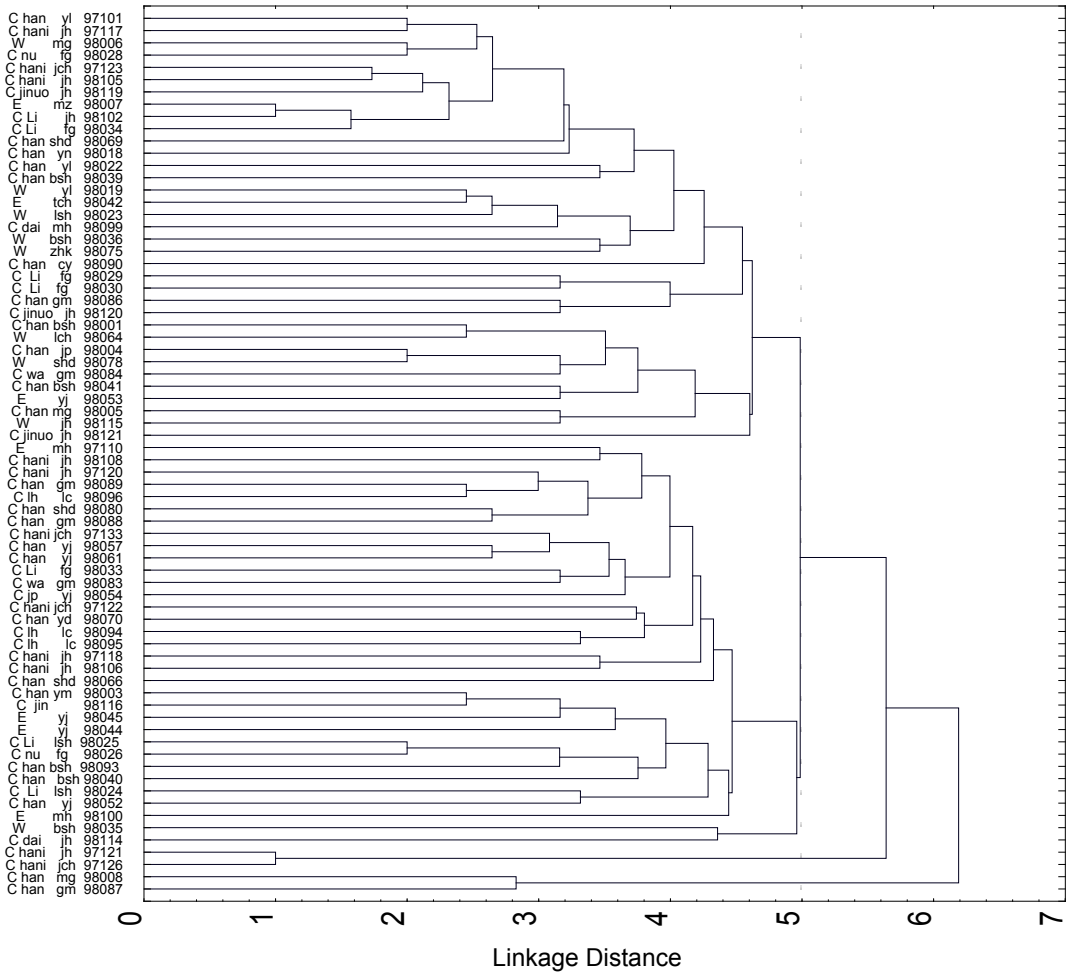


Figure 1. Tree diagram for 72 taro germplasm. Unweighted pair-group average, Euclidian distances. Nationalities: Han, Hani, Jinuo, Nu, Wa, Dai, li=Lili, jp=Jingpo, lh=lahu. Status: C=cultivated; W=wild; E=escaped.

Nineteen bands of PPOD were recorded altogether, distributed evenly from anodal to cathodal zone. In total, 42 zymograms were shown.

The most active zone of SOD was the anodal. In the cathodal zone, there were two weak active zones. Nineteen bands were detected, of which 4 were common to all accessions. The 70 accessions were grouped into three zymograms.

In total, five isozymes for 72 taro accessions produced 87 bands. Among them, 83 are polymorphic bands, accounting for about 94.3% of all bands.

The data based on the matrix of 72 accessions (zymotypes) × 87 electromorphs from all isozymes were used for cluster analysis. Using the genetic distance from the raw data, a diagram for 72 accessions was produced by the UWPGA method. In Figure 1, high levels of genetic diversity can be observed among taro accessions. Three great groups could be distinguished. The first group, 98087 and 98008, were quite far from other accessions (genetic distance of about 6.0). In the second group, 97121 and 97126 were also far from the remaining accessions (genetic distance of 5.6), but the two accessions were the closest in linkage distance. Remaining accessions all belonged to a third group, in which great diversity could also be observed. Accessions could be further subdivided into three subgroups at a genetic distance of 5.0. The first subgroup had only two accessions (98035 and 98114). The second subgroup included 33 accessions. The closest and the furthest distances among them were 2 and 4.9, respectively. The third subgroup had 35 accessions; with 98007 being the closest to 98102, at a genetic distance of 2.0. The furthest distance was 4.8.

Isozyme differences between taro accessions with different status

Fifteen wild and naturalised taro accessions were respectively distributed in different collecting locations, except for the counties of Yiliang and Jiangcheng. In the tree diagram for 72 accessions (Figure 1), the wild and naturalised accessions could not be distinguished from all other cultivated taros. They were classified into different groups. However, among 15 wild and escaped taro accessions, 11 (98007, 98006, 98019, 98042, 98036, 98075, 98064, 98078, 98053, 98115 and 98100) were grouped into subgroup 3, and three (98045, 98044 and 98035) were classified as subgroup 2. It could be calculated that the dissimilarity among cultivated taro accessions was 94.3%, which was greater than that of wild (56.3%) or escaped accessions (44.8%).

Isozyme polymorphism of taros in different locations and ethnic regions

The number of taro accessions collected in different counties or cities, managed by different ethnic groups and their dissimilarities are reported in Tables 3 and 4, respectively. The Han people managed the greatest diversity of taro accessions (Table 2). The diversity of taro accessions cultivated by the Hani was also great. As for the different locations, the taro accessions in Jinghong were the most diverse. Greater diversity existed in taro accessions from the counties of Maguan, Baoshan and Jiangcheng. Some accessions from different communities or nationalities were classified into the same groups (Figure 1). This may indicate that there was a similarity between taro accessions from different counties or nationalities. It could be inferred that the cultural communication and germplasm exchange among the peoples in different counties have influenced taro genetic diversity in the Yunnan Province.

TABLE 3. Dissimilarity of taro germplasm accessions originating in different counties.

Location name	No. of access.	Dissimilarity (%)	Location name	No. of access.	Dissimilarity (%)
Yiliang	1	—	Tengchong	1	—
Jinghong	14	73.6	Yingjiang	7	48.3
Jiangcheng	4	51.7	Nongcun	1	—
Baoshan	6	56.3	Shidian	4	46.0
Yimen	1	—	Yongde	1	—
Jingping	1	—	Gengma	5	40.2
Maguan	3	58.6	Zhenkang	1	—
Mengzi	1	—	Cangyuan	1	—
Yunnong	3	29.9	Lancang	4	32.2
Lushui	3	47.1	Menghai	2	35.6
Fugong	6	43.7			

TABLE 4. Dissimilarity of taro germplasm managed by different ethnic groups.

Ethnicity	No. of accessions	Dissimilarity (%)	Ethnicity	No. of accessions	Dissimilarity (%)
Han	53	82.8	Jingpo	1	—
Jinuo	4	46.0	Wa	2	29.9
Hani	13	66.7	Lahu	4	29.9
Lili	6	49.4	Dai	2	34.5
Nu	2	29.9			

Comparison of genetic diversity among 28 taro accessions detected by RAPD and isozyme analysis

A total of 100 primers were screened using template DNA of representative taros; 19 primers among them produced polymorphic bands.

The 19 randomized primers selected were employed to amplify genomic DNA of 28 representative taro accessions. A total of 183 producible bands appeared. Among them, 22 bands were shared by all accessions, and 161 bands were polymorphic. The number of total bands amplified per primer was 9.6. The number of polymorphic bands amplified per primer was 8.5. The polymorphic rate was 88.5%. The size of all bands ranges from 2500bp to 300bp. The bands amplified by different primers can be seen in Table 5. Among 19 primers, the number of detected bands by OPN07 is the

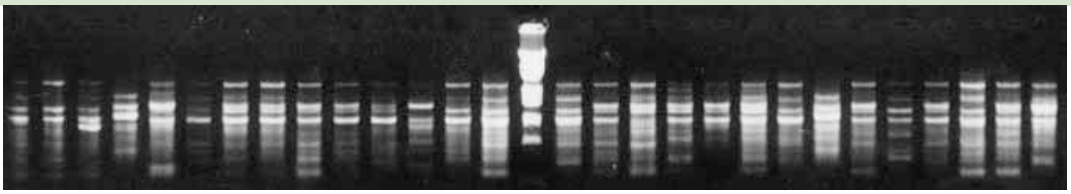
greatest (16 bands) and no bands were shared by all accessions. Polymorphism shown by OPQ06 was the least. Three bands among the detected five bands were common to all accessions. These showed that the genetic diversity of taro germplasm from distinct regions and ecological conditions in Yunnan was rich (Figure 2).

TABLE 5. The total bands and the specific bands amplified by different primers

Primer	Bands			Primer	Bands		
code	Total	Common	Specific	code	Total	Common	Specific
OPN07	16	0	16	OPP03	7	0	7
OPN09	9	1	8	OPP14	7	0	7
OPN10	6	1	5	OPP15	4	1	3
OPN14	10	1	9	OPP16	11	1	10
OPO01	12	2	10	OPP20	10	1	9
OPO03	15	0	15	OPQ04	7	1	6
OPO07	11	1	10	OPQ05	11	2	9
OPO18	11	1	10	OPQ06	5	3	2
OPO19	12	0	12	OPQ20	13	3	10
OPP02	6	3	3				

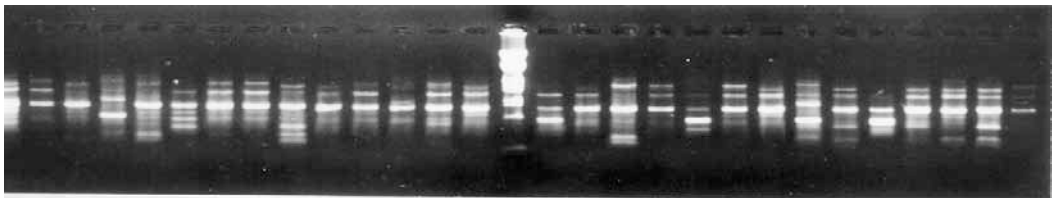
The data based on the matrix of 28 zymotypes × 183 electromorphs from all RAPD markers were used in cluster analysis. Using the genetic distance from the raw data, a diagram for 28 accessions was produced using the UWPGA method. In Figure 3, great genetic diversity can be seen among the taro accessions. The 28 taro accessions were clustered into two groups at a distance of about 10. The first group included 98087 and 98008, which were a great distance from other accessions. In the second group, 26 taro accessions were divided into two subgroups at a distance of about 6.5. The first subgroup contained four accessions (98075, 98108, 98042, 98019), of which 3 were wild taros. The rest were included in the second subgroup. In this subgroup, four accessions (97126, 98069, 98057, and 98024) were farthest from the rest, at a distance of about 5.5. Their petioles are all red. Six accessions (97122, 98089, 98114, 98061, 98033, and 98030) have purple petioles. Eleven accessions (from 98040 to 98001, Figure 3) have green and white petioles. There appears to be no rational explanation for why 98030 with stolons and 98033 without stolons go together, or why escaped 98044 with stolons and cultivated 98003 without stolons are close to each other. The other puzzle is why cultivated 98108 is grouped with wild 98075, 98042 and 98019.

For 28 taro accessions, 86 isozyme bands were displayed by five isozyme analyses; 17.2 bands per isozyme were shown. There were 81 polymorphic bands between different accessions, with a polymorphic rate of 94%.



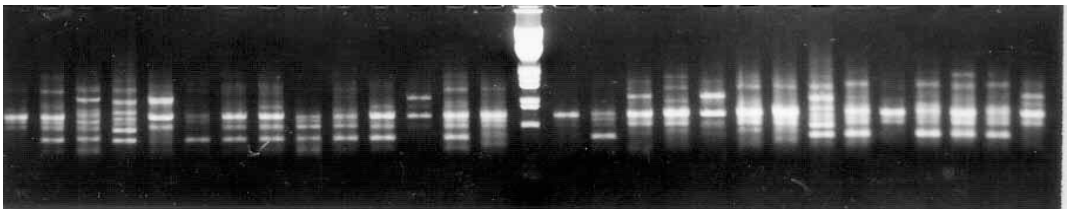
Primer OPP20

98001 98003 98006 98008 98019 98024 98025 98026 98030 98033 98040 98042 98044 98053 Marker 98057 98061 98069 98070 98075 98083 98084 98087 98089 98108 98114 98121 97122 97126



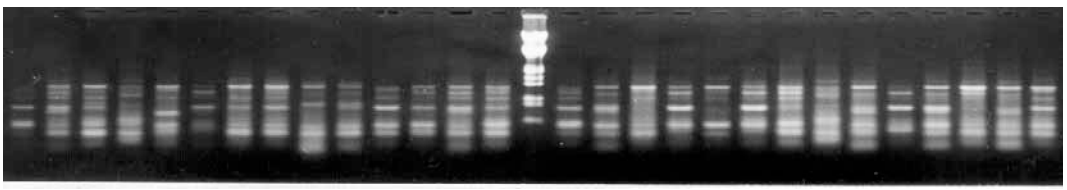
Primer OPO01

98001 98003 98006 98008 98019 98024 98025 98026 98030 98033 98040 98042 98044 98053 Marker 98057 98061 98069 98070 98075 98083 98084 98087 98089 98108 98114 98121 97122 97126



Primer OPO10

98001 98003 98006 98008 98019 98024 98025 98026 98030 98033 98040 98042 98044 98053 Marker 98057 98061 98069 98070 98075 98083 98084 98087 98089 98108 98114 98121 97122 97126



Primer OPQ05

98001 98003 98006 98008 98019 98024 98025 98026 98030 98033 98040 98042 98044 98053 Marker 98057 98061 98069 98070 98075 98083 98084 98087 98089 98108 98114 98121 97122 97126

Figure 2. DNA polymorphism of 28 taro accessions revealed by RAPD.

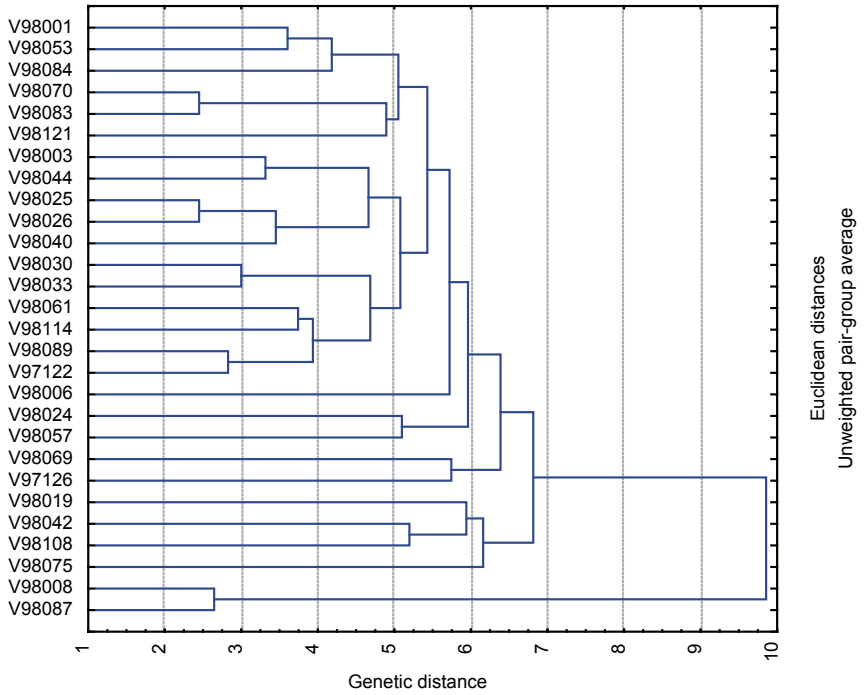


Figure 3. Tree cluster diagram of RAPD markers for 28 taro accessions.

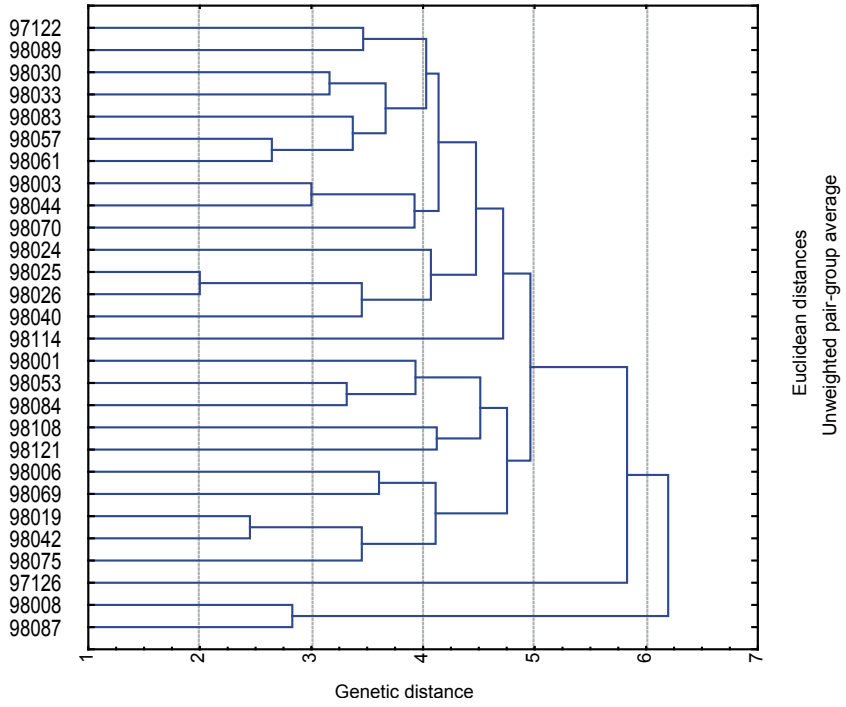


Figure 4. Tree cluster diagram of isozyme markers for 28 taro accessions.

The cluster result from a matrix of 28 accessions × 86 electromorphs from five isozymes can be seen in Figure 4. The groupings and distances for taro germplasm changed, between Figure 3. andFigure 4. In Figure 4. , which shows the results of isozyme analysis, 21 taro accessions were grouped together at a genetic distance of less than 4.0, whereas for RAPD analysis (Figure 3.), 17 taro accessions were grouped together at the same genetic distance. The RAPD tree cluster diagram decreases gradually from left to right as the genetic distancedeclines. This may be because variance revealed by RAPD analysis is based directly on DNA structure and could be accumulated in taro accessions. The isozyme cluster diagram remains horizontal at the fourth level of linkage. This may be because the variance in isozymes reflects the combination of variation in structural genes, and variation in how those genes are expressed.

In the two cluster diagrams, both the first groups contained 98087 and 98008. These two taro accessions were far from other taros. The genetic distance between the first group and other accessions was circa 6.0 at protein level. The genetic diversity between the first group and the other accessions was about 10.0 at DNA level. Accession 97126 was specific in POD and COD isozyme expression. Eleven bands were specific for this accession in the two isozymes analysis. So, it followed that the first group was a single group and was far from the rest at a distance of about 5.8. The remaining accessions in the third group were clustered into two subgroups at 5.0. The places and distances for 97126, 98069, 98057, 98024, 98006, 98114, 98061, 98121, 98108, 98083 and 98070 were different in RAPD marker analysis and isozyme analysis. In particular, 98001 and 98053 (both multi-head taros), 98030 (both white petiole and purple tuber) and 98033 (both red petiole and purple tuber), 98025 and 98026 (both white taro), 98089 and 97122 (both purple taro) were classified into the same group respectively in the two methods. Accessions 98075, 98042 and 98019 (all wild) were clustered together in two methods. Accessions 98040, 98025 and 98026 always went together. The situations of 98089 and 97122, 98033 and 98030, 98044 and 98003 were similar. These show that their relatedness in each pair or group is near both at DNA structure level and at expression level (morphological characters and isozyme).

Combining the data from isozyme and RAPD analysis, a matrix of 28 accessions × 270 electromorphs was obtained. In the cluster diagram (Figure 5), 98087 and 98008 are still together in the first group, far from other accessions at a distance of almost 12.0. Remaining accessions in the second group were clustered into two subgroups at a distance of nearly 9.0. In the first subgroup, 98075, 98042 and 98019 were wild and stood together; cultivated 98069 and 97126 with flowers and purple petioles were close to the three wild taro accessions. In the second subgroup, most accessions were cultivated except for wild accessions 98006 and 98044. Accessions 98025, 98026 and 98040, all with green petioles and multi-tillers, were close. The clustering situation for other accessions was similar for both isozyme and RAPD analyses.

Conclusion

The present condition of taro genetic diversity in Yunnan and future alternative strategy for taro conservation

Greater variation in Asia than in Oceania has been observed in taro. Asia is thought to be the area of the greatest genetic diversity for taro (Lebot 1991). At the time of Lebot's study, few taro accessions from China could be included. In this study, a broad genetic base of taro crop in Yunnan has been presented through morphological identification, as well as isozyme and RAPD analysis of 72 accessions. These accessions represent typical germplasm collected from different geographical conditions, different ethnic groups and different agroecosystems. Of course, they cannot represent all Chinese taro accessions.

In other parts of China, taro is a crop only of local importance or is cultivated in marginal and disused fields. Many traditional local varieties have been discarded because taro is difficult to conserve and has low economic value. However, the situation in Yunnan is different. Many wild and escaped taro accessions are extensively distributed, and many local taro varieties are still planted, used and conserved on-site by different ethnic people using different methods. The first reason for this is the diversity of ecological and geographical environments. The second reason is the diversity of people and their cultures. The third reason is the adaptability of taro to wet, hot tropical environments and its productivity under less intensive cultivation and management systems.

Nevertheless, more attention must be given to taro conservation as the crop is facing threats and challenges from urbanization, industrialisation and tourism development in Yunnan. The main effects are as follows.

1. Indigenous people plant taro mostly for their own use. Taro with low output cannot bring them economic benefits. As the population increases and land area per person decreases, taro crops will be replaced by other crops with high economic value. For example, traditional agricultural crops have been replaced by rubber, tea and fruit crops in Jinghong County. In what was previously the main taro-production region, only a few outstanding taro varieties still remain, while other local varieties are gradually disappearing.
2. Cultivation systems have changed the ecological environments. For instance, swampland was converted into dryland and terraces.
3. Under a market economy, the type of crops being cultivated will shift with shifting market demand and vegetatively propagated taro will most likely be excluded.

From the results of this study, we conclude that an on-site conservation model of planting, utilization and conservation is worth recommending for this vegetatively propagated crop. We should develop a feasible strategy and take effective measures to support and encourage farmers' by increasing the added value of original taro products through varietal improvement and product processing.

Difference in genetic diversity measures at different levels

Representation of taro genetic diversity by isozyme analysis was similar to that by DNA markers for most germplasm, but there was some discrepancy. For example, some taro varieties are close at isozyme level but far apart at DNA level or vice versa. Isozyme polymorphism of taro is the genetic diversity shown at protein level. It is a type of biochemical phenotype. It can reflect the expression and regulation of genes during different developmental stages of a plant. The expression of genes is regulated usually by many internal and external conditions. RAPD analysis of taro directly reflects the variance among accessions in the nucleotide sequence of DNA. The functional genes in different varieties can be expressed and regulated in different ways, in different environments, even if they have the same DNA structure.

Correlations between morphological patterns and molecular markers could not always be found. Gene expression and regulation are complicated processes. A character may be controlled by more than one gene. The structural genes determining an enzyme may be different from that affecting a morphological character. Therefore, it is necessary to study genetic diversity at different levels and analyze the data comprehensively to reach more reliable conclusions.

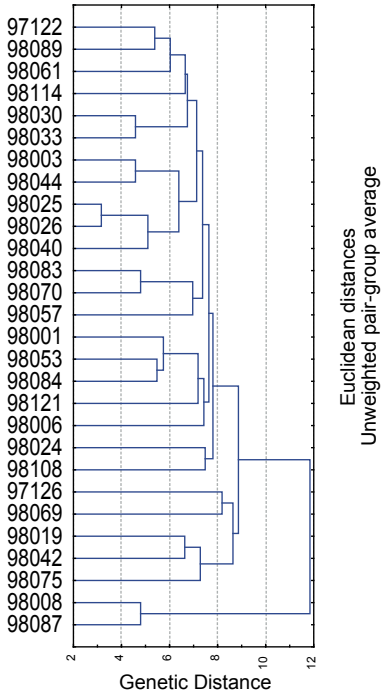


Figure 5. Tree diagram for 28 cases using integrated data from isozyme and RAPD analyses.

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Taro genetic diversity and its use in taro improvement

V. Ramanatha Rao

Introduction

Taro, *Colocasia esculenta* (L.) Schott, ranks fourteenth among the staple/vegetable crops worldwide, and about 5.5 million tonnes of taro are produced from about 1 million hectares. Taro is one of the oldest crops, said to have originated in India or Southeast Asia (Barrau 1965; Plucknett 1976; Kuruvilla and Singh 1981), though this is still debated (Matthews 1990; Yen 1991, 1993; Loy *et al*. 1992). An attempt is made here to describe the status and challenges of conservation and utilization of taro genetic diversity.

In world agriculture, root and tuber crops, including taro, play a major role and feed millions of people. While species like potato, sweet potato and cassava can be considered the major root and tuber crops, about 100 other root and tuber crop species have significance for agricultural or medicinal purposes, including taro. Most of these may be important only locally, but play a significant role in the subsistence economies and crop diversification. Root and tuber crop production has been steadily increasing and in 1999 it was 700 million tonnes from about 1100 million ha, while 7 years earlier, it was 586 million tonnes from about 478 million ha (FAO 1984). About 268 million tonnes of potato were produced from 180 million hectares, 128 million tonnes of sweet potatoes from 92 million hectares, 152 million tonnes of cassava from 15 million hectares and 27 million tonnes of yams from 2.8 million hectares.

Though other minor root and tuber crops are not the subject of this presentation, some information on them and other crops will be used to highlight the need for increased action at all levels to improve the situation of all these minor species because of the agroecological similarities in their production systems. Data on production and area of the minor species are not available, although these crops are very important in world food production and for industry, fodder and medicines, as well as in subsistence agriculture (Prescott-Allen and Prescott-Allen 1990). Many so-called minor species have great potential to become major crops, for example the material coming from the Andean region (Sperling and King 1988). The world's rapid population growth is demanding increased production and greater diversification of crops. Roots and tubers can play a major role in addressing this issue, including taro, which in many countries is treated just as a vegetable. Research on these crops is rarely high on the agenda of many countries. Therefore, there is a need to intensify activities that relate to better conservation and efficient use of these root and tuber genetic resources. I present here the status and challenges of conservation for taro, with some examples, including other root and tuber crops, of the work carried out by some institutes, including that of Bioversity International.

Bioversity International

Bioversity International (formerly IPGRI) is one of 15 centres of the Consultative Group on International Agricultural Research (CGIAR). Bioversity's mandate is to advance the conservation and use of plant genetic resources (PGR) for the well-being of present and future generations. Its mission is

to encourage, support and undertake activities to improve the management of genetic resources worldwide so as to help eradicate hunger and poverty, improve human nutrition and health, and protect the environment. Bioversity focuses on the conservation and use of genetic resources important to developing countries. To undertake and to fulfil its mandate, Bioversity does not seek to conserve and use PGR itself; rather, it works with a variety of partners, including national agricultural research systems (NARS), universities, regional organizations, other international agricultural research centres (IARCs), private organizations and non-governmental organizations (NGOs). Through these partnerships, which can constitute a sort of network of different stakeholders, Bioversity supports countries' efforts to effectively conserve and sustainably use their own PGR, fosters international collaboration, undertakes joint research and supports information exchange and training on PGR.

Bioversity's work on underutilized crops

Underutilized species often have comparative advantages for growing in marginal lands where they have been selected to withstand poor soil conditions and where they are capable of providing a sustainable production with low agricultural inputs. They contribute significantly to maintain a diversity-rich and hence more stable agroecosystem. They represent strategic crops for particularly fragile ecosystems, such as those of arid and semi-arid lands, where salinization and desertification are major obstacles for the successful growth of major crops (Ramanatha Rao and Riley 1995; Padulosi *et al.* 2000).

Underutilized species also can be found in urban and peri-urban agricultural land, where their better deployment and more efficient commercialization can bring additional income for the increasing population of urban poor. In the APO (Asia, Pacific and Oceania) region, underutilized crops such as buckwheat, sesame, safflower, *Lathyrus* and taro have been identified as having immense potential for improvement. As such there is a need to care for their genetic resources with emphasis on their conservation and utilization.

Like most other root and tuber crops, taro is generally propagated vegetatively (although seed production is possible) and this poses unique challenges for germplasm conservation and use. Some challenges include the effects of vegetative propagation on genetic diversity; the need for complementary conservation strategies that include field genebanks, *in vitro* conservation, cryopreservation and *in situ* conservation, and the problems associated with each method and with safe movement of vegetative germplasm. It is also possible in the case of taro, and a few other root and tuber crops, to conserve true seed in conventional cold storage conditions and to exploit the advantages of seed conservation. Over the last two decades, Bioversity has supported a number of activities related to conservation and use of taro germplasm. Most of these activities have been carried out in collaboration with different national, regional or international programmes. Bioversity itself does not maintain any germplasm, so most of the work we do is through our partners, such as the national programmes.

Conservation and use of taro genetic resources

Germplasm collecting

There are many organizations involved in collecting and conservation of root and tuber crop germplasm. It will not be possible to go into detail at the species level, but the existing collections around the globe are summarized in Table 1. There are about 5900 *Colocasia* spp. accessions

conserved in 53 different institutes. The information provided here is from the Directory of Root and Tuber Crops (IBPGR 1986) and many accessions have been lost since then in many collections. Table 2 presents the status of taro collections in individual countries. Bioversity has supported germplasm-collecting, including that of root and tuber crops. About 1520 accessions of taro have been collected in Bioversity-supported missions and the details are given in Table 3. Most of these missions have been multi-crop collecting expeditions. For example, from December 1982 to November 1983, collecting expeditions of 5–7 days each month resulted in the collection of 646 accessions of *Colocasia*, 239 of *Dioscorea* spp. and 528 accessions of *Ipomoea batatas* in 11 states of peninsular Malaysia. The accessions are maintained in a field genebank for evaluation and use (Hussain 1986). However, over the years there have been reports of total loss of certain collections (Jackson 1994; Ramanatha Rao *et al.* 1998). A significant amount of duplication may exist among and between the collections; hence there is a need to focus on rationalization of collections to make them more manageable.

Besides supporting germplasm collecting work, Bioversity has developed a taro germplasm-collecting strategy for the Pacific countries (Ramanatha Rao *et al.* 1998). TaroGen (Taro Genetic Resources: Conservation and Utilization) partner countries have collected material based on this strategy. There is also a need to determine the pace of genetic erosion occurring in the case of taro landraces. There is hardly any information on this topic (Yen 1979; Kesevan and Aburu 1982) and on the toll that biotic and abiotic stresses are taking on the genotypes that have evolved over hundreds of years.

Genetic diversity

It is generally believed that the particular root and tuber crop cultivars/clones have been linked to specific agricultural systems for hundreds of years, as the planting material passes on from one generation to the next. Consequently, in comparison with other crops, which generally are exchanged among farmers more frequently, taros grown by farmers often harbour a unique genetic diversity in landraces. It has been suggested that agriculture might have begun with the digging of roots or tubers of wild plants (Sauer 1965); in some places, this is supported by archaeological records (Hawkes 1989). On the other hand, there is a clear tendency worldwide to prefer grain to root and tuber crops because of differences in taste, status and quick post-harvest deterioration of the latter (Heiser 1990).

However, root and tuber crops produce more calories per unit area and time than cereals. As the world population increases and the need to produce more food on finite land area becomes more acute, the importance of root and tuber crops is amplified. In anticipation of this problem, the need to capture, conserve and use the genetic diversity of root and tuber crops, including taro, becomes imperative.

A better understanding of the extent and distribution of genetic diversity in any species is basic to conservation effort, and taro is no exception. While considering the issues in conservation of taro germplasm, it is important to note some major differences between root and tuber crops and other groups of crops like cereals (Ramanatha Rao *et al*. 1994). In taro, as in other vegetatively propagated crops, there is a delay in the processes leading to meiosis and seed formation, but seed fertility is not critical to its survival because the root, tuber, corm, cormel or rhizome also functions as a propagule. The delay in seed production encouraged farmers to collect and plant other plant parts.

In a system where biomass (other than seed) production is important, and ploidy level, mutations and heterosis can be maintained easily, farmers look carefully for specific genotypes. The factors such as mutations, transposable elements and the occasional full sexual cycle resulting in seed production are the keys to understanding the genetic diversity structure. It is equally necessary to understand reasons why farmers maintain morphological variation and test it.

TABLE 1. Details of germplasm holdings of root and tuber crops

Species	No. of accessions	No. of organizations
Alocasia spp.	231	18
Amorphophallus spp.	327	17
Arracacha spp.	418	6
Calathea spp.	6	3
Canna spp.	31	13
Coleus spp.	54	6
Colocasia spp.	5944	53
Cyrtosperma spp.	117	9
Dioscorea spp.	10661	53
Helianthus spp.	56	4
Ipomoea spp.	26020	83
Lepidium spp.	6	1
Manihot spp.	25835	73
Maranta spp.	78	11
Mirabilis spp.	16	4
Oxalis spp.	1317	7
Pachyrrhizus spp.	364	17
Polymnia spp.	42	3
Solanum spp.	60284	92
Trapaeolum spp.	225	5
Ullucus spp.	471	5
Xanthosoma spp.	1035	41

Source: Directory of Root and Tuber Crops (IBPGR 1986).

Germplasm characterization and evaluation provide information on the genetic variability present in the material. Bioversity has supported many such activities and has helped national programmes in documenting the information generated through such efforts. Bioversity has also supported the work on describing and documenting root crops in the South Pacific, which produced country catalogues containing descriptions and evaluations of root crop cultivars (Guarino and Jackson 1986), and a revised descriptor list for *Colocasia* (IPGRI 1999). As indicated earlier, the Directory of Root and Tuber Crops (IBPGR 1986) gave information on collections existing at that time and efforts to update and revise it are underway.

Several workers have attempted to study the genetic variation in taro, mainly on the basis of morphological variation, karyotype differences and by using isozymes (Coates *et al*. 1988; Lebot and Aradhya 1991, Tanimoto 1990; Tanimoto and Matsumoto 1986; Yen 1968). Kuruvilla and Singh (1981) concluded that the forms they studied have diverged at morphological, karyotypic and genotypic levels. Most of the *Colocasia esculenta* grown in the Pacific are diploid with 2*n*=28, but a few triploids, thought to be recent introductions, have been identified. Lebot and Aradhya (1991) studied isozyme variation in about 1400 cultivated and wild accessions of taro from Asia and the Pacific. Accessions from Polynesia and Micronesia exhibited very low variation. However, the phenotypic diversity in the region is fairly high and this might be an indication that these characters are controlled by very few major genes. Much more work in this area needs to be carried out. Likewise, there is a need to initiate studies leading to the use of molecular techniques to assess genetic diversity. These will be more accurate, and in the long run, may also be cost effective. Such methods may also lead to molecular marker-assisted improvement of taros.

The meeting reported in this proceedings presented more studies on taro genetic diversity than any time in the past. From various studies reported, it can be concluded that farmers maintain numerous morphotypes with different uses, and in many cases their quality traits are reflected in vernacular names. From the studies reported so far, there seems to be little correlation between morphological and isozyme variation. Molecular studies indicate significant genetic diversity as well as geneflow in taro. Morphological variation may be more 'adaptive' in nature and hence is of significance while conserving and utilizing taro genetic resources. Some studies also indicate that the triploids might have originated from diploids and it is possible to assume early divergence of two groups. Taro in Africa and then the Caribbean could have had multiple origins through introduction from South and Southeast Asia.

TABLE 2. Details of collections of taro genetic resources in different countries

Country	No. of accessions				
	C. esculenta	Colocasia spp.	C. esculenta var. esculenta	C. esculenta var. antiquorum	C. gigantea
Australia	193	–	–	–	–
Bangladesh	130	–	–	–	–
Brazil	–	17	–	–	–
Burkina Faso	–	1	–	–	–
Cameroon	70	24	–	–	–
Colombia	–	36	–	–	–
Cook Islands	–	–	57	–	–
Costa Rica	15	13	–	–	–
Cuba	42	–	–	–	–
Ethiopia	–	1	–	–	–
Fiji†	–	–	73	2	–
France	–	60	–	–	–
Great Britain	50	–	–	–	–
Guadeloupe	6	–	–	–	–
Guatemala	–	65	–	–	–
India‡	480	–	–	170	–
Indonesia†	82	350	–	–	–
Japan†	120	–	–	–	–
Malaysia§	–	1352	–	–	–
Nigeria	67	–	–	–	–
Nicaragua	11	17	–	–	–
Niue	52	–	–	–	–
Nepal	67	75	–	–	–
Panama	–	5	–	–	–
Peru	–	11	–	–	–
Philippines†	380	–	–	–	–

Country	No. of accessions				
	C. esculenta	Colocasia spp.	C. esculenta var. esculenta	C. esculenta var. antiquorum	C. gigantea
Papua New Guinea†	747	168	–	–	–
Samoa†	–	–	48	–	–
Seychelles	–	12	–	–	–
Solomon Islands†	267	–	–	–	1
Sri Lanka	–	7	–	–	–
Thailand‡	68	–	–	–	–
Togo	1	–	–	–	–
Tuvalu	14	–	–	–	–
USA	468	31	–	–	–
Vanuatu†	–	138	–	–	–
Vietnam†, ‡	215	10	–	–	–

Source: Bioversity International and Jackson 1994. †Definite indications of some increase in the numbers conserved owing to new collecting missions under TaroGen and TANSAO. ‡Definite indications of some loss in the numbers conserved in recent times. §Most of the collections went to different institutions, and may have been lost, status not clear.

Conservation

Field genebanks

Owing to the mostly asexual nature of reproduction and the value of genotypes (clones), germplasm of most of the root and tuber crops, including that of taro, is maintained in field genebanks. Though the field genebanks make the germplasm readily available for use, there are a number of problems with them. Destruction by natural calamities, pest epidemics, the large numbers of plants that need to be maintained and the high cost of maintenance are some such problems. Jarret and Florkowski (1990) considered conservation of germplasm of *Ipomoea batatas*. They compared the technique of conservation in field genebanks with *in vitro* conservation in terms of security, availability and cost. They concluded that *in vitro* conservation was generally more secure and less expensive and labour-intensive than maintenance in the field. The problem of somaclonal variation is not considered a major obstacle when genetic instability is monitored regularly. However, this needs thorough investigation. It can be assumed that this may be true for taro as well. One of the advantages quoted for field genebanks is the continuous opportunity to evaluate and characterize germplasm. Production of planting material of taro is very slow. A single plant gives only about 2-10 daughter plants after 7-10 months of growth. A rapid propagation method was developed by Pardales (1993)

which would be useful in continued maintenance of field genebanks containing fairly large numbers of plants per genotype.

TABLE 3. Details on Bioversity-supported germplasm-collecting missions

Country	No. of samples collected	No. of missions
Bangladesh	20	2
Burkina Faso	1	1
Cuba	1	1
Guatemala	29	1
Indonesia	87	3
Liberia	5	1
Malaysia	646	1
Nigeria	9	1
Nepal	34	2
Philippines	41	1
Papua New Guinea	378	3
Solomon Islands	167	2
Thailand	95	3
Total	1523	22

Source: Directory of Root and Tuber Crops (IBPGR 1986).

In vitro conservation and cryopreservation

Because maintenance of field genebanks is often problematical and costly, *in vitro* conservation is increasingly being considered as the safer and more practical option for crops that produce recalcitrant seeds or are propagated clonally (Withers 1993). Slow growth of shoot cultures offers a method of medium-term conservation. For long-term conservation of shoot cultures, cryopreservation in liquid nitrogen is becoming available for some species. Research has led to the development of routine cryopreservation protocols for cell suspensions of some species, but regenerating fully differentiated and complex structures, e.g. shoots and embryos, still presents problems. *In vitro* techniques have a role to play at other stages of the conservation process, such as for the distribution of germplasm or for the collection of samples from the field. For several crops, including potato and cassava, most of the components of an *in vitro* conservation system are in place. Much research is still needed in monitoring genetic stability and ways of conserving diversity through *in vitro* conservation. Krikorian (1994) reviewed the work done so far on taro and raised a number of questions on certain basic methodological issues that need to be addressed in the case of taro tissue culture work.

Florkowski and Jarret (1990) examined the relative monetary costs of different technologies for conservation of sweet potato germplasm. These methods included repeated field planting and propagation every season, *in vitro* culture and cryopreservation of tissues or organs that can regenerate. *In vitro* and cryopreservation technologies offer the highest quality preservation, but the cost involved may require repeated evaluation. Simple and inexpensive systems have to be developed. There is an urgent need to assist developing countries in *in vitro* conservation through training. Work needs to be intensified in taro.

Cryopreservation protocols are now available for cell suspensions, calli, apices, zygotic and somatic embryos of several hundreds of species of temperate and tropical origin (Kartha and Engelmann 1994; Engelmann 1997; Engelmann and Dussert 2000). In the last 3–4 years, new cryopreservation procedures for apices and embryos (encapsulation-desiccation, desiccation, pregrowth-desiccation and vitrification) have been developed, and reports involving a larger number of genotypes/varieties are becoming more frequent (Benson 2000; Engelmann and Engels 2000; Engelmann and Takagi 2000). These new freezing procedures generally lead to satisfactory survival rates with a wide range of genotypes using the same technique. Work on taro is in progress in a few laboratories, including the Regional Germplasm Centre in Suva, Fiji (Taylor 2000).

In situ conservation

In situ conservation involves conservation of diversity in natural habitats where the plant species evolved or occur. *In situ* conservation can be carried out either in natural ecosystems or on-farm, depending on the material under consideration. This type of conservation is dynamic, as opposed to the semistatic nature of *ex situ* conservation, and allows evolution and change in species or populations. For some forms of biodiversity, *in situ* conservation is the only option. One of the main reasons given for choosing *in situ* conservation over *ex situ* is the need to maintain the evolutionary potential of species and populations (Frankel 1970; Frankel and Soulé 1981; Ledig 1988). This view is not only the plant breeder's but also, of late, the conservation biologist's, who is concerned with maintaining the variability in small populations and endangered species.

For successful operation of any *in situ* conservation programme we also need information on the following genetic aspects: (1) genetic erosion due to the introduction of new varieties, (2) identification of regions rich in genetic diversity, (3) effects of land fragmentation on genetic diversity, (4) temporal and spatial changes in genetic structure of populations, (5) biogeographic studies, especially when introgression is involved, (6) minimum viable populations sizes and areas, and (7) effects of inbreeding and occurrence of natural seed banks. In the case of agrobiodiversity, the effects of farmers' practices, cultural preferences and environmental factors are the most important ones. There is much scope for *in situ* conservation of taro genetic resources, which needs to be exploited. At the time of writing, Bioversity was developing frameworks for the *in situ* conservation of agrobiodiversity (Hodgkin *et al*. 1993; Jarvis 1999; Jarvis *et al.* 2000; Sthapit and Jarvis 1999) most of which will be applicable to taro as well.

Complementary conservation strategy

Although the genetic issues as outlined above are important, there is increasing understanding that successful *in situ* conservation depends first of all on farmers' management of the diversity of crops that they grow. Therefore, programmes on *in situ* conservation should focus on farming systems to conserve diversity on-farm. This is especially true in the case of crops like taro and yam,

which are, basically, subsistence crops. This approach is also essential because it combines social, biological and agroecological aspects that interact and affect *in situ* conservation in different ways. This approach will also assist in making the development compatible with diversity, in involving the farming community in research and development, and in enhancing diversity on farms. Thus, farmers will truly participate in the management and use of genetic diversity that they helped develop over the centuries through selection of adaptation, quality and other traits. So, a number of management questions need to be addressed, which will help to focus on the genetic questions raised earlier on, as well as questions of access. It is understood that a national *in situ* conservation programme should be closely linked to, and complementary with, the *ex situ* approach. While some genepools or parts of genepools in some areas can be conserved best using *in situ* methods, *ex situ* conservation will be needed in cases where *in situ* conservation is not sufficiently secure, or where there is a need to conserve specific genotypes which might be lost *in situ* because of evolution and change.

Taro leaf blight and virus diseases

Taro Leaf Blight (TLB) (*Phytophthora colocasiae*) is a severe fungal disease in Melanesia, Papua New Guinea, the Solomon Islands, Micronesia and Hawaii. By 1993 it had spread to Western Samoa and American Samoa. The Taro Leaf Blight Seminar held during 22–26 November 1993 in Alafua, Western Samoa, highlighted the problems of this disease in the region. The absence of the disease in several Pacific countries (Lebot and Aradhya 1991) indicates a possibility of the existence of resistant genotypes in those countries. However, recent experiences in both Samoas may counter this argument. It is important to survey all those countries that have not reported the occurrence of TLB and look for resistant sources, and also to practise strict quarantine in the movement of taro to these countries. Among the viruses, the taro bobone rhabdovirus appears to be the most difficult one to work with and it is imperative that better identification and testing methods be developed. Without such techniques the movement of taro germplasm may virtually come to a standstill. Furthermore, the development and use of rapid propagation methods would help in promoting the distribution of disease-free materials and, with a good programme, in flushing out diseased material in the field.

Safe movement of germplasm

It is important that all accessions in a genebank are available to all those who wish to use them, either in crop improvement or for other studies. Often there may be some restrictions on the availability of germplasm, because of either the cost of seed production or national priorities. Nevertheless, the general principle should be free and unrestricted availability of germplasm seed material. As far as possible, any information on the accession must accompany the seed material. While distributing seed or propagules of any PGR material it is important to observe the principles of safe movement of PGR so that pests and diseases are not exchanged along with the seed material.

Taro, like other root and tuber crops propagated vegetatively, poses special problems not only in collecting and storing, but also from a germplasm health point of view (Rodoni *et al.* 1995). In vegetatively propagated crops, for example, viruses present in the mother plant inevitably will be found in derived vegetative parts, such as tubers and roots. In cassava, the causal agent of bacterial blight (*Xanthomonas manihotis*) is present in the xylem tissue of planting material, and is difficult to detect. An example of a fungal pathogen that is spread through tubers is the causal agent of the infamous late blight disease, *Phytophthora infestans*. Although occurring worldwide nowadays, it is of quarantine concern because of the existing physiological races, some of which are resistant to modern fungicides such as metalaxyl. IPGRI (now Bioversity International) has, jointly with FAO,

published a series of Guidelines for the Safe Movement of Germplasm including the one on "Edible Aroids" (Zettler *et al*. 1989) which contains information on taro and may require a revision, based on the current work of TaroGen-related activities.

Use of germplasm

One of the major objectives of conservation of PGR is to make genetic diversity available for immediate or future use. Abundant evidence exists that it is necessary to preserve a wide range of diversity in order to meet the current crop-improvement needs. However, it is evident that the widest possible range of genetic diversity has to be conserved in order to meet future, as yet unknown, needs (Hodgkin and Debouck 1992). Taro PGR programmes are expected to promote and facilitate the use of germplasm through maintenance of sufficient healthy, readily accessible and adequately characterized/evaluated material, and proper documentation of the relevant information. As indicated earlier, there is a need to initiate genetic diversity studies using molecular techniques. Such studies will lead to molecular marker-assisted improvement of taros.

One of the first uses of taro genetic resources in varietal development was in early the 1980s in the Pacific (Sivan and Tavaigia 1984). After the Samoan debacle, through TaroGen and TANSAO networks, much more work is now in progress and will be the subject of other papers in this volume. An excellent review of the use of taro genetic resources was published in 2000 by Ivancic and Lebot.

Future prospects

The following are some general possibilities. These are equally applicable to taro genetic resources and other root and tuber crops.

Some serious drawbacks for effective conservation and use of taro genetic resources are: insufficient knowledge on structure of genetic diversity among genepools, the lack of investment for research on basic biology, plant geography and short-sighted collecting (i.e. focusing only on a few landraces and ignoring the genepool as a whole) (Ramanatha Rao *et al*. 1994). These drawbacks need to be urgently remedied. This essentially means that any significant effort to conserve taro genetic resources should pass through genetic diversity assessment of the materials in *ex situ* collection(s). Whenever possible, this should be compared with what is still present in the wild or in the farmers' fields. In particular, we need to take a closer look at the other wild *Colocasia* species, as well as its close relatives.

The nature of conservation, to be really meaningful and effective, has to be interactive (back and forth, between *ex situ* and *in situ* germplasm collections) and should not be limited to a small collection at the local (or national) level (Ramanatha Rao *et al*. 1994). The effort should span genepools that most often move across national boundaries. A true international effort, well in line with the Convention on Biodiversity, will be required. This is where the establishment of a network of taro genetic resources workers would be extremely useful.

Understanding of the extent and distribution of genetic diversity will also assist in identifying gaps in diversity and will help in planning strategic collecting missions. These efforts can be further helped by increased focus on ecogeographic surveys in areas of diversity, and this is particularly significant in the context of *in situ* conservation. There is a general need to strengthen the evaluation and documentation efforts in many national programmes and to promote the sharing of technologies

and resources among countries. The international and regional organizations can play a strategic role in helping the national programmes in this area.

The tendency to prefer grains to underground parts may extend into the conservation scene. Perhaps it is particularly so if the process of domestication of certain root and tuber crops is still incomplete. This seems to be especially true if a narrow view of plant genetic resource conservation prevails (Ramanatha Rao *et al*. 1994). However, in the recent past there has been a renewed interest owing to the increasing recognition of economic and social significance of root and tuber crops in rural development. We should be able to make use of this opportunity for the development of taro. Globally there is an increased interest in the cultivation of indigenous species by local communities and the conservation community needs to capitalize on this increased interest.

There is a need to determine the choice or choices of conservation methods for PGR. We need to identify the reasons that motivate the farmers/communities to conserve and the situations under which *in situ* conservation would be self-sustaining. We must understand that *in situ* conservation is possible only when the farmers or communities derive benefit from conserving diversity and that such conservation is not possible in all areas. If we choose other options, especially if we wish to conserve genotypes, when the method of propagation is mostly vegetative, there will be hundreds and thousands of genotypes, both in cultivation and in the wild. This will require rationalization of extant collections as well as serious studies on *in vitro* and cryopreservation of taro. We have seen the problems of field genebanks. Cryopreservation may not be the complete answer or technique because several developing countries have little or no access to it. Additionally, any *ex situ* conservation approach is basically static in terms of crop species evolution. So, there is a need to pay increased attention to *in situ* conservation of taro (Ramanatha Rao *et al*. 1994). This should also consider the appropriate balance of field collections, where possible, to complement other methods. And it should have a component of *in vitro* conservation, as a means for safe germplasm transfer and for medium-term conservation. Cryopreservation can be used, when possible, as a safety back-up. We also should look at the possibility of seed conservation in long-term storage conditions. Most taro genotypes set seeds in many locations. If seed can be produced and stored, we can capture and conserve genetic diversity as populations for future generations. Some work has been done on seed germination and storage (see Shaw 1975; Strauss *et al*. 1979; Strauss and Griffin 1983; Sivan and Tavaigia 1984, and others), but much more needs to be done before this can become a viable option within the context of a complementary conservation strategy.

The current level of use of conserved taro germplasm could be further improved through promoting and facilitating the use of taro germplasm. This requires the maintenance of sufficient healthy, readily accessible, adequately characterized and evaluated material. Accessible and appropriate documentation, which includes traditional knowledge, will further help in this process.

To conclude, the improved conservation and better use of PGR will require a more thorough and multidisciplinary understanding of the genetic diversity, agroecosystems and the methods that could be applied to the conservation of genetic diversity. It will need identification of areas rich in genetic diversity and knowledge on the causes and rate of genetic erosion or of its absence. An example of such studies is the one by Brush (1992). As indicated earlier, the conceptual basis for such an approach is being developed (Hodgkin et al. 1993). These efforts to conserve and use the root and tuber crops better will need collaboration at various levels, both national and international.

This will facilitate a successful complementary conservation of the genetic resources of root and tuber crops.

Dwindling resources for most agricultural research activities will probably be a fact for some time to come. Hence it is important that the countries interested in taro genetic resources work together, even establishing a common genebank, to make the best use of limited funds available. Bioversity International will seek to facilitate these actions and our success will depend mainly on our collaboration with our partners and their interest. So, it is important that we work together to promote better conservation of taro genetic resources along with more efficient use of the available genetic diversity.

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Taro (*Colocasia esculenta*) and tannia (*Xanthosoma sagittifolium*) crops in the Republic of Cuba

Sergio Rodríguez Morales, Miguel Portieles Rodríguez, Luis Ruiz Martínez, Magaly García García, Maryluz Folgueras Montiel, Ramón E. Pérez Hernández and Julia Albert Llerena

Abstract

Scientific results on aroids (*Colocasia* and *Xanthosoma*) from the Research Institute of Tropical Root and Tuber Crops (INIVIT) are presented. References are shown in relation to the evolution on both genera, including studies on genotype environment interaction, which were the bases for a clonal regionalization as `Isleña Japonesa` clone; the word *Japanese* is related with the origin of the crop.

Characterization and evaluation work was carried out in the National Germplasm Bank by INIVIT. The refinement of biotechnological techniques for mass micro propagation and somatic embryogenesis as a base to obtain genetic variability and further researches on transformation are presented. Results from studies on consumption and utilization of fertilizers where N increased yields at rates of 200 kg/ha and a mean utilization efficiency of 40 kg yield/kg of applied N are also shown. Different technologies for applying fertilizers and the most suitable methods for a more efficient utilization of them are discussed, including appropriate recommendations to give solutions to chlorosis, which is present in calcareous soils with pH higher than 7.5. In relation to planting materials, methods with a multiplication rate ranging from 1 to 5 (traditional methods) up to 1:60 (accelerated conventional methods) are also mentioned. All other aspects related with crop culture to obtain yields up to 70 t/ha in a growing cycle from 9 to 11 months are described, as well as, post harvest management of corms and cormels including storage for their availability 7 months after harvest.

Introduction

Aroids (*Colocasia* and *Xanthosoma*) are among the most-widely consumed root and tuber crops, used as a source of energy, by Cuban people. Their nutritional values, cooking and digestive qualities make them high-demand crops in the national markets and they are included in hospital diets and diets of the elderly.

In Cuba, both genera are grown commercially, including *Colocasia* which is grown on plantations, because the available clones were from the *Xanthosoma* genus. Research carried out at INIVIT permitted us to identify varieties that guarantee availability in the market for a minimum of eight months, if good management practices are adopted.

Over the past few years, planting areas for *Colocasia* and *Xanthosoma* have increased in Cuba, as corms and cormels can be stored under natural conditions for long periods of time without losing their quality. In addition, techniques have been developed that allow *Colocasia* to be grown on plastic (clay) soils with poor drainage - soils that cannot be used for other root and tuber crops. There are no pests and diseases causing serious damage to plantations. As a result, aroids are

considered a safe crop that does not cause damage to the environment because no pesticides are used. *Colocasia* and *Xanthosoma* have become valuable components in food security for Cuban families.

Development

A group of commercial and promising clones from the *Colocasia* and *Xanthosoma* genera is available in Cuba, including the following:

Genus	Clones	Potential yield (t/ha)
Colocasia	'MC-2'	90
	'Rosada Habana'	90
	'Camerún 14'	104
	'INIVIT'	106
Xanthosoma	'Macal Sport'	20
	'Amarilla Especial'	50
	'Morada'	30
	'Japonesa'	30
	'Selección INIVIT'	35
	'México 1'	30
	'México 8'	30

The main germplasm of *Colocasia* and *Xanthosoma* genera, with 45 and 73 clones respectively, is found at the Research Institute of Tropical Root and Tuber Crops (INIVIT). Since 1972, INIVIT have been working on principal directions that is, prospecting, characterization, evaluation and conservation.

Prospecting missions and introduction of clones have been performed nationally and abroad. *Colocasia* germplasm has included clones from Asia and Africa, such as 'Isleña Japonesa' considered a commercial clone since 1972 due to its high potential yield. In Cuba, the *Colocasi*a genus is named 'Malanga isleña' and 'Japonesa' because the crop was introduced by a group of Japanese who were living on the Cuban Isle of Youth. At present, the 'Camerún 14' clone (introduced from Cameroon) is the commercial clone with the highest potential yield. However, new native genotypes with high potential yields and very good cooking qualities have been recommended from selections of natural mutants or after mutation via irradiation. In relation to the genus *Xanthosoma*, INIVIT supports the biggest germplasm collection in America, including *X. sagittifolium*, *X. violaceum*, *X. caracu*, *X. atrovirens* and *X. brasiliensis*. This genus originates in America, and the collection brings together clones from the American continent. These include clones with qualities (high yield and

excellent cooking) that have permitted their distribution through Bioversity International to different countries in the American continent.

Our germplasm has been characterized according to the list of Bioversity descriptors and results will be known soon. We have been able to group possible mutants according to various criteria. After evaluating prospected and introduced clones and mutants, multiplication of propagules was started, and the resulting plants were used in studies on genotype–environment interactions in the different edapho-climatic conditions in Cuba. This survey was of fundamental importance for the regional adoption of clones. Genetic resources were not only maintained but also evaluated and there has been a direct link between breeders and growers carrying out participatory research and developing a programme to validate and select genotypes. Growers participate directly in the validation and selection programme.

Germplasm is conserved in field conditions and *in vitro*. For field conservation, a methodology developed at INIVIT is used and an *in vitro* technique for medium term storage has been refined. In collaboration with Bioversity International and Havana University a micro-corm cryo-conservation system was developed. The genetic resource programmes for both genera have been top priorities in Cuba, and new genotypes obtained in different ways are incorporated to the genebank every year.

The use of biotechnological techniques at INIVIT has produced advanced results. Mass propagation techniques in both genera have been refined. Cuba has produced more than 1,000,000 *in vitro* plants, which are the starting point in the process of obtaining original 'seed' (vegetative planting materials) for a certified seed programme aimed at providing clean planting materials for growers. Somatic embryogenesis and plant regeneration are also being developed in order to obtain new mutants via irradiation, and free-virus (Dasheen Mosaic Virus) plants through transformation. We are also using Temporal Immersion Systems with great success.

Diagnosis and plant cleaning have been important efforts. A cleaning methodology is now available, using electrotherapy, and permits the production of high quality *in vitro* plants.

Corms and cormels are still used as planting materials, but commercial *in vitro* propagation methods and Temporal Immersion Systems have recently become important alternatives. The latter represent a revolution in the field of mass propagation, as multiplication rates are increased ten-fold in comparison with traditional tissue-culture methods. The following seed production structure has been established for both genera.

Starting from healthy *in vitro* plants, we could establish national seed certification programmes and, in four years, high-quality 'seed' with higher potential yield became available for growers. This has been one of the reasons for increased yields in our country.

Surveys have shown that the best planting times in these two genera are from December to May for *Colocasia*, and from May to June for *Xanthosoma* clones, mainly *X. sagittifolium, X. caracu* and *X. atrovirens* species for earlier plantations and *X. violaceum* from May to June.

Seed (*Colocasia* genus) production strategy from original *in vitro* plants produced at INIVIT. M/U = ha

Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7
25000.0 VP†	9.0 (ha)	63.0 (ha)	315.0 (ha)	1575.0	–	–
	B	R-I	R-II	C-I	–	–
	25000.0 VP	9.0	63.0	315.0	1575.0	–
		B	R-I	R-II	C-I	–
		25000.0 VP	9.0	63.0	315.0	1575.0
			B	R-I	R-II	C-I
			25000.0 VP	9.0	63.0	315.0
			VP	B	R-I	R-II

† VP=Vitroplants; B=Basic; R-I=Registered I; R-II=Registered II; C-I=Certified I.
Multiplication Rates: from Original VP = 1:10; from Basic Original = 1:7; Others 1:5.
Recommended clones: 'Camerún 14'.

Seed (*Xanthosoma* genus) production strategy from original 'seed' (*in vitro* plants) produced at INIVIT. M/U = ha

1998	1999	2000	2001	2002	2003	2004
25000.0 VP†	5.4	43.2	259.2	1555.0	–	–
	B	R-I	R-II	C-I	–	–
	25000.0 VP	5.4	43.2	259.2	1555.0	–
		B	R-I	R-II	C-I	–
		25000.0 VP	5.4	43.2	259.2	1555.0
			B	R-I	R-II	C-I
			25000.0 VP	5.4	43.3	259.2
			VP	B	R-I	R-II

† VP=Vitroplants; B=Basic; R-I=Registered I; R-II=Registered II; C-I=Certified I.
Multiplication Rates: from Original VP = 1:10; from Basic Original = 1:8; from Basic R-I = 1:6.

Good soil management is the basis for obtaining high yields in these crops. In Cuba, plastic (clay) soils are preferred for *Colocasia* and well-drained soils in mountainous regions are preferred for *Xanthosoma*. *Colocasia* is more demanding of organic matter.

Planting may be conducted in the furrow bottom or on ridges if it is done manually or semi-mechanically, respectively. Planting distance for *Xanthosoma* is 0.90 m × 0.35–0.40 m, and 0.90 m × 0.30–0.40 m for *Colocasia,* depending on 'seed' types, and planting depth should always be 20–25 cm. In areas under irrigation systems, Gesagard 50% HP at a rate of 3.0–4.0 kg/ha (Prometrine 1.5–2 kg/ha) is applied to control annual weeds in pre-emergent applications, and prior to sprouting Gramoxoma 20% at 1.2 pounds/ha c.p. (Paraquat 2.4 kg/ha of a.i) is also applied in a guided form.

The following fertilizer rates for *Xanthosoma* and *Colocasia* clones are recommended.

Elements	*Colocasia* (kg/ha)	*Xanthosoma* (kg/ha)
N	260–340	100–130
P_2O_5	80–100	40–50
K_2O	280–380	130–190

Split nitrogen rates in four applications for *Colocasia,* and two applications of K_2O and P_2O_5 are very important for nutrient intake. In the case of *Xanthosoma,* two-thirds of the total is applied prior to planting and the remaining fertilizer is supplied 80 days after planting.

Alternative sources of nutrition have been important for these crops. Organic matter is placed in the furrow bottom at a rate of 20-30 t/ha depending on the quality of organic material. Biofertilizers may be applied as follows:

Mycorrizae: In the furrow bottom and under tubers (3 t/ha).

Azotobacter: 20 L/ha at planting time and 60 days after planting with a final solution of 400 L/ha.

Phosphorine: 20 L/ha prior to planting with a final solution of 200 L/ha.

In our conditions, the total evapotranspiration on aroids ranges from 10.000 to 12.000 m^3/ha in a 9–11 month period. Maximum values are obtained 5–7 months after planting and minimum values at the initial and final stages of the crop, depending on soil types. *Colocasia* clones are irrigated with a net partial pattern of 250 m^3/ha each 7-8 days up to 120 days. After 120 to 180 days, intervals are reduced to 4 days, but they are increased with the first frequency until 20 days before harvesting. *Xanthosoma* clones are irrigated at 10-12 day intervals in the first months and weekly during the highest vegetative development, but intervals are increased again in the last months.

The most significant disease in Cuba is a corm rot caused by *Fusarium esclerotium*, which does not cause much damage. Aphids and mites are the most common pests on leaves, but neither group has caused serious problems.

Semi-mechanized harvesting is carried out and corms are collected manually; in some cases harvesting machinery is used.

Corms and cormels are stored in shady and ventilated places. Pile height cannot exceed 20-30 cm and storage time depends on corm health (soft and dry rot).

Storage is not only carried out in natural conditions. Studies were performed for storage under controlled temperature; that is, in cold stores to keep tubers in good conditions during 10 months.

Conclusions

INIVIT has the biggest collections of *Colocasia* and *Xanthosoma* germplasm in the Americas and these are maintained using *in vitro* and field methods for medium-term storage. For *Colocasia,* steps are being developed together with Bioversity International for cryo-conservation. Technological progress has allowed the valuable corms of both genera to reach the market for at least eight months per year. Certified 'seed´ programmes and refined biotechnological techniques, especially Temporal Immersion Systems (permitting production of 200 000 in vitro plants in an 8-month period at minimum cost) have significantly contributed to these outstanding scientific results. Thus, over the past few years, growers have been able to greatly increase the growing areas and yields of these two genera.

Techniques for the mass propagation of taro corms (*Colocasia esculenta*)

Motomu Akita and Yoshimoto Ohta

Introduction

Tissue culture is an important tool for mass propagation of vegetative crops of high quality. Mass propagation of storage organs created *in vitro* has great advantages because such organs are easy to acclimatize, store and transfer. We have developed a mass propagation system using taro (*Colocasia esculenta*) as a model plant.

The *in vitro* efficiency of propagating storage organs is, in general, considerably lower than for shoots. The number of storage organs formed is usually much smaller than the number of buds. Storage organs also require a longer culture period than do shoots. Low efficiency in propagation can be compensated for by enlarging the culture scale with bioreactor techniques (Levin and Vasil 1989) but it is still difficult to shorten the culture period. This usually means that expensive apparatuses and/or culture rooms are occupied during the prolonged culture period. For example, Hulscher *et al.* (1996) reported that 1600 to 1700 potato tubers can be produced by using a 10-L culture vessel and the total culture period was 18 wks (8 wks for shoot multiplication followed by 10 wks for tuber production). One way to achieve cost reduction is to make the bioreactor simpler. Since forced aeration is the most costly and troublesome operation in bioreactor culture, we tried to design a bioreactor without forced aeration and evaluated it for propagating corms (the storage organs of taro).

Acclimatization is required for the practical use of most *in vitro* derived plantlets and it is one of the most laborious steps for producing larger plants. Although some *in vitro* derived storage organs could be directly transferred to the field (e.g. Akita and Takayama 1993), we found that taro corms derived *in vitro* should be acclimatized before transfer to the field. A number of excellent methods have been developed for acclimatization; however, the quality of plant materials, as well as other factors, are important for efficient and stable production of plants in the field. For example, phytohormones needed for culture may inhibit root formation during the early stage of acclimatization. In our study, we also tried to improve efficiency of acclimatization.

Materials and methods

Plant material and culture medium

An *in vitro* stock culture of taro (*Colocasia esculenta* cv. Ishikawa-wase) was kindly provided by Dr S. Takayama of Tokai University. This was maintained by serial subculture on a modified MS solid medium (Murashige and Skoog 1962) with the concentrations of major inorganic salts (KNO_3, NH_4NO_3 and $CaCl_2$, abbreviated as 'A' components) reduced by half, and 1 mg/L of N-phenyl-N'-(4-pyridyl)urea (4-PU) and 30 g/L of sucrose. The medium was solidified using 0.8% (w/v) of agar.

Culture conditions

To maintain the plants in static culture, a test tube (25 mm diam. × 165 mm height) containing 10 ml of the modified MS medium was used. Test tubes were capped with spongy silicone plugs. A single plantlet was inoculated in the test tube and cultured at 25°C. Photosynthetic photon flux density at the top of the test tube was 140 mol m^{-2} s^{-1}.

For shaking culture, five plantlets were transferred and subcultured using a 100-ml glass flask containing 40 ml of the same medium as in static culture but without agar. Corms were developed using the same basal medium supplemented with 60 g/L sucrose. About 20 plantlets were cultured in 300-ml flasks containing 200 ml of the liquid medium (30 g/L sucrose) to prepare explants for the bioreactor. The flasks were shaken at 100 rpm on a rotary shaker (Model SCS-225NP, Iwashiya-Sanki, Osaka) under illumination from the bottom of flasks (180 mol m^{-2} s^{-1}) at 25°C.

For bioreactor culture, a rotary drum bioreactor (10 L, 20 cm diam.; Shibata-Kagaku, Tokyo) was used. A cylindrical stainless steel mesh (15 cm diam.) was placed in the centre of the reactor (Figure 1), and the mesh was covered with polyurethane foam (1 cm thick). Two litres of the liquid medium containing 60 g/L sucrose was put in the reactor and autoclaved (120°C, 20 min). The reactor was then capped with either a spongy silicone plug or an air filter. The plantlets were transplanted and cultured for 6 wks. The drum was constantly rotated at a speed of 1 rpm at 25°C under dim light (5 mol m^{-2} s^{-1}) for 12 h/day.

Application of reducing agent

Corms were obtained by shaking the culture for 1 month in the medium supplemented with 60 g/L of sucrose. The medium was replaced with sterilized water containing one of the reducing agents tested, and the corms were cultured for another week.

Dithiothreitol (DTT), n-propyl gallate (GNP), glutathione (reduced form, GSH), 2-mercaptoethanol (ME), tiron, ethoxyquin (EX) and butylated hydroxytoluene (BHT) were tested reducing agents. Each agent was dissolved in dimethylsulfoxide (DMSO) and 1 ml of the solution was added to the sterilized water. One millilitre of DMSO was added to the control culture.

Acclimatization

Plantlets were removed from the culture vessel, washed with water and transplanted to plastic containers (20×30×6 cm) filled with a mixture of vermiculite and peatmoss (1:1). For acclimatization, the containers were placed in a growth cabinet (Model FLI-300N, Tokyo-Rika, Tokyo) with a relative humidity of about 67%. Humidity of the soil surface, without cover, was about 75%. Temperature was 25°C and photosynthetic photon flux density was about 85 mol m^{-2} s^{-1} (12 h/day). The container was covered with plastic film during the first week of acclimatization and then the cover was removed. The number of surviving plants was counted 1 month after removal of the cover.

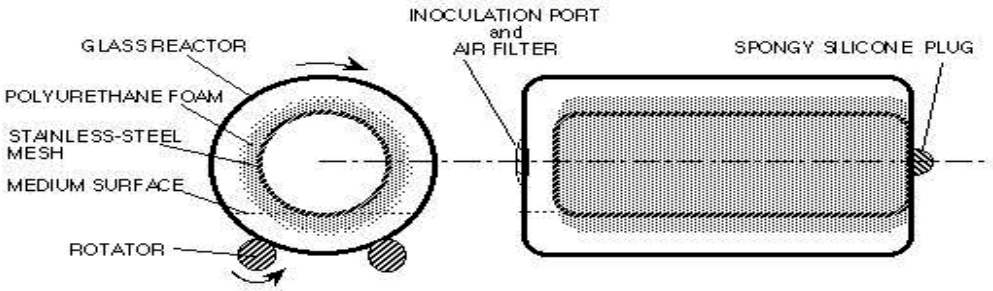

Figure 1. Diagram of bioreactor for mass propagation of taro corms.

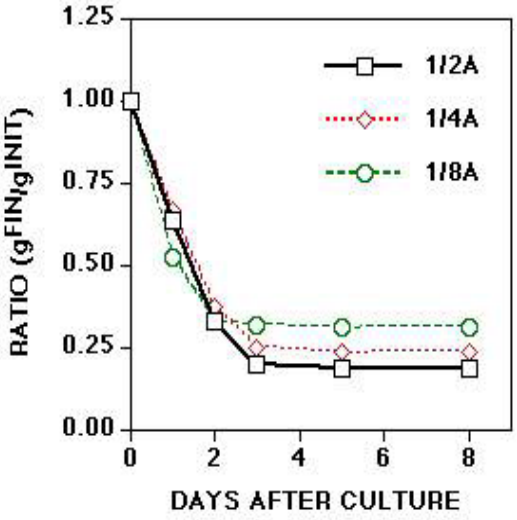

Figure 2. Effect of concentration of 'A' components in the MS medium on reduction in weight of corms after culture. Corms were stored at room temperature and the weight change was measured.

'A' components = KNO_3, NH_4NO_3 and $CaCl_2$; g^{FIN} = weight of corm at each measurement; g^{INIT} = initial weight of corm.

Results and discussion

Previously we examined the necessity of forced aeration for the survival and growth of taro. When explants were cultured under completely submerged and static conditions using liquid medium, growth as measured by the increase in dry weight was not significantly different compared with the control where the plants were grown on the surface of the liquid medium, although proliferation was suppressed by 30% (Akita and Ohta 1996). This result indicated the possibility of using a bioreactor system without equipment for forced aeration (compressor, air lines and sparger) to produce taro corms. Proliferation of corms is achieved during shaking culture; corm growth then occurs in the bioreactor.

For large-scale culture, we tested a rotating drum type reactor with a cylindrical polyurethane foam to trap the explants. This bioreactor had no equipment for forced aeration but the air inside the reactor could be passively exchanged through the spongy plug and/or an air filter. Explants trapped on the surface of the polyurethane foam were exposed to light and immersed intermittently in the medium throughout the culture period.

When explants were transferred to the reactor, they were trapped on the polyurethane foam. Distribution was not uniform and several dense areas of corms were observed. By the end of the culture, about 330 corms had formed in the most dense area (76.5 cm^2). Since the total area of the polyurethane foam was about 1800 cm^2, the theoretical maximum number of corms can be calculated as about 7800 for the reactor used in this experiment.

This result indicates the possibility that our system could be useful for mass production of taro corms. One of the major problems in using our reactor is how to distribute the explants uniformly on the polyurethane foam. This problem may be solved by using smaller segments of the explants: as etiolated stems with several adventitious buds can be induced from taro explants, stem cuttings of 2 to 3 mm long could be used. In fact, efficient development and growth of corms from such small cuttings was confirmed under shaking culture conditions (data not shown).

Plantlets grown as above wilted easily when they were removed from culture vessels. Figure 2 shows the time course of reduction in weight of corms under room temperature. Corms quickly lost their weight irrespective of the composition of the medium used in the shaking culture. This means that it is necessary to acclimatize *in vitro* taro corms before transferring them to soil. Medium composition affected the ease of acclimatization. In the case of the corms shown in Figure 2, for example, the survival rate of plants during acclimatization was about 72% and 38% following the use of 1/2A and 1/8A media, respectively (data not shown).

We have reported previously that the efficiency of acclimatization could be improved when the roots were removed, because the *in vitro* generated roots rotted easily irrespective of the composition of medium (Akita and Ohta 1996). The positive effect of root removal for acclimatization indicated that the abundance of roots formed during culture is not important; rather it is root induction during acclimatization that matters.

Since root induction is known to be stimulated by auxins and/or reducing agents such as dithiothreitol (DTT) (Lis-Balchin 1989; Standardi and Romani 1990; Auderset *et al.* 1996), the effect of reducing agents on the efficiency of acclimatization of taro was examined. *In vitro* corms were

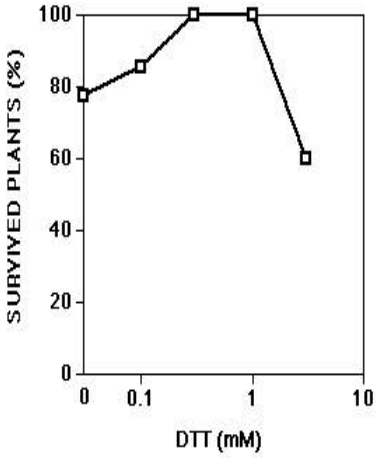

Figure 3. Effect of DTT (dithiothreitol) in the shaking culture medium on survival rate of plants in soil.

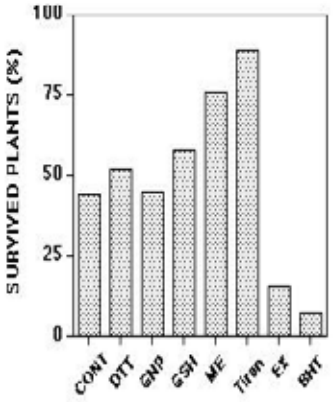

Figure 4. Effect of reducing agents in shaking culture medium on survival rate of plants in soil. Reagents were dissolved in dimethylsulfoxide (DMSO) to which was added: DTT – dithiothreitol 1mM; GNP – n-propyl gallate 0.1mM; GSH – glutathione (reduced form) 1mM; ME – 2-mercaptoethanol 1mM; Tiron – tiron 1mM; EX – ethoxyquin 0.1mM; BHT – butylated hydroxytoluene 0.1mM. One ml of DMSO was added to the control.

cultured for an additional week in water containing DTT and treated with 3mM of indole-3-acetic acid (IAA) for 1 h after removal of the roots. As shown in Figure 3, the efficiency of acclimatization of corms treated with 0.3 to 1mM DTT increased by approximately 20% compared with the control. Stimulation of the root formation during acclimatization was clearly observed on these plants (data not shown).

Figure 4 shows the effects of application of reducing agents other than DTT on acclimatization. Since the treated corms and control were merely placed on the surface of soil without additional auxin treatment in this experiment, the rate of survival of the control plant was significantly decreased compared with the previous experiment (Figure 3). Some reducing agents such as 2-mercaptoethanol (ME) or tiron clearly enhanced the rate of survival as well as root development, whereas ethoxyquin (EX) and butylated hydroxytoluene (BHT) had an inhibitory effect.

These results indicated that efficiency of the acclimatization is improved by using appropriate reducing agents *via* stimulation of the root development. Details of the mechanism of this stimulative effect are not clear but may be correlated to their activities as plant growth regulators (Siegel and Porto 1961).

Conclusion

We attempted to design a simple and economical system for production of taro corms using a bioreactor technique. This type of culture system could be applied for mass propagation of other storage organs. We have reported previously the propagation of potato (*Solanum tuberosum* L.) microtubers using the same type of system (Akita and Ohta 1998).

The corms propagated in a liquid culture medium could be used after additional culture in water containing appropriate reducing agents. Since exchanging the medium is quite easy in a bioreactor, the requirement of the additional culture does not cause any serious problem in our system. The elimination of the *in vitro* developed roots before transferring corms to the soil seems to be an inevitable requirement for acclimatization. This process is a laborious one, but could be simplified in our bioreactor because most of the roots were torn off when corms were scraped from the polyurethane foam.

In this study, we evaluated a method for supplying *in vitro* propagated seedlings by using a simple bioreactor and subsequent treatment of corms with reducing agents. Further studies are necessary to improve the production of planting materials for specific economic and social purposes.

Acknowledgements

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Taro collecting and conservation in the Pacific Region

Mary Taylor, D. Hunter, V. Ramanatha Rao, G.V.H. Jackson, Param Sivan and Luigi Guarino

Introduction

Taro [*Colocasia esculenta* (L). Schott] is a vegetatively propagated crop with edible tubers and leaves belonging to the family Araceae. It ranks fourteenth among staple vegetable crops of the world, with about 9.2 million tonnes produced globally from 1.8 million hectares with an average yield of 5.1 t/ha (TANSAO 2001). In the Pacific, where it is of greatest significance, taro has special cultural, dietary and economic importance. In many Pacific Island (PI) countries it is considered an essential component of every meal. Corms are baked, roasted or boiled, and the leaves are eaten as "palusami". The leaves represent an important source of vitamins, especially folic acid. It is a plant with high prestige value and has great importance as a presentation on formal occasions. It is also favoured for its considerable productivity in the fertile and high-rainfall environment of many of the islands. In addition to being an important traditional food crop, taro is a significant export commodity in a number of countries, such as Fiji and Cook Islands and it was in Samoa prior to 1993.

Genetic diversity of taro in the Pacific

Taro is one of the most ancient cultivated crops. There is now evidence to suggest that most cultivars found in the Pacific were not brought by the first settlers from the Indo-Malayan region (Plucknett *et al.* 1970), or from India/Bangladesh (Lebot and Aradhya 1991), and subsequently dispersed as a domesticate (Matthews 1990), but rather were domesticated from wild sources existing in the Melanesian region (Lebot 1992).

Isoenzymes of 1417 cultivars and wild forms from Asia and Oceania (Lebot and Aradhya 1991) showed that taro from Indonesia differed from those of the Philippines and Oceania. This division into two groups is supported by DNA studies. Furthermore, the cultivated taro in Cook Islands, Easter Island, Hawaii, New Zealand, Niue, Samoa and Tonga exhibit an extremely narrow genetic diversity. Greatest variation was found in taro from Indonesia, more than in Papua New Guinea (PNG), Solomon Islands and New Caledonia. Variation in Vanuatu taro was also low. The substantially higher levels of variation found in New Caledonia were probably due to recent introductions from Indonesia and Vietnam.

However, there is extensive phenotypic variation present in Pacific Islands. This is due to somatic mutation, occasional sexual recombinations, and intense selection by isolated human communities for suitability in diverse environments and for diverse purposes. The importance of taro genetic resources in PI countries may reside, therefore, in the adaptation of cultivars to local conditions and uses, irrespective of the genetic diversity which exists, compared with that present in the larger taro genepool.

TABLE 1. Collections of taro in nine Pacific Island countries in 1986, 1994 (Anonymous 1999) and 2000 (AusAID/SPC 2001)

Country	1986	1994	2000
American Samoa	—	12	—
Cook Islands	57	0	18†
Commonwealth of the Northern Marianas	—	6	—
Fiji	72	78	72
French Polynesia	—	34	—
Federated States of Micronesia	—	0	—
Guam UG	—	6	—
Hawaii	—	>400‡	—
Kiribati	—	6§	—
Marshall Islands	—	20	—
New Caledonia	—	86	82
Niue	52	0	25†
Palau	—	6	—
Papua New Guinea	—	—	—
Lowlands Agricultural Exp. Station	—	48	—
Laloki	135	0	—
Bubia	120	450	859
Aiyura	52	—	—
Saramandi	—	23	—
Samoa	—	—	—
Ministry of Agric., Forests, Fisheries & Meteorology	20	17	15†
USP	28	106¶	—
Solomon Islands	31	2	692
Tonga	14	21§	9†
Tuvalu	13	13	—
Vanuatu	138	0	502

† In tissue culture at SPC, Fiji.
‡ Local, as well as accessions from Asian and Pacific countries.
§ Importations from USP/IRETA (Univ. of the South Pacific/Institute for Research, Extension and Training in Agriculture). Tonga also has importations from Hawaii.
¶ In tissue culture.

These findings have obvious bearings for genetic resource conservation and use:

- PNG and/or Indonesia appear to have been areas of domestication and therefore remain important centres of diversity and potential sources of germplasm for improvement programmes
- genetic variation in taro from Polynesia is low, but phenotypic diversity is high
- pacific taros form a distinct group separate from Southeast Asian material
- wild taro may be phenotypically similar to, but genetically different from, the cultivated material.

Genetic erosion of taro in the Pacific

Although there is no guide as to the pace of genetic erosion of taro in PI countries, there are several reports indicating significant levels of erosion. Before the advent of Taro leaf blight (TLB) in Solomon Islands, taro and yam were the principal crops. Today, sweet potato accounts for most of the land devoted to food production. Anecdotal evidence suggests that in both Solomon Islands and PNG, many cultivars are no longer grown. It is not known whether these can still be found as feral populations, or in areas where TLB is not present. Kesevan and Aburu (1982) suggested that in the New Guinea islands, as well as on the mainland, taro production declined in response to population pressure, plant diseases and the introduction of higher-yielding species such as *Xanthosoma*, sweet potato and cassava. Declining taro production also has been recorded in Micronesia. In the Federated States of Micronesia, this decline has been attributed to pests and diseases and today taro ranks behind breadfruit, yam, banana and imported rice as a staple food (Raynor and Silbanus 1992). In Palau, too, production has been decreasing (Ngiralmau 1992).

Where taro is still being cultivated, there is a trend toward the replacement of traditional cultivars with a smaller number of varieties selected or bred for high yield in monoculture (Guarino and Jackson 1986). This loss of traditional diversity can have serious repercussions. It may mean that in the face of serious pest outbreaks, or the need for a new ecological adaptation (e.g. as a result of climate change), or quality characteristic, cultivars will not be available for evaluation. Nowhere can this have more meaning than in Samoa where, to meet domestic and export demands, one variety predominated nationwide and where, in 1993, the introduction of TLB devastated production. A further concern in PI countries is that, with a decline in production, traditional knowledge of varieties and cultivation practices also will be lost, and this impacts on cultural values since food production and social structures are inextricably entwined (Coursey 1977).

Collecting efforts in the Pacific

The threat of genetic erosion was recognized early on, and root crop collections were established in the 1980s with project funds through UNDP/FAO. As Table 1 shows, many of these collections were partly or completely lost by 1994. The Taro Genetic Resources: Conservation and Utilization (TaroGen) project was established in 1998 largely in response to the impact of Taro leaf blight on taro cultivation in Samoa, as well as the loss of taro genetic resources conserved *ex situ* and the continuing vulnerability of other PI countries to the disease. With the start of the TaroGen project, collections had to be re-established in all the participating countries. A regional approach was adopted to enable a large part of the taro genepool in the Pacific to be sampled at one time. The strategy adopted promoted the use of common methods for collecting, describing and documenting germplasm (including indigenous knowledge about the material). It was agreed that this approach would assist in the later rationalization of national collections and the identification of a core sample representative of taro diversity in the region, to be conserved *in vitro* in the Regional Germplasm

Centre (RGC) of the Secretariat of the Pacific Community (SPC). Collecting strategies and plans for PI countries were devised and agreement reached on a set of standard descriptors to be used for germplasm characterization. Training was also provided by Bioversity International (formerly the International Plant Genetic Resources Institute -IPGRI) on recording of information on the agreed descriptors, documentation of information and database development.

It was considered that collecting was justified in a number of cases, including when diversity was "missing" (gap filling), threatened, or required for a specific use (drought tolerance, early maturity), or when an estimation of diversity was necessary to develop a cost-effective conservation strategy. For most of the countries, gap filling was the main reason for collecting taro germplasm, with many countries having to re-collect the entire collection. A collecting strategy was defined at a TaroGen Collecting Strategy workshop (Lae, PNG, December 1998), in which participating countries agreed to follow a decision-making process based on the following:

- assemble a planning team reflecting the different stakeholder groups
- determine the extent of taro cultivation in different agroecological zones
- analyze passport and characterization/evaluation data on existing collections
- determine and prioritize present user needs
- predict future needs
- gather information on main threats of genetic erosion
- identify and prioritize areas within the area of cultivation based on lack of coverage, present needs and threat of erosion
- target specific diversity or specific taro types, if such information is available
- determine whether final collection size is manageable, and if not, adapt sampling parameters
- decide on routes necessary to achieve desired sampling and if necessary, use decentralized and/or *in vitro* collecting in some areas.

Experience has shown that national resources are often insufficient to maintain large national field collections over a long period of time, and so morphological descriptor data were used on the national collections to select a 20% core subset. This core subset would be further analyzed using microsatellites to obtain the final core of 10%. This approach was used with all national collections, excluding those from Polynesia. In the Polynesian countries, field collections could not be established, and consequently collections were made from each country and established *in vitro*. Microsatellites were then used to select 10% of these *in vitro* collections as a core subset (Mace *et al*. 2010, this volume).

Within three years, almost 2274 taro accessions with well-documented data were held in PI collections (Table 1). In PNG, collecting was carried out by four teams, with each team comprised of two research staff and an extension officer familiar with the area. Accessions were forwarded to Bubia Agricultural Research Centre (BARC) in Lae, where the national collection was established. A similar strategy was adopted in the Solomon Islands, but the resulting collection was severely affected by alomae and bobone virus diseases and the majority of accessions were lost before morphological characterization could be completed. The TaroGen Taro Genetic Resources Committee (TGRC) therefore decided to re-collect taro in Solomon Islands and, because the Ministry's resources and capacity were severely limited as a result of the civil unrest, to involve

an NGO—the Planting Materials Network (PMN)—in the collecting process. Awareness-raising and planning workshops were subsequently held in four provinces. The workshops included farmers, local agriculture extension staff, research division resource people and PMN facilitators. Workshop participants were trained in collecting, community field genebanks and diversity fairs, and also planned the collection process. After the workshops, teams of PMN members, assisted where possible by Research Division and Agriculture Officers, established collections in the four provinces. In both countries, ethnobotanical information was also collected during the missions and entered into a database. In PNG, the collection was replanted five months later so that accessions could be compared at the same stage of growth. Morphological description of the accessions was completed and a database prepared using the IPGRI descriptors. Synchronous replanting was not possible with the four separate collections in Solomon Islands. However, it was necessary to repeat the description process. Describing the taros at the time of collecting (*in situ* characterization) proved difficult, because many were not mature, and were obtained from locations too remote for the collecting team to personally visit. Consistency in the use of descriptors was also a problem, as each province had a different collecting team. Consequently, these collections were described again when the sample was taken for the 20% core subset.

Vanuatu established a collection of 502 accessions under its national programme. Similarly, a New Caledonia national collection of 82 accessions also was collected and maintained by its national programme. Although both collections were described using the Taro Network for Southeast Asia and Oceania (TANSAO) descriptors, which differed from the set used by TaroGen, these data were used by IPGRI (now Bioversity International) to select a 20% core subset from these collections. Fiji also had a national collection of 72 accessions, established under its national programme. This collection was described using the IPGRI descriptors.

In all, 67 accessions were collected from Cook Islands, Niue, Samoa and Tonga. Ethnobotanical information collected during collecting was entered in a database. The Polynesian accessions required virus indexing at Queensland University of Technology (QUT) before they could be planted out in Fiji for morphological characterization.

Rationalization of collections

As genebanks around the world amassed collections of germplasm, many faced major problems of size and organization. Realizing that the large size of some collections could deter use, Frankel and Brown (1984) proposed that a limited or "core collection" could be established from an existing collection. Frankel defined a core collection as "a limited set of accessions representing, with a minimum of repetitiveness, the genetic diversity of a crop species and its wild relatives".

Collecting activities under TaroGen have resulted in assembling 2274 accessions. Such large collections are not sustainable as national collections over the long term in the PI countries. Similarly, unless cryopreservation protocols achieve acceptable recovery rates, this large number of accessions could not be maintained under slow growth conditions in the SPC RGC. With this in mind, the TaroGen project recognized the need to establish a core collection, so that the genetic diversity captured in these collections could be conserved long-term. This core collection would be maintained *in vitro* in the SPC RGC. While it was recognized that a core collection is an aid to use, rather than an alternative to conserving the entire collection, the approach outlined here was justified given the limited resources available in the region.

In order to develop the Pacific taro core collection, 20% of country collections (PNG, Vanuatu and New Caledonia), was selected on the basis of morphological data. In the case of Fiji and the Polynesian collection (Cook Islands, Niue, Samoa and Tonga), since no morphological data were available, entire collections from these countries were subjected to molecular analysis. Either leaf samples or tissue cultures of the selected accessions were sent to the University of Queensland (UQ) for DNA analysis. Microsatellite markers were developed to enable further rationalization of the taro collections, to approximately 10% of the whole (Mace *et al*. 2010, this volume). The process of selection of the core collection is summarized in Table 2.

From the DNA analysis, the recommended final core collection for the PI region (not including the Solomon Islands collection) consists of 165 accessions. Although this final core relied heavily on genetic analysis, where possible at least one representative from each morphological group was included. In addition, between-countries duplication was avoided by cross-checking to the cluster analysis for the entire data set.

Approaches to conservation

There have been several attempts to collect and conserve taro genetic resources in the Pacific over the past 20 years. Conservation in field genebanks has been the main option used, but there are problems with this method. Other options exist, however, and these have been explored by the TaroGen project as part of developing an integrated approach to conservation applicable to the Pacific region.

Ex situ conservation

SEED STORAGE

Seed storage of taro, and other root crops, has received little attention because these crops are usually vegetatively propagated and many species are difficult to propagate by seed. However, seed can be produced by taro and preliminary studies have shown that they have orthodox storage characteristics (Jackson *et al*. 1977). Therefore, the potential exists to develop seed storage as a useful method of conservation of taro genes, though not genotypes. Wilson (1989) has demonstrated that taro seed can be stored for up to 2 years in a conventional refrigerator in an airtight container with desiccant. There are also reports of seeds being stored in the freezer compartment of a domestic refrigerator for 1 year, and 100% germination being obtained after storage. Prior to storage the seeds were dried at room temperature (Lebot, pers. comm.).

Initially TaroGen attempted to work with seeds imported from the Samoa and PNG breeding programmes. However, all seeds had poor viability, and so no storage experiments could be established. Since then, work has continued in PNG, where seeds can be easily accessed from the breeding programme. Preliminary results have indicated that the germination of taro seeds is affected by genotype, environmental factors, harvesting conditions, storage conditions and germination protocols (Singh *et al.* 2001). Germination rates of 75-80% were obtained with seeds less than 3 months old, but only 40-50% was obtained when the seeds were over 12 months old. In the same study it was noted that a system using special waterbeds was more supportive of germination than petri dishes. This all points to the need to identify best practices for handling seeds on harvesting, and to optimize germination methods. In addition, the orthodox behaviour of seeds should be confirmed using the protocol described by Hong and Ellis (1996). If conditions

TABLE 2. Process of selecting the taro core collection in the Pacific

	Initial number	Basis of initial selection	No. initially selected	Basis of final selection	No. finally selected
PNG	859	Morphological data: (i) classification into 12 groups according to combinations of states for 3 descriptors; (ii) followed by clustering of the 7 largest groups using 22 descriptors. †	151	Microsatellites: 151 of initially selected accessions classified into 23 clusters.	83
Vanuatu	502	Morphological data: (i) classification into 8 groups according to combinations of states for 3 descriptors; (ii) followed by clustering of the 3 largest groups using 22 descriptors. †	89	Microsatellites: 89 of initially selected accessions classified into 21 clusters.	45
New Caledonia	82	Morphological data: classification into 10 groups using 22 descriptors. †	18	Microsatellites: 18 of initially selected accessions classified into 8 clusters.	8
Solomon Islands	692	To be done			
Fiji	72	Microsatellites: accessions classified into 7 clusters	70	-	8
Palau	11	Microsatellites: accessions classified into 4 clusters	11	-	5
Cook Islands	18	Microsatellites: accessions classified into 3 clusters	13	-	3
Niue	25	Microsatellites: accessions classified into 6 clusters	22	-	6
Samoa	15	Microsatellites: accessions classified into 4 clusters	13	-	4
Tonga	9	Microsatellites: accessions classified into 3 clusters	9	-	3

† Using Ward's method of cluster analysis. PCA scores used for selection of accessions within each cluster

can be optimized, seed storage will provide a convenient method for long-term conservation of taro genes/populations, though not specific gene combinations characteristic of different landraces, because the recombination occurring during sexual reproduction would break these up. In addition, it could offer an alternative method for the exchange of taro germplasm (especially breeding lines), assuming transmission of viruses by seeds is shown to minimize quarantine risks. If this is the case, seed storage and exchange would be very useful for national taro improvement programmes.

FIELD GENEBANKS

As taro is vegetatively propagated, field genebanks have been the most utilized method of conservation adopted in the region. However, there are several disadvantages associated with field genebanks, including their vulnerability to climatic extremes, pest and disease outbreaks and limited resources. As shown in Table 3, these have been responsible for the very significant losses that have occurred in many collections in the region.

While taro field collections do not require particularly large areas of land, they do demand a considerable amount of labour and thus expense. In addition, the plant material remains exposed to biotic and abiotic stresses (Florkowski and Jarret 1990; Jarret and Florkowski 1990; Ramanatha Rao 1998). Little information is available on costs, though CIAT has shown with cassava that the costs are comparable for field and *in vitro* genebanks (Epperson *et al*. 1997). A study carried out by TaroGen also showed the costs of field and *in vitro* genebanks to be similar.

TABLE 3. Losses of taro (and yam) in Pacific Island collections (dates are indicative only; in many cases, losses occurred over several years)

Country	Year	Collection	Reason for Loss
FSM (Pohnpei)	1994	Taro	Lack of staff
Papua New Guinea	1980	Taro (partial)	Alomae disease
Solomon Islands	1974	Taro	Alomae disease
Solomon Islands	1991	Taro	Cost of maintenance
Solomon Islands	1978	Yam	Anthracnose disease
Samoa	1986	Taro & yam	Cost of maintenance
Tonga	1985	Taro	Drought
USA (Hawaii) – Lyon Arboretum	1988	Taro (most)	Unknown
Vanuatu	1976	Taro	Drought
Vanuatu	1994	Taro & yam	Lack of staff

For many vegetatively propagated crops, alternative methods to field genebank conservation have not been fully developed (Chin *et al*. 1999), and even when they are, field genebanks will continue to play a major role in any conservation strategy. Conservation in field genebanks ensures

the maintenance of the genetic integrity of specific genotypes, and easy access for characterization, evaluation, multiplication and breeding. In addition, field genebanks do not depend on complex technology, can be cheap to maintain in some circumstances, and can be used to foster linkages between germplasm use and improvement (Nissilä *et al.* 1999).

IN VITRO METHODS

In vitro methods include slow-growth storage and cryopreservation. Slow-growth storage is generally used for short- to medium-term storage, whereas cryopreservation is a long-term option. Like field collections, *in vitro* methods are particularly suited to vegetatively propagated species and those with recalcitrant seeds. However, *in vitro* storage has certain advantages over field collections, which include security from climatic extremes and pest and disease outbreaks, ease of distribution and the ability to exchange pathogen-tested material.

Slow growth. Subculture intervals of 3 years for taro stored in the dark at a temperature of 9°C have been reported (Bessembinder *et al*. 1993). However, the maintenance of such low-temperature storage facilities in PI countries is impractical. In addition, genotypic differences are known to occur with taro *in vitro,* and only a few cultivars were studied in this investigation. Research carried out under the EU-funded Pacific Regional Agriculture Programme (PRAP) at the University of the South Pacific (USP), Samoa, showed that *in vitro* cultures of taro could be maintained at a temperature of 20°C with subculture intervals of 9 to 12 months, depending on the variety. TaroGen conducted a study comparing *in vitro* and field maintenance of some 50 local Fijian varieties of taro. Using the same temperature of 20 C, subculture intervals with these varieties were only extended to 6–9 months. This demonstrates the influence of genotype on slow-growth storage methodologies. Additional studies investigating the effect of culture container size and reduced total nitrogen in the medium as potential slow-growth methodologies for taro are underway. The methodology selected must take into account genetic integrity and available resources. Somaclonal variation can be a problem with slow-growth storage systems, but studies at IITA on sweet potato and at CIAT on cassava did not detect any morphological changes in plants that had been in culture for 6 to 7 years (Ng 1991). Low-temperature storage (at a reasonable temperature) is generally considered as the least likely of the slow-growth methods to lead to genetic change.

Cryopreservation. Long-term *in vitro* storage employs cryopreservation, storage at ultralow temperatures using liquid nitrogen (–196°C). At this temperature, all cellular division and metabolic processes are suspended, with minimal impact on genetic stability. Theoretically, plant material can thus be stored without alteration or modification for an unlimited period of time. Moreover, cultures are stored in a small volume, protected from contamination and require very limited maintenance. Owing to the various problems and limitations encountered with other approaches (Withers and Engels 1990; Maxted *et al.* 1997), cryopreservation currently offers the only safe and cost-effective option for long-term conservation of genetic resources of problem species.

Successful cryopreservation methods have been reported for a number of root crops, including sweet potato (Towill and Jarret 1992), yams (Mandal *et al.* 1996) and cassava (Escobar *et al*. 2000). However, the level of success varies, and is often limited to a small number of different varieties. Recovery rates between 80 to 100% have been reported for taro using a vitrification procedure (Takagi *et al*. 1997). TaroGen has been using this cryopreservation protocol with some modifications, and recovery rates of 70 and 100% were obtained with three varieties. However, these high success

rates have not been reproducible and on average 20–30% recovery rates are obtained with the varieties tested. This investigation has demonstrated that cryopreservation of Pacific Island taro is possible. Research is continuing to increase recovery rates by improving the quality of the source plants, and optimizing conditioning treatments. Encapsulation-dehydration is also being evaluated for its applicability to taro.

BOTANICAL GARDENS

There are about 1600 botanic gardens and arboreta worldwide (Bramwell *et al.* 1987; WWF–IUCN–BGCS 1989), managing almost 4 million accessions of 80–100 000 species. However, their role in plant genetic resources conservation—particularly of crop plants—has been relatively limited. The emphasis is more on number of species than on genetic diversity within species, which means that few plants per species are usually maintained. For example, the National Tropical Botanical Gardens in Hawaii has only nine taro accessions. This limitation generally restricts the role of botanic gardens in genetic resources conservation to one of public awareness and education, helping bring people and plants together. Botanic gardens will be particularly interested in rare and endangered wild plant species, including crop wild relatives. For example, there is currently research interest (mainly taxonomic and ethnobotanic) in *Colocasia* in botanic gardens such as Kunming Botanical Garden in China.

In situ conservation

In situ conservation is dynamic, as opposed to the semi-static nature of *ex situ* conservation. It enables the evolutionary potential of species and populations to be maintained, and increases control by local communities over their genetic resources (Frankel 1970; Ledig 1988, 1992; Jarvis 1999; Sthapit and Jarvis 1999; Jarvis *et al.* 2000). However, it is necessary to complement *in situ* conservation with *ex situ* conservation, because it is difficult to conserve large numbers of populations *in situ* and make them easily available to users such as breeders.

PROTECTED AREAS

Various types of protected areas (IUCN 1994) are in place throughout the world, in areas identified as rich in diversity of ecosystems and/or species or focused on ecosystems or species of particular value. This is a useful approach for conserving genetic resources of wild fruit, forestry and other species. However, genetic resources conserved in protected areas are often not easily accessible for use by breeders and others, and accessibility also can present problems for monitoring, management, characterization and evaluation. The reserves are also vulnerable to natural and anthropogenic disasters. Although some protected areas are home to wild relatives of crop species, so contributing to conservation of their genetic diversity, this is often not a specific aim. Generally, Ministries of Environment, rather than Agriculture, are responsible for implementation of protected areas, and future consideration of wild relatives conservation will require more input and collaboration from other stakeholders (Sharrock and Engels 1996). However, it is possible that this is now more achievable in the Pacific region with the development of the National Biodiversity Strategy Action Plans under the Convention on Biological Diversity (CBD). The implementation of these strategies requires the establishment of national committees with representation from both environment and agriculture sectors. With the recent establishment of the PGR network in the Pacific, there should be increased interaction among the different sectors involved in agrobiodiversity, and investigation of

the potential role of protected areas in the conservation of wild relatives such as *Colocasia esculenta* var. *aquatilis* will be a priority.

ON-FARM CONSERVATION

In situ conservation of agrobiodiversity, or on-farm conservation, is the maintenance of traditional crop cultivars (or landraces) and farming systems by farmers within traditional agricultural systems (Hodgkin *et al*. 1993; Jarvis 1999). Farmers have of course been managing genetic resources on their farms for centuries, but supporting their practices has been gaining importance as an approach to conservation in recent years (Sthapit and Joshi 1996). This can be achieved by adding value to germplasm that is managed on-farm (for example through participatory crop improvement), building awareness of its importance, strengthening market and non-market incentives and also through policy interventions. It is increasingly clear that supporting the continued cultivation of traditional varieties on-farm can be an effective strategy to improve nutrition, reduce poverty and foster sustainable livelihoods.

Conservation of taro on-farm is potentially an important component of a complementary conservation strategy. Several studies have been carried out in the region with support from TaroGen to evaluate the approach. In 1998, a pilot study in on-farm conservation was carried out by the Farm Support Association (FSA), Vanuatu. This preliminary work indicated that if on-farm taro conservation is to be successful, the areas selected should be remote and isolated, with limited access to markets. Awareness of the benefits and value of maintaining taro landraces also needs to be increased among farmers.

A TGRC meeting held in May 2001 considered the results of this study, and agreed that the on-farm conservation work was important and should be continued. Funds were made available for further pilot projects in Solomon Islands and Vanuatu. In both countries, NGOs took the lead, working closely with the relevant government institutions. Funding was also provided to undertake a survey to determine the extent of genetic erosion of taro landraces in Fiji.

Solomon Islands: The study was carried out by a farmers' organization—Solomon Island Planting Materials Network (PMN)—supported by an NGO—Kastom Gaden Association (KGA)—in cooperation with the Ministry of Agriculture and Primary Industries (MAPI). Data were collected using a rapid rural appraisal approach over a period of 4 weeks in conjunction with the collecting activities in four provinces. The study found that four major factors were influencing the production of taro, leading to a decline in genetic diversity through loss of landraces, and a loss of taro indigenous knowledge. These factors were:

Cultivation and production constraints: Other crops such as sweet potato are easier to cultivate. Pressure on land availability means that crops suited to shorter fallows will be preferred to taro, which traditionally is cultivated with a long fallow.

Pests and diseases: Taro cultivation has become a relatively risky activity owing to threats of virus (alomae) and pests, mainly taro beetle.

Changing consumption patterns: In urban and semi-urban parts of the country, there is a shift from traditional crops and green vegetables toward rice, tinned fish and biscuits.

Economic and market forces: Taro is significantly more expensive than sweet potato in all the markets surveyed, though prices decline with distance from the main urban centres.

The study showed that many issues are involved in any consideration of on-farm conservation in the Solomon Islands, and that genetic conservation and food security needs will have to be balanced in an environment of limited resources for agriculture and nutrition improvement. It was difficult to say from the information obtained whether genetic erosion of taro landraces had stabilized in areas of high land pressure, or if the erosion was continuing. However, support for taro cultivation could be achieved with the appropriate interventions, such as improved pest management, improved farming systems and practices to improve soil fertility in short fallow areas, and participatory variety selection of virus-resistant cultivars. In bush and low population density areas, taro remains an important crop, and these areas would seem suited to promote continued on farm conservation. As part of this study in the Solomon Islands, community-level field genebanks were established in the four provinces. Taro from the field genebanks will be distributed back to farmers who donated taro and recorded in a community diversity register that can be monitored over time. Strong support for this work has been experienced in the four provinces, especially with regard to the local field genebanks.

Vanuatu: The study was again carried out by FSA, because they have been working with smallholders on commercial agricultural projects for a number of years, and had established a network of associates throughout the country. Although taro is planted almost throughout Vanuatu, traditional farmer knowledge in conservation, variety and characteristic description and identification is very limited in many areas. Therefore, in order to get a better idea of the existing traditional knowledge of cultivation and management of taro, FSA surveyed two isolated areas (remote to very remote) where taro is considered a staple food. The specific objectives of the study were to: determine the extent of taro diversity and its distribution in targeted areas; understand the process whereby taro diversity is maintained and managed; understand the factors that influence the maintenance of diversity, and use the information gathered to develop a national *in situ* strategy.

These objectives were addressed by collecting information through field surveys and farmer interviews. The information gathered was mainly for wetland taro. This more in-depth study came to the same conclusions as the previous study. Generally, it was felt that taro cultivation is not a declining practice, or in danger of declining in the future, possibly because of the lack of any severe pests or diseases. Further, farmers conserve as many taros as they find necessary, maintaining those for which they have a specific need. As indicated in the previous study, for on-farm conservation to work, care would have to be taken in identifying the most appropriate sites and attention given to expanding the benefits to be gained from maintaining increased taro diversity.

Fiji: The survey was conducted by the Ministry of Agriculture in three provinces, selected according to their proximity to Koronivia Research Station (KRS), where hybrids were developed, accessibility to market centres, and size, topography and infrastructure of the province. The survey showed that erosion of taro landraces had occurred and was occurring within the selected sites, mainly because of proximity to KRS, and accessibility to markets.

The work in the three countries has demonstrated that some potential for on-farm conservation exists. Consequently, a workshop was held to determine how to proceed, and possibly formulate

a project proposal for on-farm conservation. The main outcome of the meeting was consensus on the need for further work in this area, but with broader focus than merely on-farm conservation. It was agreed that any project should address on-farm conservation of traditional crops for the health, national security and income-generation of rural people, with diversification, at both species and genetic levels, being the underlying philosophy. A number of recommendations were made, and these are currently being considered by participating countries. Once national-level discussions have been held with all stakeholders, further decisions will be made on the project proposal.

Home gardens

Home gardens, especially in rural areas, tend to contain a few plants of a wide spectrum of species used in small amounts, such as vegetables, fruits, medicinal plants and spices (Evenson 1986; Michon *et al*. 1986). Surveys have shown that taros are among the most common plants found in home gardens in PNG, Fiji and Tonga (Thaman 1982). Little is known about the extent of taro diversity in Pacific home gardens, but there is evidence from other regions suggesting that they are important sites for maintaining and experimenting with diversity. Twenty-four taro landraces were reported in a baseline survey of home gardens in Nepal, with eight being the highest number of landraces maintained by a single household (Rana *et al*. 2000). Similar surveys in the Philippines recorded 14 taro landraces in home gardens (Pardales *et al*. 1999). Encouraging home garden and urban taro production could provide immediate nutritional, economic and social benefits in addition to enhancing genetic conservation and could be achieved through community, school or youth groups or other networks. Within a community or region, a network of home gardens could contribute significantly to the conservation and direct use of genetic diversity.

Integrated approach to taro conservation

It is important to emphasize that neither *ex situ* nor *in situ* conservation can meet all needs and that the two approaches or strategies are complementary in nature. This is emphasized by the CBD (Article 9), which promotes complementary approaches for the conservation biodiversity. Therefore, for any genepool, there is a need to employ a combination of methods, ranging from nature reserves to genebanks, to ensure as much genetic diversity as possible is conserved and made available for use. The balance between the different methods employed depends on factors such as the biological characteristics of the genepool, the available infrastructure and human resources, the levels and partitioning of genetic diversity, and the intended use of the conserved germplasm (Ramanatha Rao 1996; Maxted *et al.* 1997).

At the TaroGen Conservation Strategy Workshop, held at SPC, Suva, in September 2001, a complementary conservation strategy (CCS) for taro was considered. All aspects of conservation from rationalization of existing collections through to utilization were discussed, and several recommendations made (Taro Conservation Strategy Workshop Report 2001). As some conservation methods were still being evaluated, for example seed storage and on-farm conservation, suggestions were made as to how these approaches could contribute to the overall strategy.

The taro core collection has captured the diversity present in the collections currently maintained either in the field and/or at the RGC. This core collection must be readily available for distribution. It has to be evaluated by countries so that more information can be obtained about the accessions, thereby promoting their further use. Its distribution must not involve any quarantine risks. *In vitro* techniques, namely meristem culture with thermotherapy, can eliminate viruses, so *in vitro* storage

and virus indexing can facilitate distribution. Slow-growth storage *in vitro* satisfies these requirements, so this was the approach recommended for the core. Countries could also maintain their own core collections, but this would depend on the available resources. They might also choose to maintain other, different varieties in field genebanks, knowing that the SPC RGC was conserving the core collection for the region as a whole. The establishment of the RGC at SPC in Suva has provided the region with the means to conserve the genetic diversity of its major crops in tissue culture. Having the RGC within a long-established regional organization eliminates some of the arguments often put forward against *in vitro* conservation, such as the level of technical skills and long-term resources required.

Having an *in vitro* taro collection at SPC also allows further research into cryopreservation. As stated earlier, this is the only effective long-term method of genotype conservation for vegetatively propagated species. With an effective and reproducible cryopreservation technique, large collections could be maintained in a small space with low maintenance costs. In addition, the risk of losing accessions due to contamination or somaclonal variation would be minimized. Cryopreservation was recognized as the most appropriate approach for maintaining base collections. Countries were concerned that the taro accessions currently held in national collections could be lost. The meeting therefore recommended further research so that cryopreservation protocols can be extended to all taro accessions in national collections.

Field genebanks have played a major role in the work of TaroGen in developing a regional taro collection, and although their use on a long-term basis has not been recommended, national field genebanks will continue to play a role in a taro CCS. Field genebanks could be used for a number of specific functions:

- conserve varieties most difficult to recollect, for example, those from geographically inaccessible areas in PNG
- maintain frequently utilized varieties
- maintain working collections for breeding and evaluation work in the country
- maintain elite or farmer-preferred varieties, many of which are not included in the core collection.

PI countries could therefore maintain relatively small field collections for breeding, evaluation and/or distribution to farmers, which would require minimal resources. Such an approach would help broaden the genetic diversity conserved in the region. Spreadsheets have been used by PNG and Fiji to determine the costs of field genebanks. Spreadsheets for *in vitro* storage are also available. These are available for all countries to use and compare costs between the two methods, enabling countries to determine whether sufficient resources are available to sustain field genebanks and/or *in vitro* storage.

Storage of taro seeds provides a method that is more dynamic. Seeds represent new genetic combinations that could be evaluated by countries to provide new varieties suited to their specific needs. However, as indicated by preliminary studies, more information is required on harvesting, handling, storage and germination protocols. Studies are continuing in this area in PNG. There are also plans to produce seeds in Fiji, enabling the RGC to carry out research in this area. Once effective protocols have been determined, seed conservation will provide countries with another

option and the means by which taro genes can be stored at low cost. Seeds from uncommon varieties and crosses could be stored in this way.

In situ approaches will promote the conservation of taro landraces that are not, and cannot be, part of *ex situ* collections and therefore allow greater genetic diversity to be conserved in the region. *In situ* also allows for the continued evolution and adaptation of these particular landraces to changing environments, needs and tastes, for example through somatic mutations and selection, and occasional seed production. Preliminary studies have shown there is a need to determine the natural and human factors influencing on-farm conservation. For farmers to maintain taro diversity, they must see benefits through increased income or other advantages, for example as a result of improved methods of processing or marketing. Through TaroGen, considerable experience in participatory plant breeding has been developed in some countries, and this could be used with on-farm conservation to directly improve landraces by increasing disease resistance or quality. It is also important to document the diversity on-farm (what, where and how long it may last) for the diversity to be effectively a part of CCS. Work in this area will continue to determine in which countries on-farm conservation would be most applicable, and what role this approach can play in an overall CCS for taro.

Reserves are an important means of conserving wild relatives of crop species. Wild taros have been reported in Australia, PNG, Solomon Islands and Vanuatu, but have not been thoroughly studied. They may be feral taros as opposed to true wild types. Breeders have largely avoided wild taros because of agronomically negative traits, but wild genotypes could be an important source of genes, especially with impending climate change and its associated environmental and physical stresses (Ivancic and Lebot 1999). Wild genotypes in New Caledonia, for example, can survive long periods of drought. *In situ* conservation of wild taro genotypes in natural habitats, allowing continued evolution and possibly introgression with cultivated material, should be considered when investigating the possible implementation of protected areas for taro conservation. However, like other wild relatives of crop plants, more information on distribution, minimum habitat size and population dynamics is required (Sharrock and Engels 1996).

Summary

This paper has attempted to describe recent activities in taro collecting and conservation in the Pacific region. The current status of taro conservation has been reviewed, highlighting the advantages and disadvantages of various *ex situ* and *in situ* methods as they apply in general, and to taro in particular. The paper has outlined the need for a complementary conservation strategy for taro genetic resources, and suggested how the various approaches can be managed so taro diversity is conserved and utilized most efficiently and effectively. Establishing national taro field genebanks and a regional core collection at the SPC RGC have been important steps in the conservation and use of taro genetic resources. However, they will not be sufficient. Since it is impossible to collect and conserve all taro genetic diversity *ex situ*, on-farm conservation needs to be further investigated to see if it can assist in accessing a much wider genetic diversity and at the same time benefit taro growers, thereby strengthening the link between conservation and use.

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Improving taro production in the South Pacific through breeding and selection

D. Singh, D. Hunter, T. Iosefa, T. Okpul, P. Fonoti and C. Delp

Introduction

The Pacific region is unique in that all its traditional staples are vegetatively propagated; therefore, domestication appears to have captured a limited proportion of genetic diversity. The genetic base of these crops is relatively narrow (Lebot *et al*. 2001) and as a consequence, many of these crops are vulnerable to biotic and abiotic stresses. This was highlighted by the recent devastation of Samoan traditional taro (*Colocasia esculenta*) cultivars with leaf blight disease (caused by the pathogen *Phytophthora colocasiae*). This was a timely reminder of the need for genetic improvement of taro and other traditional Pacific staples to broaden their genetic base.

Taro leaf blight (TLB) has been present in the Pacific region since the early 1900s (Weston 1918; Carpenter 1920). It is a disease highly adapted to the wet humid environment of the region and is a major constraint for taro production in those countries where present. The most recent introduction of the disease was to the Samoan islands in 1993, but it has been present in Papua New Guinea (PNG) since the Second World War. In Samoa, over 90% of taro plantations were devoted to the local cultivar, *Niue*, the choice for commercial production. The disease spread rapidly, severely affecting all local cultivars and within a few months TLB reached epidemic proportions. As a result, Samoa lost an export market estimated to be worth around US$ 4 million per year, with a similar decline in domestic supplies. TLB continues to be a major constraint in PNG, and many countries, including Fiji, Vanuatu, Tonga, Cook Islands and Niue, remain vulnerable to this devastating disease.

Taro can be genetically improved by breeding and has potential for new product development and export to countries like Japan, New Zealand and the United States. Despite its global importance, there is no international institute or programme devoted to its improvement, making it very much an orphan crop. However, in recent years, two regional projects—TaroGen (Taro Genetic Resources: Conservation and Utilisation) and TANSAO (Taro Network for Southeast Asia and Oceania)—have made considerable progress in taro breeding programmes commenced by PNG, Samoa and Vanuatu. Participatory plant breeding (PPB) has been initiated in Samoa since 1999 through the Taro Improvement Project (TIP), a farmer group that evaluates taro germplasm at the University of the South Pacific (USP). Although still in its infancy, it has pioneered PPB approaches appropriate elsewhere in the Asia-Pacific region. Decentralized evaluation of cultivars is underway in PNG, with future efforts concentrating on the involvement of farmers at earlier stages of the breeding programme.

This paper reviews developments in taro breeding under the TaroGen project, focusing on the conventional approach in PNG and the more recent PPB approach in Samoa.

Taro breeding in Papua New Guinea

Since TLB first arrived in PNG in the 1940s, the country has considered two main strategies in an attempt to control and prevent its spread. Some fungicides can be used (Cox and Kasimani 1988),

but are generally too expensive for the majority of growers. Breeding plants with resistance to the disease offers the best long-term strategy for the control of the disease, but breeding programmes that commenced in the 1980s and early 1990s were not successful owing to lack of funds and staff changes. In 1993, PNG re-established its taro breeding programme (then under the Department of Agriculture and Livestock). This programme was revived at the National Agricultural Research Institute (NARI) with further support from the TaroGen project to develop taro lines with resistance to TLB, high yield potential and good eating quality. TLB resistance sources in the base population included a wild variety from Thailand (cv. Bangkok), hybrids from a Solomon Islands breeding programme and three semi-wild taro accessions (Ph 15, Ph 17 and Ph 21) from PNG. The entire methodological approach used by the breeding programme for the release of new varieties to farmers is outlined in Figure 1.

Advances of PNG Breeding Programme

Cycle 1 was developed in 1994 by crossing the resistant base population with superior local taro varieties. The evaluation results of Cycle 1 superior lines are presented in Okpul *et al.* (1996). Although several TLB-resistant lines were selected from Cycle 1, no recommendations were made since most lines retained undesirable wild characteristics. Cycle 1 was then advanced to Cycle 2 in 1996 by intercrossing superior genotypes. In September 1998, 32 lines were selected from Cycle 2 on the basis of preliminary assessment of yield, TLB resistance and eating quality. Further testing enabled selection of 12 TLB-resistant lines for inclusion in a replicated advanced yield trial at Bubia. A popular local variety (*Numkowec*) was included for comparison. The results of this trial are summarized in TaroGen (1999) and Singh and Okpul (2000).

Adaptability (G×E) Trials

Adaptability trials are a prerequisite of the PNG breeding programme before any varieties can be recommended or released. Seven lines (C2-E1, C2-E3, C2-E4, C2-E7, C2-E8, C2-E10 and C2-E11) were selected for advancement to genotype × environment (G×E) trials in 2000. Other lines were dropped because of poor acceptability by tasters during routine eating-quality tests. The G×E trials were conducted at seven sites, ranging from very dry (Gabadi) to very wet (Finalbin). All sites except Finalbin were in lowland areas (<600 m asl). Two sites (Gabadi and Ragiampun) have a strong seasonal rainfall pattern. The trial methodology is outlined in Okpul *et al.* (2002). Corm yields for all sites are presented in Table 1.

All lines except C2-E11 yielded better than *Numkowec* when averaged over all sites. Four lines (C2-E3, C2-E4, C2-E11 and *Numkowec*) gave stable yield across all sites (Table 2) on the basis of the Eberhart and Russell (1966) stability model.

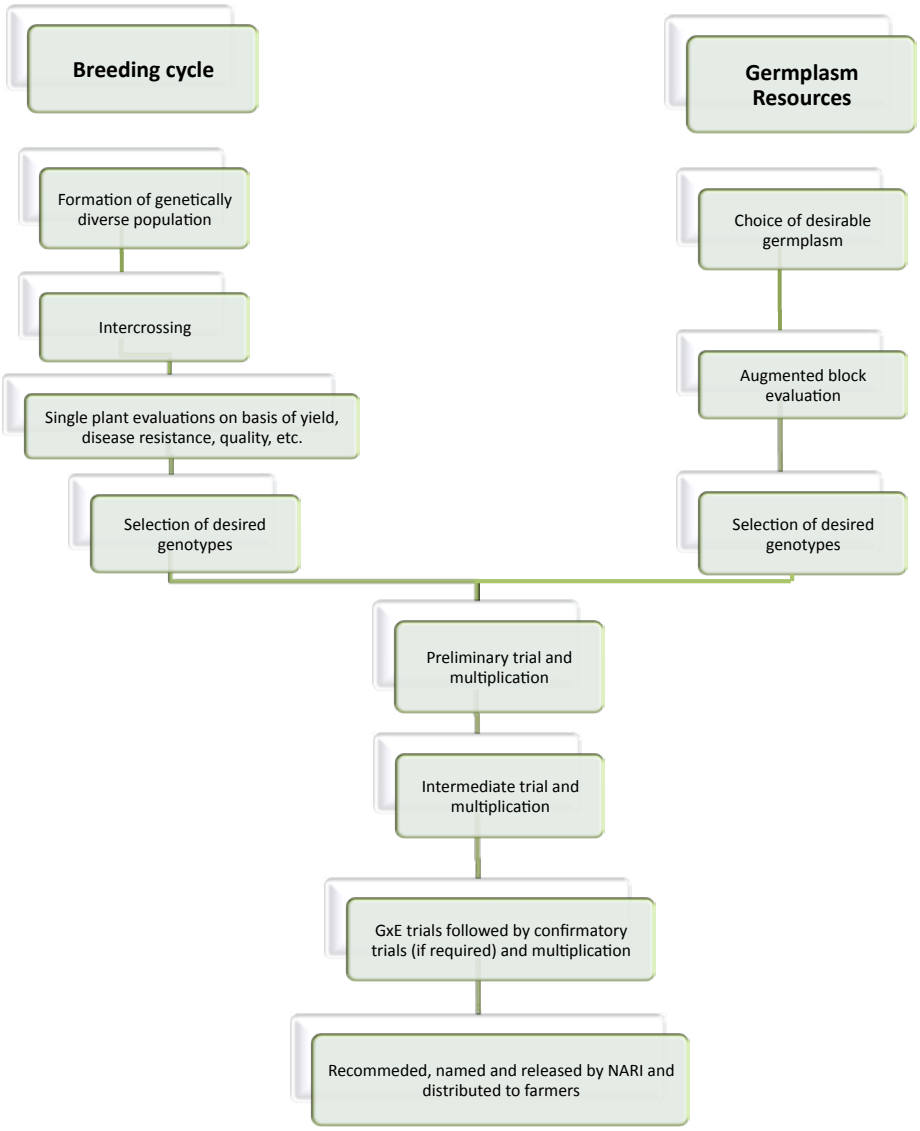

Figure 1. Schematic representation of evaluation, development and release process for promising taro varieties.

TABLE 1. Mean corm yield (t/ha) of selected elite genotypes at seven different agroecological sites in PNG

Genotype	Site							
	Finalbin	Gabadi	Murunas	Ningrum	Ragiampun	Sikut	Tikeleng	Mean
C2-E3	7.94	6.04	6.27	8.18	19.38	14.25	7.16	9.89
C2-E10	9.38	5.14	9.10	8.51	10.08	10.70	3.89	8.11
C2-E1	8.13	6.09	3.35	11.10	14.21	7.42	6.46	8.11
C2-E7	7.86	3.77	6.23	8.30	16.68	7.89	4.33	7.87
C2-E8	4.87	5.14	6.69	3.23	13.64	9.24	7.49	7.51
C2-E4	7.13	5.01	4.81	6.66	11.78	10.17	5.32	7.27
C2-E11	5.97	4.45	6.09	6.14	9.60	6.81	2.58	5.95
Numkowec	2.95	4.58	6.25	3.72	12.44	8.54	3.13	5.94
Mean	6.78	5.31	6.10	6.98	13.48	9.38	5.04	
LSD (5%)	1.54	0.84	ns	2.46	2.27	0.61	2.41	

All lines showed significantly better resistance to TLB than the susceptible control *Numkowec*. There was little difference between the elite lines in their response to TLB (Table 2).

Lines differed in their suckering ability and their tendency to flower (Table 2). C2-E8 and C2-E10 expressed frequent flowering and low suckering ability, respectively. Eating quality was considered acceptable to good. All lines were susceptible to taro beetle. There was no significant difference among lines for beetle damage (Table 2). The overall characteristics of the eight tested genotypes indicated that genotypes C2-E1, C2-E4, C2-E7, C2-E8 and C2-E10 produced significantly higher corm yields than control *Numkowec*. However, the corm yields of genotypes C2-E1, C2-E7, C2-E8 and C2-E10 were unstable across sites. The performance of genotypes C2-E3 and C2-E4, conversely, was characterized by general adaptability. Under different environments they yielded medium and high respectively, showed moderate resistance to the TLB pathogen, rare flowering ability and adequate sucker production.

Recommendations

The G×E trial results were reviewed by a panel of scientists and were presented to the national Taro Improvement Coordinating Committee (TICC) with a recommendation to release two lines, C2-E3 and C2-E4. These lines were selected on the basis of high yield, yield stability over a range of environments, resistance to TLB and good eating quality. After carefully considering the performance of the seven lines, the TICC endorsed the recommendation to release the two lines, and considered that a third line (C2-E8) also was promising, and therefore should be released. C2-E8 has been identified to have very good eating quality and its yield is comparable to C2-E4. However, its yield was found to be unstable in the G×E trial and it yielded rather poorly at the Western Province sites. It also had a tendency to flower frequently, which could adversely affect corm shape and quality.

TABLE 2. Some important traits of Cycle 2 elite taro genotypes assessed by the PNG breeding programme

Genotype	Yield stability (S^2d)†	TLB (% DLA)‡	Eating quality (score)§	Flowering	Average no. of suckers	Taro beetle (% damage)¶
C2-E1	Unstable (2.41)**	Resistant (8.02)**	Acceptable (2.34)	Rare	3	Susceptible (18.92)
C2-E3	Stable (0.04)	Resistant (8.24)**	Good (2.64)	Rare	3	Susceptible (19.70)
C2-E4	Stable 0.53	Resistant (7.34)**	Good (2.59)	Rare	3	Susceptible (19.04)
C2-E7	Unstable (1.05)*	Resistant (8.32)**	Acceptable (2.43)	Rare	5	Susceptible (20.40)
C2-E8	Unstable (4.45)**	Resistant (7.19)**	Good (2.54)	Frequent	5	Susceptible (18.74)
C2-E10	Unstable (2.51)**	Resistant (8.39)**	Acceptable (2.32)	Rare	1	Susceptible (17.77)
C2-E11	Stable (0.31)	Resistant (6.31)**	Good (2.51)	Rare	3	Susceptible (20.93)
Numkowec	Stable (0.58)	Susceptible (15.76)	Good (2.52)	Rare	3	Susceptible (19.53)

†Mean square deviation from stability (*, ** at P<0.05 and P<0.01, respectively).
‡Mean of 5 sites where significant disease pressure occurred excluding Gabadi and Ragiampun (** = significantly lower Diseased Leaf Area (DLA) than the control, *Numkowec*, at P <0.01).
§Score out of 3; >2.0 considered acceptable and >2.5 good.
¶A genotype with more than 15% damage was considered susceptible.

However, these disadvantages were not considered sufficiently serious to prevent its release. Given its possibly greater consumer acceptability, it was considered that farmers should have the opportunity to assess and adopt this genotype. Therefore, it was agreed to release C2-E3, C2-E4 and C2-E8. Full descriptions of the three lines are given in Table 3.

A final release document paper was prepared and presented to NARI management. The release of lines was endorsed by NARI, and the lines were officially named and released on 13 December 2001. This is a first report of any taro varieties released in PNG.

The varieties were registered under NARI. Under current PNG regulations, breeders' rights have not been established in the country and breeding is confined to public sector research. However, the breeders (TaroGen and NARI) should be acknowledged and recognized for any commercial usage.

Awareness, multiplication and distribution of material

The released lines were promoted through press releases, rural radio, television, popular publications, national periodicals, extension booklets and pamphlets. The released material was multiplied at four different sites covering different regions of PNG, and was distributed nationally to farmers in

collaboration with extension workers, NGOs and church groups. National TICC also played a vital role in distributing and promoting the material to farmers. As part of the promotion, an open day was conducted and more than 200 farmers and 10 community groups attended and received planting material. Participants had an opportunity to taste new varieties before distribution and the taste was considered highly acceptable. Approximately 1500 plants of each line were distributed. The farmers also were given basic training/ demonstrations and answers to their queries regarding taro improvement and conservation.

TABLE 3. Descriptions of PNG-released taro varieties and a popular standard cultivar Numkowec

Feature	Variety			
	NT 01 (C2-E3)	**NT 02 (C2-E4)**	**NT 03 (C2-E8)**	**Numkowec (standard)**
Yield (t/ha)	10.49	7.68	7.65	5.89
Average corm weight (g)	525 g	380 g	380 g	300 g
Yield stability	Stable	Stable	Unstable	Stable
Taro leaf blight (TLB)	Resistant	Resistant	Resistant	Susceptible
TLB diseased leaf area (%)	8.24	7.34	7.19	15.76
Taro beetle	Susceptible	Susceptible	Susceptible	Susceptible
Taro beetle damage (%)	19.70	19.04	18.74	19.53
Eating quality	Good	Good	Good	Good
Eating quality score	2.64	2.59	2.54	2.52
Time to maturity (c. months)	6	6	6	6
Sucker production	3-4	2-3	5-6	6-8
Growth habit	Erect	Erect	Erect	Erect
Plant height	Tall	Medium	Tall	Medium
Leaf lamina	Light green	Dark green	Dark green	Dark green
Petiole colour	Light green	Purple green	Purple	Light green
Petiole junction	Purple	Purple	Purple	Purple
Flowering	Rare	Rare	Frequent	Frequent
Corm shape	Cylindrical	Elliptical	Conical	Conical
Corm skin	Smooth	Smooth	Smooth	Smooth
Flesh colour	Pink	Pink	Pink	Pink
Corm dry matter content (%)	35	41	41	38

Ongoing and future breeding programme

The PNG breeding programme has advanced to its fourth cycle. Cycle 3 was created by intercrossing 21 C2 lines in a half-diallel design. More than 300 crosses were attempted and a population of over 10 000 seedlings was created and evaluated. Forty-nine superior lines in terms of TLB resistance, high yield and good eating quality were recovered in Preliminary trials. Twenty-six lines were selected from Intermediate trials and finally twenty-six lines were selected from Advance trials. The selected six lines are being evaluated in G×E trials for adaptability.

As recommended and endorsed by the national TICC, the breeding strategy was modified for wider adaptation for Cycle 4 selections. Initially all trials were performed at one site (Bubia) and all selections prior to G×E trials were made at this site. However, recently a new strategy has been adopted in which a higher number of clones would be tested at three different agroecological sites. The new strategy has been implemented and 300 superior TLB-resistant clones have been identified using this strategy.

Taro breeding in Samoa

One of the problems contributing to the devastation of taro in Samoa in 1993, when TLB was introduced, was the relative uniformity of the crop. Increasing cultivar diversity and disease resistance on farmers' fields was identified by researchers at the University of the South Pacific (USP) as an important future disease-management strategy. Researchers were concerned that lessons had not been learned and that production might revert to the pre-1993 situation if only one or two improved cultivars were widely distributed and promoted, which had been the case prior to 1999. Discussions between researchers and farmers also revealed that resistant cultivars identified by an earlier project (Samoan Farming Systems Project) had some shortcomings including susceptibility to the disease in wetter parts of the country, low yields and poor storability. Farmers also raised concern about the length of time to get access to resistant germplasm evaluated through the formal screening programme.

Researchers at USP were also apprehensive of the slow rate at which resistant taro was released through formal taro release programmes and the rigorous testing over several years trying to identify a few cultivars that might be of limited relevance to farmers. There is evidence from elsewhere that much of the germplasm officially released through conventional plant breeding programmes is of limited relevance to farmers, and much of the material that is rejected has been found to have subsequent acceptance among farmers (Maurya *et al*. 1988).

The farmer participatory approach to plant breeding, adopted in Samoa, involved researchers, farmers, and Ministry of Agriculture (MAFFM) extension staff and was considered a means to achieve a number of objectives, including: learn more about farmers' criteria; promote farmer participation in the technology development process; provide a range of options to suit diverse environments; ensure that farmers gain quick access to resistant taro; increase the diversity of taro cultivars grown, strengthen institutional linkages and make more effective use of limited resources.

At the start of the programme, farmers in Samoa had access to a moderately resistant cultivar (PSB-G2) which was widely grown (Iosefa and Rogers 1998). They also had limited access to resistant cultivars from the Federated States of Micronesia (Hunter and Pouono 1998) and Palau (Iosefa and Rogers 1998). Evaluation of a Cycle 1 population was also underway by MAFFM researchers, but this involved minimal farmer participation (Fonoti *et al*. 1999).

Taro Improvement Project (TIP)

TIP, a farmer focus group, was initiated at USP in early 1999. The aim was to bring together taro farmers and provide them with more options for improving production and managing TLB. TIP represents a partnership between USP, Ministry of Agriculture Forestry and Fisheries (MAFFM) research and extension staff, and farmers from the islands of Upolu and Savai'i. Membership was open to all farmers who agreed to compare taro cultivars using the Participatory Plant Breeding (PPB) approach and take part in focus group discussions (FGDs) on their performance and other issues. Efforts were made to ensure good geographical coverage of the islands when initially selecting farmers. Men tended to dominate the group, which is a reflection of the gender balance in taro cultivation in the country. Farmers completed an information form on attendance at their first TIP meeting that provided researchers with information on farming systems, farmers' profiles and needs.

Participatory Rural Appraisals (PRAs): Crop-focused PRAs were conducted with farmer groups to learn more about taro production problems, perceptions of taro cultivars and criteria important in the selection of a cultivar. PRA techniques included FGDs, farm visits and observation, key informants, informal interviews and scoring and ranking exercises. The PRAs were conducted by a facilitator based at USP. The PRAs highlighted problems related to the cultivars that were available to farmers at the start of the TIP project (Table 4).

For example, taro variety PSB-G2 was identified as low yielding and having a short shelf-life, making it unsuitable for export. Because Palau cultivars had been imported illegally from nearby American Samoa, farmers experienced difficulty with identification and were unsure about what was being supplied to the market. This contributed to problems with consumer reaction to quality. Some Palau cultivars had poor palatability but were still finding their way to the market. The PRAs also revealed that palatability, TLB resistance and high yield were the most important criteria for farmers when selecting new taro cultivars (Table 5).

TABLE 4. Key problems in taro production perceived by taro farmers and extension officers identified through PRAs in Samoa

Rank	Problem identified
1	Poor quality of taro sold in local market
2	Control of taro leaf blight
3	Inappropriate use of fungicides to control taro leaf blight
4	Short shelf-life of PSB-G2 means it is not suitable for export
5	Shortage of improved taro planting materials
6	Palatability of some Palau cultivars is not acceptable for export
7	Difficulty in identification of different Palau cultivars
8	Expense involved in maintenance of taro plantations
9	Cultivar PSB-G2 is low yielding

TABLE 5. Ranking of important criteria for selection of new taro cultivars perceived by Samoan taro farmers

Rank	Criteria identified
1	Good palatability
2	Resistance to TLB
3	High yielding
4	Tender leaves for *luau* (traditional vegetable dish)
5	Long shelf-life
6	Vigorous growth

Farmer-managed trials: The planned programme of evaluations was described at TIP meetings. Researchers provided farmers with taro cultivars with a range of characteristics and TLB resistance. It was up to each farmer to identify those cultivars that he or she preferred and that were most suitable for their environment. Farmers had the opportunity to visit a demonstration site at USP to observe the cultivars close to harvest. The data accumulated on each cultivar were discussed with farmers. The evaluation process was described and appropriate, simple on-farm trial layouts discussed. Farmers were given up to eight cultivars with 10 planting suckers per cultivar. Trial design was a simple non-replicated layout using single rows of each cultivar with farmers' traditional spacing. The importance of labelling, plot maintenance and a layout plan were stressed, with no use of fungicides. Ongoing management of trial plots was based on normal farmers' practices and the responsibility of farmers. Farmers were advised to establish plots in an area where taro was already growing to ensure exposure of the cultivars under test to TLB. *PSB-G2* was included as the reference cultivar, as it was regarded as the best available cultivar at the time. The first farmer-managed trials were planted in July 1999.

Evaluation of the trials: Monthly TIP meetings held at USP were the main fora for FGDs and other PRA exercises, although a few farmer-to-farmer visits were organized to allow participating farmers to observe other trials for comparison. Simple data sheets for vigour and disease score were explained and distributed to each farmer. The importance of data-collecting was highlighted as well as the requirement for feedback information at monthly FGDs as a learning experience for the group. In FGDs, criteria such as vigour, yield, TLB resistance, suckering ability and palatability were scored using a ranking system based on 1 to 4 (unacceptable to outstanding). Farmers were also requested to notify researchers as cultivars matured so accurate yield data could be collected. All corms and planting material remained the property of farmers. All household members were encouraged to prepare and cook taro corms at home and provide information on quality. Farmers were also requested to bring corms of cultivars to monthly FGDs for assessment of quality in blind taste tests. This allowed accurate evaluations to be carried out on the effect of location and date of maturity on corm quality. A summary of the results of the preliminary trials using this approach to evaluate introduced exotic cultivars is presented in Table 6. PSB-G2 was ranked highest by farmers for palatability scoring; as high as *Niue* would have in the pre-TLB period. Farmers also ranked *Toantal* and P20 high for palatability followed by *Palau 10*. However, PSB-G2 and *Toantal* ranked

relatively poorly for the next two important criteria—yield and resistance to TLB—whereas *Palau 10* scored high for these two criteria. *Palau 20* also scored well for yield. *Palau 10* scored highest for overall plant vigour followed by *Pastora*, *Palau 20* and *Palau 7.* PSB- G2 ranked eighth for this criterion while *Pastora* scored very poorly for palatability.

Researchers made irregular visits to farmer-managed trial sites to collect data on the criteria outlined in Table 7. The data are based on two to three visits to each of 30 farmer-managed trials and blind taste evaluations carried out at Alafua Campus, USP. This allowed some comparison between farmers' and researchers' evaluations. It is interesting to note that there was general agreement on the rankings of the top cultivars in terms of palatability. PSB-G2, *Toantal, Palau 20* and *Palau 10* were the top-scoring cultivars in both evaluations. Among these 4 varieties, farmers ranked *Palau 10* highest for overall vigour, yield and resistance to TLB, which corresponds to the data collected by researchers in Samoa and elsewhere. Both sets of data also demonstrate that for criteria other than palatability, PSB-G2 ranks poorly.

Clone selection: Farmers have been largely involved with evaluation and selection of introduced cultivars using a participatory varietal selection (PVS) approach. More recently, researchers and farmers have selected the best clones from two segregating populations (Cycles 2 and 3) of taro based on the top three criteria highlighted in Table 5. These clones have been extensively tested by farmers for palatability and multiplied for further on-farm evaluation by TIP. Thirty farmers on Upolu are currently carrying out clone evaluations following the approach outlined above. As the programme develops, it is intended that farmers will become more involved in earlier stages of the breeding programme and participate in the wider selection of clones, even screening their own seedling populations. The participatory approach used in Samoa is outlined in Figure 2.

Taro diversity fairs

Regular taro diversity fairs, under the auspices of TIP, have been held at the University of the South Pacific (USP) in Samoa. The general public as well as children from local schools have attended these fairs. Fairs provide an opportunity to highlight the diversity of resistant cultivars and clones now available in Samoa. Information is provided on resistant taros and participants have the opportunity to observe displays of cultivars and clones. Hands-on demonstrations also are given on how to make crosses between cultivars as well as displays of taro seeds and seedlings. Information on leaf blight also is disseminated and the need to promote diversity as a disease-management approach highlighted. Fairs provided an opportunity for wider community assessment of taro cultivars and clones. Those attending these fairs tasted the various taros on display and their opinions were recorded. This feedback was an important component of the programme. It also strengthened the gender component of the evaluation since many of those who attended were women.

TABLE 6. Summary of farmers' ranking of exotic taro cultivars introduced to Samoa

Cultivar	Vigour	Yield	TLB resistance	Sucker production	Palatability
PSB-G2	3.1†	2.4	2.0	3.4	4.0
Pastora	3.8	3.3	2.9	3.2	1.6
Pwetepwet	3.4	2.9	2.7	3.8	2.2
Toantal	3.3	2.3	1.7	2.7	3.5
Palau 3	3.3	3.0	2.6	3.1	2.9
Palau 4	3.1	2.1	2.6	3.9	3.1
Palau 7	3.5	3.0	2.8	2.8	2.4
Palau 10	3.9	3.8	3.5	3.2	3.2
Palau 20	3.7	3.5	2.6	2.9	3.6
Niue (post-1993)‡	1.9	2.0	1.1	1.9	1.9
Niue (pre-1993) ‡	3.9	3.9	—	3.1	4.0

†Ranking for all criteria are based on 1 = unacceptable; 2 = okay, but not good; 3 = good; 4 = outstanding.
‡Farmers were asked to rank *Niue*, the preferred cultivar of Samoans, for the criteria highlighted before and after the arrival of TLB in the country.

TABLE 7. Summary of data collected by researchers from farmer-managed trials and palatability evaluations carried out at Alafua Campus, USP

Cultivar	Vigour†	Yield‡	TLB severity§	Sucker production¶	Palatability††
PSB-G2	4.2	0.6	9.7	4.0	3.1
Pastora	4.4	0.7	6.0	3.0	1.6
Pwetepwet	4.9	1.0	5.3	3.0	2.2
Toantal	4.5	0.7	9.0	3.0	2.8
Palau 3	5.2	1.1	3.6	4.0	2.3
Palau 4	4.8	0.6	5.8	4.0	2.3
Palau 7	4.3	-	5.0	-	2.2
Palau 10	5.2	1.0	3.4	4.0	2.3
Palau 20	4.2	1.0	6.0	3.0	2.6

†Average number of leaves per plant. ‡Average corm weight (kg) per plant. §Average % TLB per leaf.
¶Average number of suckers per plant. ††Average of 20 blind taste tests by teams of 10–15 tasters. Ratings are 1=unacceptable, 2=okay, 3=good and 4=outstanding.

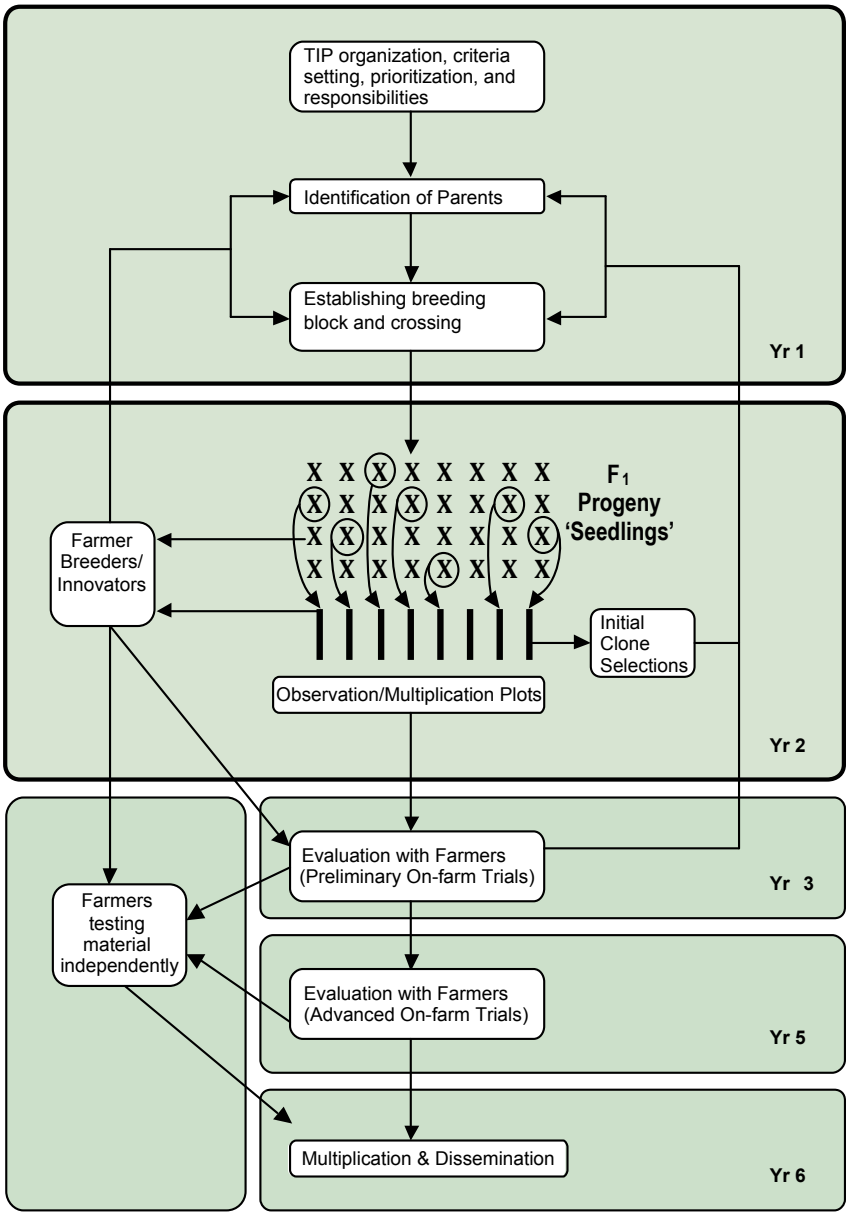

Figure 2. TIP Participatory Plant Breeding Scheme in Samoa (adapted from Fukuda and Saad 2001).

University Taro Breeders Club

A university taro breeders club was initiated at USP in 1999. The club was an innovative approach to teaching and learning, and was considered a cheap and easy approach to breeding. Robinson (1996, 1997) proposed university breeding clubs as a hands-on approach for students to learn about breeding for horizontal resistance and a way of scaling-up farmers' PPB. The overall aim of the club was to develop high-yielding, good-quality taro cultivars with high levels of horizontal resistance to TLB and other locally important taro pests, and that were adapted to a range of diverse environments. At the same time, the club allowed students to learn about the breeding process in a practical way. The club was seen as an integral component of TIP, using selected farmers for evaluation of clones and multiplication of potential new cultivars. The club had a formal structure with elected officers. A club constitution was drawn up and it was run along the lines of a student organization. Most members were students but some were professionals, such as lecturers, crop researchers, technicians and university administrators, while a small percentage were farmers.

Discussion

Papua New Guinea breeding programme

Papua New Guinea (PNG) is one of the few countries in the Pacific region where taro varieties have been successfully bred for superior yield, TLB resistance and good eating quality. The superior traits associated with these lines should enable successful acceptance and adoption by smallholders, subsistence and semi-commercial growers. The yield of these lines should be sustainable over time, since these varieties are derived from genetic improvement and are widely adaptable. The TLB resistance will be durable since it is based on horizontal resistance relying on additive effects of multiple genes against the pathogen. Important and likely positive impacts as a result of the release of these lines in PNG are:

1. The lines will provide growers with improved taro varieties to overcome limiting factors to production, especially TLB. The greatest benefits will accrue to rural people wishing to improve taro production for subsistence purposes in order to maintain a traditional food staple with cultural significance. There also will be benefits to farmers who previously grew taro for domestic markets, but in recent years have had to abandon their production because of TLB. There is also potential for export of these lines.
2. The recommended lines also will have a beneficial impact on women. Increased yields associated with these lines will provide better nutrition for women. Commercial growing, if expanded by use of these varieties, will benefit women through increased cash income from market sale. The improved lines will reduce the amount of labour, much of it women's labour, required to produce a given yield.
3. The recommended lines are resistant to TLB disease. This has potential to reduce the use of pesticides, which is both environmentally and economically beneficial.
4. It is also likely that other Pacific countries will benefit from these lines. There are no major risks associated with release of the three selected lines. There is a possible risk of genetic erosion of existing taro cultivars as farmers replace them with the new TLB-resistant lines. However, this process is likely to be slow, since farmers prefer to grow a suite of 8 to 12 cultivars with varying attributes, and have strong attachment to some of their traditional cultivars because of their cultural significance. In addition, NARI is focusing on taro germplasm conservation.

The conservation of taro genetic resources will complement crop improvement and breeding activities and will mitigate this particular risk.

In future, more lines are expected to be released from post-Cycle 2 recurrent cycles. It is likely that those lines released from advanced cycles will be more superior in their attributes, especially eating quality because of the polygenic breeding approach (accumulation of superior genes from cycle to cycle) adopted by the PNG programme.

Samoa breeding programme

The availability of resistant cultivars in Samoa has made a significant impact and allowed farmers to return to serious taro production. Prior to the outbreak of TLB in 1993, the 1989 Agricultural Census reported the area planted under taro as 14 783 ha. It is estimated that the disease caused a drastic reduction in the availability of the crop by destroying 95% of taro plantations on the islands of Upolu and Savai'i (Brunt *et al*. 2001). However, the 1999 census reported that the area under taro had recovered to an encouraging 4253 ha and that 55% of households were using improved cultivars of taro. The figure is the same for both Upolu and Savai'i. Prior to 1993 about 93% of households were growing taro; it is equally encouraging to note that the 1999 census reports that 70% of households were growing the crop again. No doubt the area planted and the number of households growing improved varieties has increased substantially since the 1999 census. Overall, 49% of the population surveyed in the 1999 census were consuming taro. These quantitative indicators illustrate that taro production had started to recover substantially in Samoa before 1999.

Unfortunately, the 1999 census does not provide information on the proportion of different cultivars grown. An estimation of this can be made from an impact survey that was completed at the same time as the 1999 census (Iosefa and Rogers 1998). This survey reported that PSB-G2 comprised 83%, FSM cultivars 16% and Palau cultivars less than 1% of the total recorded. This demonstrates that PSB-G2 was very popular at this time despite the shortcomings highlighted in this study. The dynamics of cultivar turnover in Samoa are interesting and deserve more study. Hunter (2002) indicated that the cultivar Palau 10 replaced PSB-G2 as the most popular cultivar grown by farmers. The information was inferred from visits around the country and to local markets. Although its taste is not considered as good as PSB-G2, its larger and rounder corm ensures more demand in the market. However, at this stage it is difficult to estimate to what extent the Taro Improvement Project (TIP) has contributed to the popularization of *Palau 10*. It must be stressed that farmers had access to this cultivar before commencement of the programme from unknown sources and that MAFFM had started to recommend it as well. If one claim can be made on behalf of TIP regarding this cultivar it is that the programme worked closely with farmers to systematically evaluate Palau cultivars. The collaboration resulted in a considerable body of knowledge regarding all Palau cultivars and was able to address some confusion regarding identification of good Palau cultivars. Although *Palau 10* is now the dominant cultivar grown in Samoa, field visits to farmers involved in TIP show that farmers continue to grow small numbers of the other cultivars that were evaluated such as *Pwetepwet*, *Toantal*, *Pastora* and *Palau 20*. The reasons usually given are suitability to their location, they like the taste or they can vary the harvest time to influence quality. Unfortunately, at this stage there are no quantitative data on the diversity of cultivars maintained by the farmers involved in TIP although a follow-up to the previous impact survey (Iosefa and Rogers 1998) is planned. A comparison of the cultivars maintained by farmers involved with TIP with that of the wider farmer population would be interesting. This would confirm if farmers presented with a

range of cultivars are likely to adopt different types, or if market forces determine that farmers adopt only the most acceptable variety.

There is a need for continued assessment of the impact of the participatory plant breeding in Samoa, as outlined above. It is also worthwhile to note that farmers are now involved in the evaluation of a greater range of clonal material from segregating populations and this germplasm represents an improvement in terms of quality and disease resistance. We assume that farmers will continue to experiment with new germplasm as there is no current variety capable of fulfilling the requirements of the export market and there is a considerable expectation within the country that Samoa will recapture this market. In such a situation, continued breeding will be essential. TIP has certainly been successful in achieving most of its other objectives. Researchers now know considerably more about farmers' needs and criteria when selecting germplasm, including performance in diverse environments. Farmers are getting access to germplasm quickly, usually within 2 years of initial selection of clones from a segregating population. TIP has contributed significantly to strengthening of linkages between researchers, MAFFM extension and farmers. This is manifested in the improved relationship between farmers and extension officers involved in the programme. Involvement in the technology-development process has contributed to a growing confidence among extension officers as they put this new body of knowledge to use with other farmers not involved in TIP.

It was felt that the university breeders club offered an opportunity to scale up the participatory breeding approach (Hunter *et al.* 2001). This has not been the case and its popularity has declined over the years since its inception. A range of factors has contributed to this outcome. In the Mexican example highlighted by Robinson (1997), students came from surrounding villages, where they could return with the progeny of the crosses they made and carry out participatory selection with farmers on family farms. Certain selections could become potential cultivars but also could be fed back into the breeding club system to become future parents. Unfortunately, the majority of student members of the USP breeders club came from countries other than Samoa and quarantine and unresolved ownership issues precluded taro germplasm leaving Samoa for evaluation on family farms. The solution to this problem was to pool all crosses together and evaluate seedlings as one population through the TIP programme. This may have detracted from a sense of ownership of activities and germplasm by club members.

It also was felt that the club offered an innovative hands-on approach to teaching and learning. Although expectations and membership were high in the first year, both eventually declined. The original idea for the club came from researchers or staff at USP, based on experiences elsewhere (Robinson 1996, 1997), with the result that it was driven mostly by staff. It was assumed that students would gradually have more input and take on ownership of the club after the first few years and that USP might adopt the club approach as part of its teaching curriculum and allow students to gain credit points for club activities. This never became reality and low motivation and high demands on student time from other areas, high staff and student turnover, a small student body to draw membership from can all be identified as contributing factors. Poor institutional support from USP and a general lack of interest in participatory and innovative approaches to crop improvement as part of the curriculum did not help matters.

The breeders club can be considered an interesting experiment with a novel approach to participatory plant breeding and one that may bear more fruit in a context where the environment is

more conducive to students linking to local villages and farmers. Progress of TIP has in no way been affected by the performance of the breeders club; indeed, it has grown considerably in recent years and farmer enthusiasm remains high. Taro diversity fairs have also complemented the programme and created wider awareness and contributed to motivating farmers to organize village and district-level taro open days and competitions.

Both PNG and Samoan breeding programmes could be used as the vehicle for a breeding network to control TLB disease in the Pacific region. Taro cultivars identified in Samoa and PNG, although successful locally, may not suit individual countries with a wide diversity of environments and cultures, and therefore regional G×E evaluations are recommended. In addition, innovative ways to increase the rate of multiplication of planting material, safe germplasm exchange, capacity to meet the needs for larger number and isolated farmers will be required for enhanced distribution of new and improved germplasm. Greater decentralization of breeding and effective networking between countries and within a country could help overcome such problems. There is considerable interest in many countries in scaling-up the PPB approach. While not all countries will have the resources to support breeding programmes, the necessary variability could be generated in selected countries for decentralized evaluation through exchange of true seed, which may create fewer quarantine problems. Farmers can evaluate segregating populations and make selections suited to their needs. Recent DNA fingerprinting of taro germplasm from Asia and the Pacific has confirmed the low level of genetic diversity within the Pacific countries and the existence of two distinct regional taro genepools. Future taro improvement programmes will require crosses between these Asian and Pacific groups to create genetically diverse progeny and further broaden the genetic base of the crop.

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Development of a regional core collection (Oceania) for taro, *Colocasia esculenta* (L.) Schott, based on molecular and phenotypic characterization

E.S. Mace, P.N. Mathur, I.D. Godwin, D. Hunter, M.B. Taylor, D. Singh, I.H. DeLacy and G.V.H. Jackson

Introduction

Taro, *Colocasia esculenta* (L.) Schott, is one of the most important staple foods of Pacific Island countries, where it plays an important role both as a root crop and a leafy vegetable. In 2000 it was the fifth most consumed root vegetable worldwide (FAOSTAT 2010) with over 25% produced in Oceania and Southeast Asia. The importance of the crop goes beyond its contribution to nutrition and income. In many Pacific Island countries, taro plays an important cultural role as it forms an integral part of customs and traditions. Taro, one of the oldest crops in this region, spread eastward into the Pacific, probably reaching the Polynesian islands 2000 years ago. There is now evidence to suggest that most cultivars found throughout the Pacific were not brought by the first settlers from the Indo-Malayan region as previously suggested (Plucknett *et al*. 1970; Léon 1977; Kuruvilla and Singh 1981), but were domesticated from wild sources existing in Melanesia (Matthews 1990, 1991, 1995; Yen 1991a, 1991b, 1993; Lebot 1992). From there, cultivars were taken eastward to Polynesia during prehistoric migrations, with a progressive decline in their number and diversity (Yen and Wheeler 1968; Lebot 1992; Yen 1993).

Two principal botanical varieties of taro are recognized: *C. esculenta* var. *esculenta*, commonly known as dasheen, and *C. esculenta* var. *antiquorum*, commonly known as eddoe. Dasheen varieties have large central corms, with suckers and/or stolons, whereas eddoes have a relatively small central corm and a large number of smaller cormels (Purseglove 1972). The genetic diversity of the crop has been characterized to date largely by morphological and cytological variation (Yen and Wheeler 1968; Kuruvilla and Singh 1981; Tanimoto and Matsumoto 1986; Coates *et al*. 1988), and it has been observed that Polynesian cultivars are highly morphologically variable in contrast to the phenotypic homogeneity of the wild populations of Melanesia. It is thought that the high level of phenotypic variation is due to a high rate of vegetative propagation and, consequently, of somatic mutations. This would suggest that the majority of the cultivars in Polynesia are clones of a common source, and a study using isozymes (Lebot and Aradhya 1991) indicated that there was very little genetic variation between the Polynesian cultivars, in contrast to the Melanesian and Asian cultivars. The results from a molecular study of taro genetic diversity, using RAPDs (Irwin *et al*. 1998) confirmed that although the cultivars in the Pacific region exhibit remarkable morphological variation, the genetic base appears to be very narrow. Such a limited genetic base leaves the crop very vulnerable to disease epidemics and insect damage and provides a limited opportunity for genetic improvement of important traits by recombination. Consequently, germplasm collecting from around the region has been undertaken to augment existing national collections and to safeguard threatened and useful germplasm for use in regional breeding programmes.

Figure 1. Map of the Pacific Island region, with countries involved in the TaroGen Network highlighted. Map modified from National Geographic website [www.nationalgeographic.com/resources/ngo/education/xpeditions/atlas/].

Some 1506 accessions are currently recognized in the Pacific and were collected by *TaroGen* (Taro Genetic Resources: Conservation and Utilisation) (SPC 2001), a regional project funded by the Australian Agency for International Development, which aims to establish a core collection maintained *in vitro* at the Regional Germplasm Centre, Secretariat of the Pacific Community, Fiji, representative of the genetic diversity within Pacific Island countries. The proposed concept of a regional taro core collection was not intended to replace the entire collections of national programmes in the Pacific, but rather to promote use and exchange among the network partners and for duplication in the cases of disease outbreaks and lack of resources with the national programmes to maintain such large collections as field genebanks. The development of regional core collections requires accessions to be well characterized in order to minimize genotypic redundancy and to identify gaps in the collections and hence maximize the genetic diversity conserved. To date, studies directed at the identification of redundant germplasm in the Pacific Island national collections have utilized biogeographic, agronomic and phenotypic characterization. However, although such data have been used traditionally for the construction of core collections (Ford-Lloyd 2001), using this information to make meaningful comparisons between taro collections grown in different Pacific Island countries has proved to be difficult (Jackson and Firman 1987), and less subjective methods are required. Increasingly, the characterization of germplasm collections also utilizes molecular techniques, e.g. Hokanson *et al*. 1998; Teulat *et al*. 2000; van Treuren *et al*. 2001. We report here the development of a regional core collection for Pacific taro based on the use of molecular markers, combined with phenotypic characterization.

Microsatellite markers have been isolated from taro (Mace and Godwin 2002) and a set of polymorphic markers identified through screening with a limited range of genotypes from the Pacific Island region. Here, we report on the use of seven of the polymorphic microsatellite markers to evaluate genetic diversity and subsequently rationalize nine national collections from the Pacific Islands: Papua New Guinea (PNG), Vanuatu, New Caledonia, Fiji, Palau, Niue, Tonga, Samoa and the Cook Islands (Figure 1).

Materials and methods

Phenotypic data collection and analysis

A total of 1506 accessions from nine Pacific countries were used in this study (Table 1). Of these, only accessions from PNG (816 accessions), Vanuatu (452 accessions) and New Caledonia (81 accessions) were characterized systematically for several morphological traits. Accordingly, these accessions from PNG, Vanuatu and New Caledonia were analyzed for initial grouping using morphological traits to identify a core subset based on 20% of the total accessions. These 20% accessions were genotyped with the SSR markers to arrive at a final core of 10% for these three countries, as discussed below (and outlined in Figure 2).

TABLE 1. Size of national taro germplasm collections in nine Pacific Island countries, as established through the TaroGen Network, together with details of number of accessions DNA fingerprinted per collection

Country	Collection size	DNA Fingerprinting subset
Papua New Guinea	816	163
Vanuatu	452	89
New Caledonia	81	18
Fiji	72	71
Palau	16	11
Niue	24	24
Tonga	12	12
Cook Islands	18	15
Samoa	15	13

Methodology used for PNG taro core subsets

The characterization data recorded for PNG taro accessions included: plant height, number of stolons, number of suckers, predominant position (shape) of leaf lamina surface, leaf blade colour, leaf blade colour variegation, type of leaf blade variegation, colour of leaf blade variegation, leaf length, leaf width, leaf lamina length/width ratio, petiole junction pattern, petiole junction colour, leaf main vein colour, leaf vein pattern, petiole colour (upper half), petiole colour (lower half), petiole stripe, petiole stripe colour, petiole basal-ring colour, flower formation, leaf blight resistance, screening for

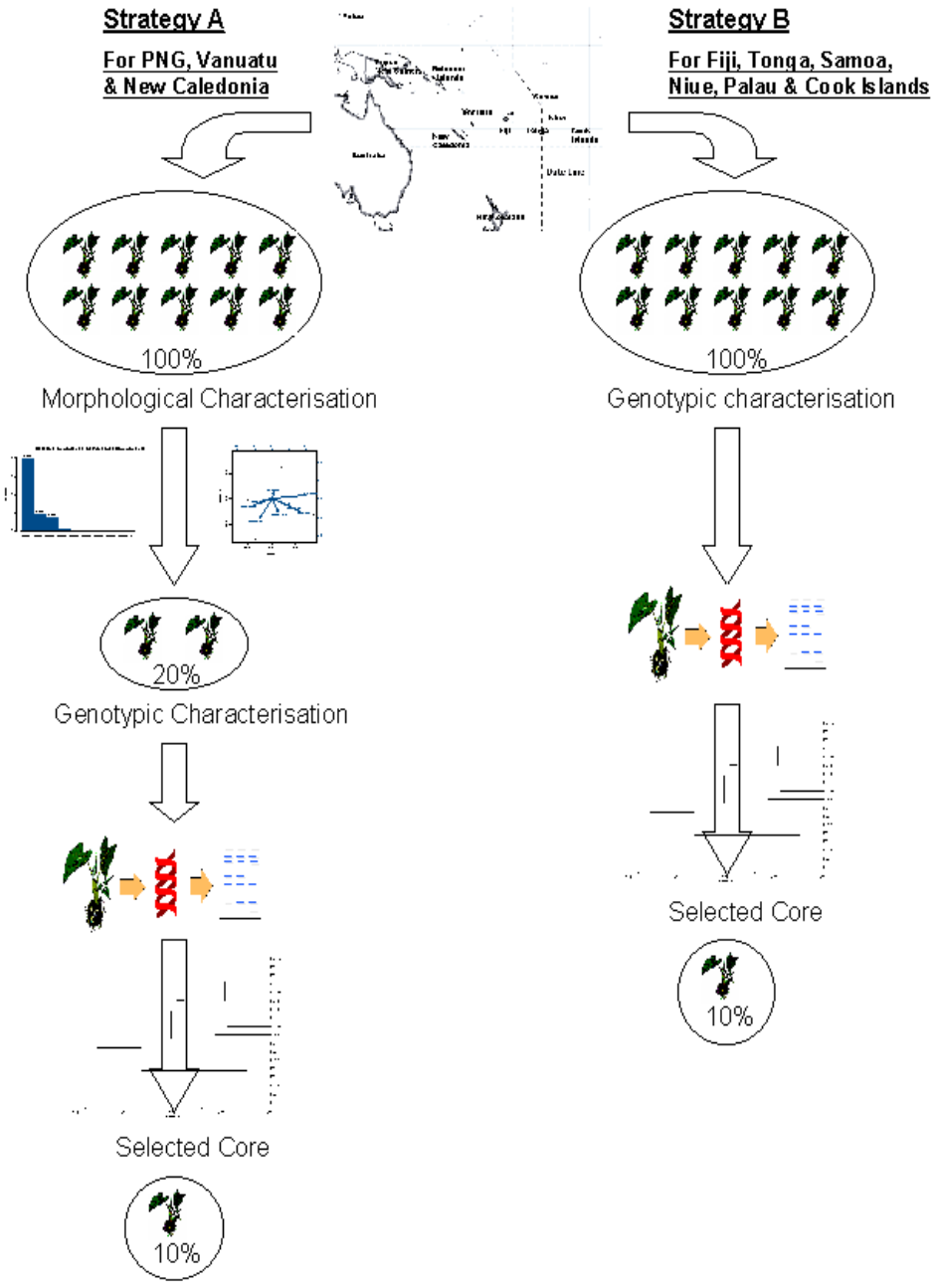

Figure 2. Strategies undertaken to select accessions for inclusion in regional core collection.

Alomae-Bobone virus complex (ABVC), corm length, corm breadth, corm shape, corm weight, corm cortex colour, corm flesh colour, corm fibre colour, number of cormels and weight of cormels.

From these morphological data, it was observed that some of the qualitative characters such as corm flesh colour, presence of stolon and leaf blade colour variegation displayed significant differences within the taro collection and, therefore, the initial grouping of these accessions was based on these three morphological traits. For groups with over 40 entries, further subgrouping was required. This was achieved by using the characterization data for cluster analysis using Ward's method based on a Euclidean distance matrix. The final number of subgroups was based on the observed clusters in the dendrogram. Final selection of the core accessions to be genotyped was based on the Principal Component Analysis (PCA) for each cluster, using the principal component scores for each accession within the cluster.

Methodology used for Vanuatu taro core subsets

For Vanuatu collections, data were available for 21 descriptors: growing conditions, altitude of growing sites, botanical variety, formation of stolon, corm shape, maturity period, growth habit, plant height, corm flesh colour, corm weight, shape of leaf lamina, position of leaf lamina, leaf lamina margin, lamina colour, lamina variegation, sinus outline, vein junction colour, basic colour of petiole, petiole variegation, taste quality of corms and dry matter yield. All these descriptors were recorded on a qualitative scale of 1 to 8, except for dry matter yield. The initial grouping was based on morphological descriptors using meaningful characters, i.e. formation of stolon (present and absent), growing conditions (flooded and rain-fed) and maturity period (early: 4–8 months and late: 8–11 months). However, for groups with over 100 entries, further division into subgroups was undertaken using cluster analysis based on Ward's method and the Euclidean distance matrix. The various descriptors used for the cluster analysis included: corm shape, growth habit, plant height, corm flash colour, corm weight, position of leaf lamina, leaf lamina margin, sinus outline, vein junction colour, basic colour of petiole, petiole variegation, taste quality of corms and dry matter yield. The final selection of the core accessions to be genotyped was based on PCA for each cluster using the principal component scores for each collection within the cluster.

Methodology used for New Caledonia taro core subsets

Data were received for 81 taro accessions collected from New Caledonia for 22 descriptors: growing type, growing conditions, botanical variety, growth habit, presence of stolon, plant height, leaf shape, lamina orientation, lamina margin, lamina colour, lamina variegation, sinus outline, vein junction, petiole colour, petiole variegation, flowering, taro leaf blight incidence, maturity, corm shape, corm weight, corm flesh colour and eating quality. However initial stratification was not possible, as for the PNG and Vanuatu data sets, since there was no variation with regard to characters such as growing type, growing conditions, botanical variety, growth habit and presence of stolon. Therefore, cluster analysis was performed using different clustering methodologies, and clusters identified based on the dendrogram patterns. Each cluster was then analyzed for PCA scores to identify the most diverse accessions within each cluster, which were then selected for genotyping.

For all the statistical analyses, SYSTAT programme was used for distance matrix construction using the Euclidean distance and clustering analysis using Ward's method of clustering and S-Plus 2000 software was used for PCA.

Plant material and DNA extraction

The national taro collections included in this study comprised, in total, 1506 accessions (see Table 1). From this entire collection, 416 accessions were genotyped (28% overall). All accessions within the national collections of Polynesian countries, and those of Fiji and Palau were analyzed. Small discrepancies between the numbers of accessions within these collections and those analyzed were due to samples being destroyed in transit to the University of Queensland or because plants were too small for analysis at the time of sampling. Prior to genotypic analysis, accessions in the larger national collections of New Caledonia, Papua New Guinea and Vanuatu were characterized using passport and morphological descriptors (see above) to select the most diverse 20% overall (Figure 2). These were further analyzed using molecular markers.

From each of the 416 accessions, 50 mg of leaf material was collected and immediately frozen in liquid nitrogen. DNA was extracted using DNeasy® 96 Plant Kit (QIAGEN). DNA was eluted in 2× 50 L sterile distilled water and stored at 4 C. DNA concentration was measured both on a fluorometer (Hoefer TKP 100) following the manufacturer's instructions, and by agarose gel (0.8%) electrophoresis.

SSR-PCR and electrophoresis

Seven SSR primers were selected for use (Table 2), based on preliminary assays of amplification and product length polymorphism in taro genotypes (Mace and Godwin 2002). PCR amplification was performed in 0.2 ml 96-well plates (ABgene, Thermo-Fast ®LP) using an MJ-Research PTC-100™. The PCR reaction mixture (10 L) contained 10 ng template DNA, 0.2 M forward primer, 0.2 M reverse primer, 0.1mM of each dNTP, 2.5mM $MgCl_2$, 0.5 unit *Taq* DNA polymerase (Promega) and 1 Ci [α-33P]dATP. The PCR regime consisted of an initial denaturation (94 C for 5 min), 35 cycles each consisting of 30 sec denaturation (94 C), 1 min annealing (ranging from 62 to 66 C, see Table 2) and 2 min elongation (72 C). Finally, an extension period of 10 min at 72 C was included. For detection via electrophoresis, the amplification products were first mixed with 5 L stop solution (98% formamide, 10mM EDTA, 0.05% (w/v) xylene cyanol, 0.05% bromophenol blue) and denatured at 94 C for 3 min. A 2 L aliquot was loaded onto denaturing polyacrylamide gels (10% acrylamide/bisacrylamide 19:1) and electrophoresed at 100W constant power for 2 h 10 min. The gels were transferred to 3 mm paper and dried in a Bio-Rad Gel Drier, model 583, at 80 C for 1 h. Once dried, the gel was exposed to Kodak Biomax MR film for approximately 20 h.

Molecular data analysis

Banding patterns observed at a particular locus were recorded as a presence/absence matrix. Similarity matrices were calculated from these data based on different measures: Nei and Li (1979), Jaccard's coefficient (Jaccard 1908) and the simple matching (SM) coefficient (Sokal and Michener 1958). Cluster analyses were performed on the similarity matrices using the unweighted pair group method with arithmetic averages (UPGMA) and dendrograms constructed from these analyses. Cophenetic correlation values were calculated to evaluate the robustness of the resulting tree topologies. All analyses were conducted using the NTSYS-pc software, version 2.02i (Rohlf 1999).

An analysis of molecular variance (AMOVA) (Excoffier *et al*. 1992) was used to partition genetic variability between and within country collections using Arlequin software version 2.0 (Schneider *et al*. 2000), and significance values assigned to variance components based on the random permutation (10 000 times) of individuals assuming no genetic structure.

TABLE 2. List of SSR primers used; repeat motif, oligonucleotide primer sequences, PCR annealing temperatures, expected PCR product size and number of alleles

SSR ID	SSR	Primer sequence (5' – 3')	Anneal. temp (C)	Allele size range	No. alleles
uq84-207	(CT)18	Fwd: aggacaaaatagcatcagcac Rvs: cccattggagagatagagagac	65.0	197-217	7
uq110-283	(TGA)6 (TGGA)4	Fwd: agccacgacactcaactatc Rvs: gcccagtatatcttgcatctcc	66.0	250-287	8
uq73-164	(CT)15	Fwd: atgccaatggaggatggcag Rvs: cgtctagcttaggacaacatgc	66.0	146-164	6
uq55-112	(CAC)5	Fwd: cttttgtgacatttgtggagc Rvs: caataatggtggtggaagtgg	65.0	112-136	3
uq88B-94	(CAT)9	Fwd: cacacatacccacatacacg Rvs: ccaggctctaatgatgatgatg	62.0	94-108	6
uq97-256	(CA)8	Fwd: gtaatctattcaacccccttc Rvs: tcaaccttctccatcagtcc	66.0	248-256	4
uq91-262	(TG)6(GA)4	Fwd: gtccagtgtagagaaaaaccag Rvs: cacaaccaaacatacggaaac	65.0	258-262	2

Results

Molecular data analyses

Thirty-six alleles were amplified from the seven SSR loci across the 416 taro genotypes included in this study. Of these, three (8%) alleles were found to be monomorphic, and 33 (92%) were found to be polymorphic. An example of SSR polymorphism is shown in Figure 3. Table 2 lists the number of alleles and the allele size ranges. An average of 5.1 alleles per locus was observed. The monomorphic SSR locus uq91-262 was excluded from the analysis. Figure 4 shows the percentage of unique DNA fingerprints obtained, across all SSR loci included in this study, for the taro germplasm collections from the nine Pacific Island countries. The taro collection from Fiji contained the lowest percentage of unique fingerprints (58.6%) compared with 92.3% for collections from the Cook Islands and Samoa. The AMOVA (Table 3) showed that the majority of variation detected (86.5%) could be ascribed to differences within country, with only 13.5% of the variation being due to differences between samples from different countries.

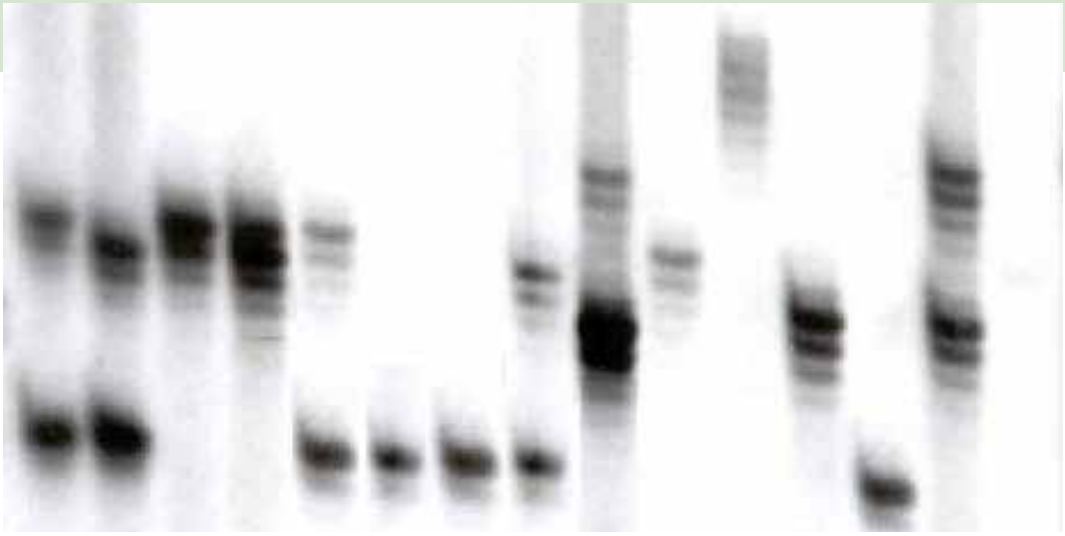

Figure 3. Example of microsatellite polymorphism detected among 14 taro genotype samples using SSR primer uq84-207. Genomic DNA was amplified from the following samples (1) *C. esculenta* var. *esculenta* from Vanuatu; (2) from Hawaii; (3) from Samoa; (4) from Fiji; (5) from Niue; (6) from Tonga; (7) from Cook Islands; (8) from New Caledonia; (9) from Palau; (10) from Papua New Guinea; (11) from Japan; (12) from China; (13) from Vietnam; (14) *C. esculenta* var. *antiquorum*; (15) *Xanthosoma*.

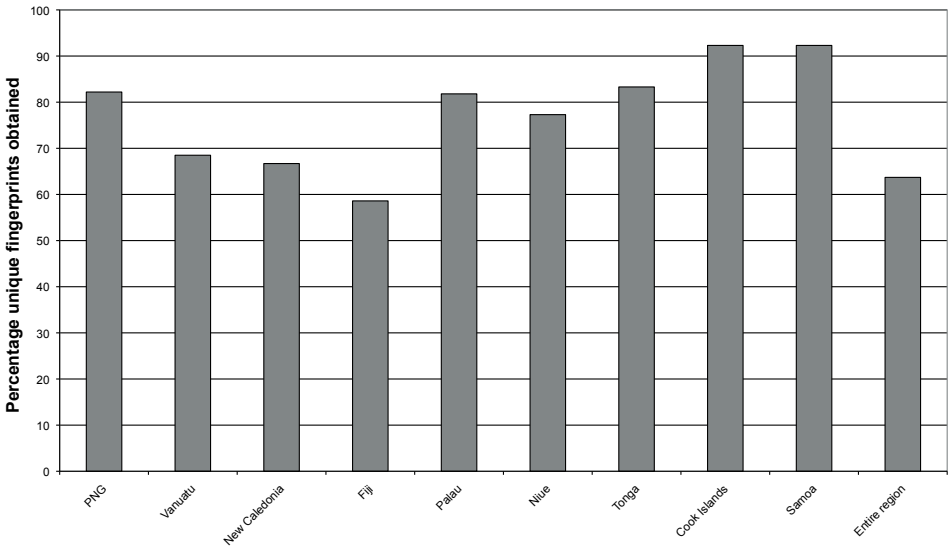

Figure 4. Comparison of percentage of unique fingerprints obtained, across all SSR loci, for the taro germplasm collections from nine Pacific Island countries.

TABLE 3. Analysis of Molecular Variance in Taro SSR Loci.

Source of variation	df	Sum of squares	Variance components	% of total variation	P values
Between countries	8	140.616	0.39382	13.59	<0.001
Within countries	390	976.655	2.50424	86.41	<0.001
Total	398	1117.271	2.89806		

Cluster analyses (UPGMA) were performed using the similarity matrices with the highest correlation coefficient based on the proportion of shared alleles across the 6 SSR loci (Table 4). The cluster analyses were carried out on SSR data sets for individual countries and additionally on a combined data set, across all countries, in order to ensure that between-country duplicates were not included in the final core set. For within-country analyses, it was found that the dendrogram constructed from Jaccard's similarity coefficient together with UPGMA resulted in the highest cophenetic correlation value for all countries except PNG and Tonga. In the latter case, the simple matching similarity coefficient together with UPGMA gave the highest cophenetic correlation value.

TABLE 4. A comparison of the cophenetic correlation values obtained from the three similarity coefficients employed, Simple Matching, Jaccard's and DICE, together with the UPGMA clustering techniques for the taro accessions in each country

	Simple Matching	Jaccard's	DICE
PNG	0.54565	0.53633	0.50982
Vanuatu	0.69993	0.72362	0.70991
New Caledonia	0.91587	0.94225	0.93582
Fiji	0.76251	0.79505	0.77388
Palau	0.84433	0.87146	0.83572
Niue	0.65574	0.68921	0.63764
Cook Islands	0.74601	0.76553	0.75938
Tonga	0.84541	0.81907	0.79421
Samoa	0.79316	0.81021	0.78292

Selection of core based on molecular and phenotypic data analyses

PAPUA NEW GUINEA (PNG)

Twelve major groups were identified based on morphological stratification of the PNG taro collection as follows:

Group 1	Corm flesh colour 1 + Stolon 0 + Leaf blade colour variegation	0 = 256
Group 2	Corm flesh colour 1 + Stolon 0 + Leaf blade colour variegation	1 = 71
Group 3	Corm flesh colour 1 + Stolon 1 + Leaf blade colour variegation	0 = 111
Group 4	Corm flesh colour 1 + Stolon 1 + Leaf blade colour variegation	1 = 22
Group 5	Corm flesh colour 4 + Stolon 0 + Leaf blade colour variegation	0 = 160
Group 6	Corm flesh colour 4 + Stolon 0 + Leaf blade colour variegation	1 = 40
Group 7	Corm flesh colour 4 + Stolon 1 + Leaf blade colour variegation	0 = 45
Group 8	Corm flesh colour 4 + Stolon 1 + Leaf blade colour variegation	1 = 21
Group 9	Corm flesh colour 99 + Stolon 0 + Leaf blade colour variegation	0 = 55
Group 10	Corm flesh colour 99 + Stolon 0 + Leaf blade colour variegation	1 = 18
Group 11	Corm flesh colour 99 + Stolon 1 + Leaf blade colour variegation	0 = 12
Group 12	Corm flesh colour 99 + Stolon 1 + Leaf blade colour variegation	1 = 3
Total		= 814

Where:
Corm flesh colour: 1 = white, 4 = pink and 99 = other colour
Stolon: 0 = absent and 1 = present
Leaf blade colour variegation: 0 = absent and 1 = present

Ward's method of hierarchical clustering, based on a Euclidean distance matrix, was used to further subdivide the larger groups 1, 2, 3, 5, 6, 7 and 9. As a result, a total of 39 clusters were identified and from these 151 accessions were selected for genotypic characterization. The number of accessions identified from each of these 39 clusters is presented in Table 5. The 151 accessions thus identified (20% of the total accessions) were then subjected to genotypic analysis, and from these 151 selected accessions, we aimed to select 50% for inclusion in the suggested final core (Table 6) based on the molecular marker analysis (Figure 2). The dendrogram created based on the genotypic data using the SM coefficient and UPGMA clustering was initially subdivided into 23 separate clusters. From these 23 clusters, 83 accessions in total were selected, based on the level of diversity within each cluster, the morphological groupings of the accessions and the cluster analysis of the entire data set.

TABLE 5. Clusters 1-39 Identified by analysis of phenotypic variation in the Papua New Guinea taro collection.

Cluster no.	Acc. no.	Acc. no identified for core	Cluster no.	Acc. no.	Acc. no identified for core	Cluster no.	Acc. no.	Acc. no identified for core
Cluster 1	33	7	Cluster 14	11	2	Cluster 27	4	1
Cluster 2	14	3	Cluster 15	41	8	Cluster 28	21	4
Cluster 3	23	5	Cluster 16	28	6	Cluster 29	37	8
Cluster 4	22	4	Cluster 17	13	3	Cluster 30	3	1
Cluster 5	18	4	Cluster 18	7	2	Cluster 31	39	8
Cluster 6	51	10	Cluster 19	18	4	Cluster 32	6	1
Cluster 7	29	6	Cluster 20	4	1	Cluster 33	24	5
Cluster 8	31	6	Cluster 21	35	7	Cluster 34	31	6
Cluster 9	22	4	Cluster 22	29	6	Cluster 35	22	4
Cluster 10	13	2	Cluster 23	18	3	Cluster 36	21	4
Cluster 11	29	6	Cluster 24	19	4	Cluster 37	18	4
Cluster 12	18	3	Cluster 25	28	6	Cluster 38	12	2
Cluster 13	13	2	Cluster 26	6	1	Cluster 39	3	1

Note: For each cluster, we list the number of accessions present (Acc. no.), and the number of accessions kept in the core taro collection(Access. no. identified for core) for further genotypic analysis.

TABLE 6. Summary of data sets of taro collections for nine Pacific Island countries

Country	Total size of collection	No. selected based on morphological analysis	No. recvd at UQ	No. to select for core	Actual no. selected for core
PNG	816	163 (20%)	163	81 (10%)	83
Vanuatu	452	93 (20%)	89	45 (10%)	44
New Caledonia	81	19 (20%)	18	8 (10%)	8
Fiji	72	—	71	7 (10%)	8
Palau	16	—	11	2 (10%)	5
Niue	24	—	24	3 (10%)	6
Tonga	9	—	12	1 (10%)	3
Cook Islands	18	—	15	2 (10%)	3
Samoa	15	—	13	2 (10%)	4
Total	1503		416	151	164 (10.9%)

VANUATU

The Vanuatu collection was first stratified using meaningful characters: formation of stolon (present and absent), growing conditions (flooded and rain-fed), indicating adaptation, and maturity period (early: 4–8 months and late: 8–11 months). The details for this grouping are given below:

1. Presence (1) or absence (2) of stolon formation:	
1	= 99 collections
2	= 353 collections
Total	= 452 collections
2. This was further subdivided based on growing conditions recorded as 1 (flooded: 69 records) and 2 (rain-fed: 383 records). The details for these subgroups are as follows:	
Stolon (1) + growing conditions (1)	= 17
Stolon (1) + growing conditions (2)	= 82
Total	= 99
Stolon (2) + growing conditions (1)	= 52
Stolon (2) + growing condition (2)	= 301
Total	= 353
GT	= 452

Further subgrouping was done based on maturity period recorded as 1 (early types, 4–8 months, 254 records) and 2 (late types, 8–11 months, 198 records). The final subclassification based on these three descriptors has resulted into eight groups as indicated below:

1. Group 1	= Stolon 1 + growing condition 1 + maturity	1	= 9
2. Group 2	= Stolon 1 + growing condition 1 + maturity	2	= 8
3. Group 3	= Stolon 1 + growing condition 2 + maturity	1	= 53
4. Group 4	= Stolon 1 + growing condition 2 + maturity	2	= 29
5. Group 5	= Stolon 2 + growing condition 1 + maturity	1	= 25
6. Group 6	= Stolon 2 + growing condition 1 + maturity	2	= 27
7. Group 7	= Stolon 2 + growing condition 2 + maturity	1	= 167
8. Group 8	= Stolon 2 + growing condition 2 + maturity	2	= 134
Total			= 452

Since group numbers 3 (53 accessions), 7 (167 accessions) and 8 (134 accessions) were large, further classification was undertaken using Ward's method of hierarchical clustering based on the Euclidean Distance. From the resulting dendrogram, a total of 22 clusters were identified and the final selection of the core accessions to be genotyped was based on the Principal Component Analysis (PCA) for each of these 22 clusters using the principal component scores for each collection within the cluster. Table 7 shows the number of collections belonging to each of the 22 clusters.

TABLE 7. Clusters 1-22 identified by analysis of phenotypic variation in the Vanuatu taro collection.

Cluster no.	Acc. no.	Acc. no identified for core	Cluster no.	Acc. no.	Acc. no identified for core	Cluster no.	Acc. no.	Acc. no identified for core
1	9	2	9	25	4	17	44	9
2	8	2	10	20	4	18	39	8
3	14	3	11	16	3	19	24	5
4	11	2	12	14	3	20	11	2
5	28	6	13	24	5	21	14	3
6	29	6	14	27	6	22	2	1
7	25	5	15	34	8			
8	27	6	16	7	1			

Note: For each cluster, we list the number of accessions present (Acc. no.) and the number of accessions kept in the core taro collection (Acc. no. identified for core) for further genotypic analysis.

In total, 89 accessions (20% of total accessions) were selected for further genotypic analysis, and from these 89 selected accessions, we aimed to select 50% for inclusion in the suggested final core (Table 6) based on the molecular marker analysis (Figure 2). The dendrogram created based on the genotypic data using Jaccard's coefficient and UPGMA clustering was initially subdivided into 21 separate clusters. From these 21 clusters, 45 accessions were selected following the same procedure as detailed for PNG.

NEW CALEDONIA

Initial stratification based on characters such as growing type, growing conditions, botanical variety, growth habit and presence of stolon was not possible owing to the lack of variation observed for these characters. Therefore, Ward's clustering method using Euclidean distance was used for classifying the New Caledonia taro collection and, based on the dendrogram pattern, the entire collection was divided into 10 clusters. Each of these clusters was than analyzed for PCA scores to identify the most diverse accessions within each cluster for genotypic analyses, and from this a total of 19 accessions were selected for genotyping. Table 8 details the number of accessions belonging to each of the 10 clusters. From these 19 selected accessions, we aimed to select 50% for inclusion in the suggested final core (Table 6) based on molecular marker analysis (Figure 2). The dendrogram created using Jaccard's coefficient and UPGMA clustering was initially subdivided into 8 separate

clusters. From these 8 clusters, 8 accessions were selected, one from each cluster, following the same procedure as detailed for PNG.

For the remaining six countries (Fiji, Palau, Niue, Tonga, Cook Islands, Samoa), the selection of the accessions for the core collection was based on genotypic data analysis only (Figure 2). For each country, 10% of the total number of accessions genotyped were selected for inclusion in the suggested final core (Table 6). This was achieved in each case by subdividing the dendrogram into subclusters, and selecting one or more accessions from each cluster, based on the level of diversity within each country and also the cluster analysis of the entire data set. The full details of the accessions recommended for inclusion in the final core can be requested from the authors.

TABLE 8. Clusters 1-5 identified by analysis of phenotypic variation in the New Caledonia taro collection.

Cluster no.	Access. no.	Access. no. identified for core	Cluster no.	Acc. no.	Acc. no identified for core
Cluster 1	9	2	Cluster 6	12	2
Cluster 2	5	1	Cluster 7	6	1
Cluster 3	7	1	Cluster 8	6	2
Cluster 4	10	2	Cluster 9	10	2
Cluster 5	5	1	Cluster 10	11	2

Note: For each cluster, we list the number of accessions present (Acc. no.) and the number of accessions kept in the core taro collection (Acc. no. identified for core) for further genotypic analysis.

Discussion

A regional core collection of taro germplasm has been successfully developed for Pacific Island countries. The selection of accessions to be included was based primarily on cluster analysis of the molecular data sets for the nine countries. However, the final selection took two other factors into account: first, comparison of selections with the entire data set cluster analyses, to ensure that duplicates were not included in the core; and second, the morphological analyses for New Caledonia, PNG and Vanuatu. At least one representative accession from each morphological data grouping was selected. Overall, the process of the taro regional core development can be summarized as below, following core development steps as defined by Brown (1995):

I. *Data assembly*: Molecular data sets generated and analyzed and considered in conjunction with all other available passport and phenotypic characterization data.

II. *Grouping accessions* (based on genetic similarity): In this case, the cluster analyses of the molecular data formed the basis of the stratified core sampling on an individual country approach. The number of groups assigned to each country was dependent on the total size of the country's collection and the level of selection necessary (i.e. 50% or 10% overall). However, the clusters from the analyses of the combined data sets from the entire region, were also referred to, in order to avoid duplication between countries, and in this sense it can be assumed that the clusters,

rather than the distinct countries, formed the basis of the stratified core sampling.

III. *Selection:* Having divided the collection into groups, the third step was to select entries from each group. If redundancy does not differ between groups, then the Kimura and Crow (1964) theoretical equilibrium model of selectively neutral mutants implies that representation should be in direct proportion to the size of each group. Consequently, the richness of diversity within each group was the major criterion for determining representation in the core, and this was achieved through the careful selection of a proportional number of unique accessions from each group, based on both within and between country analysis, which were cross-referenced to the morphological analyses in order to try and capture the broadest spectrum of morphological diversity in the regional core, in addition to genetic diversity. It was found, in a number of cases, that accessions with contrasting morphological characteristics clustered together, based on the results of the molecular analyses. This could be a reflection of the clonal nature of taro, and 'sports' type mutations that do not have any underlying genetic basis. In these cases, it was considered that the level of similarity, based on the molecular analyses, was superior to that based on phenotypic characteristics. In total, 164 (10% of Pacific Island region) taro accessions were selected following the above procedures.

IV. *Handling*: As a clonally propagated crop, the core collection will be maintained *in vitro*, under slow growing conditions, in the Regional Germplasm Centre, SPC, Suva, Fiji. It is anticipated that the collection will be duplicated at an international agriculture research centre as well as at the University of the South Pacific, Samoa. With the core established, programmes for evaluation will be considered.

Very few accessions were indistinguishable based on the SSR data set (Figure 4). Fiji's national collection had the lowest rate of unique DNA fingerprints, with only approximately 60% of accessions differentiated. This contrasted with results from the other Pacific Island countries, particularly those from Samoa and the Cook Islands, where over 92% of the accessions had unique DNA fingerprints.

There are a number of options that can be used to try to differentiate between accessions that appear to be identical. First, other polymorphic primers that have been developed (Mace and Godwin 2002) could be applied. Sixteen polymorphic markers are available, but only seven were selected in this study based on the quality of their electrophoretic banding patterns and the level of polymorphism generated. Consequently, the remaining 9 could be applied to limited data sets. Secondly, the ISSR technique, which has been optimized for taro germplasm (Godwin *et al.* 2001) and which has a higher multiplex ratio than SSRs (a large number of DNA loci that can be assayed in a relatively short period of time) could be used.

The cluster analysis of the entire data set revealed that there were numerous accessions in Pacific Island countries that were identical to PNG accessions. This indicates that originally the cultivars could have been introduced to the region from PNG. It also suggests that regional breeding programmes using lines resistant to taro leaf blight from PNG could be highly successful, as there should be no barriers to breeding since they originally came from a common source. The fact that many lines from the Pacific Island countries can be traced back to Papua New Guinea also raises the issue of whether that country is the centre of origin for taro. However, without Southeast Asia accessions included in analysis, it is difficult to draw conclusions.

Owing to political conflict, the Solomon Islands' taro collection was unavailable for inclusion in this study. However, the collection will be characterized at a later date, and 10% selected following the procedures described above for inclusion in the regional core.

Currently, the recommended core collection for Pacific Island countries consists of 164 accessions (Table 6), and this is likely to increase when the collections from Solomon Islands have been analyzed. After that, the next task will be to validate the core, which can be carried out in two ways: first, by comparing phenotypic characterization data of the core and with a randomly selected 10% of the Papua New Guinea collection, and second, by comparing the genotypic characterization of a random 10% sample from PNG (81 accessions) with the level of diversity of the core. The assumption in both cases is that the core will contain more than 75% of the genetic diversity of the regional collections, a figure which could not have been obtained by random selection alone.

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Conclusion

The chapters of this book highlight a growing number of countries and organizations involved in taro research and conservation. Preliminary efforts are described which illustrate the benefits of regional and international cooperation when tackling the challenge of taro conservation and utilization. Such efforts include regional approaches to undertaking taro collection, morphological and molecular methods for measuring and analysing diversity and innovative techniques for short to long-term *in vitro* conservation. These efforts have resulted in the development of two core collections representative of the genetic diversity of taro from the Pacific and Southeast Asia genepools, which can be readily managed and conserved *ex situ* and are available for international exchange. In fact, both the TANSAO and TaroGen core collections have now been distributed within Southeast Asia and the Pacific regions. In addition, we now have useful models of decentralized farmer evaluation and breeding that can make better use of the diversity represented in core collections for taro improvement.

Unfortunately, progress has been generally limited to the Pacific and Southeast Asia regions. There may be much to be gained by scaling-up or replicating many of the approaches reported here. Research on taro in West and Central Africa, the Caribbean and other regions that have received less attention from taro scientists and crop development agencies would lead to a deeper understanding of the many contributions that taro can make to food security. Such research would ensure that a true international effort emerges, building on the already established network of people working with taro, and providing a more thorough and multidisciplinary understanding of the genetic diversity of taro, its agroecology, and methods for effective conservation, improvement, and utilization of taro genetic diversity. An increased global interest in taro would also coincide with a growing interest in the cultivation of indigenous crop species by local communities. It is important for researchers and communities to capitalise on all the initiatives, and to recognise opportunities for benefit in the global taro genepool.

Ramanatha Rao
P.J. Matthews
P.B. Eyzaguirre
D. Hunter